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ექსპერიმენტული და კლინიკური მედიცინა

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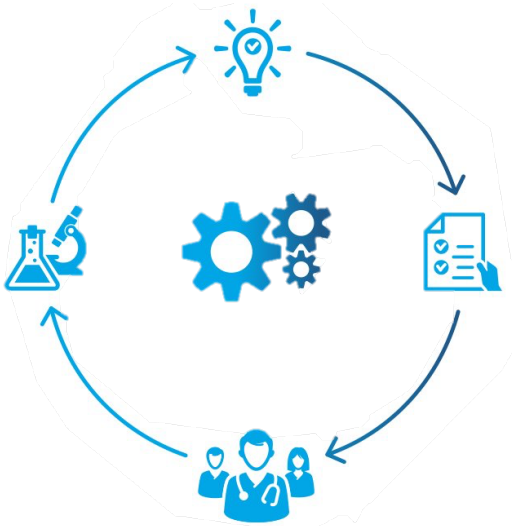

DISCOVERY PHASE OF MEDICAL RESEARCH

Since 2012, Tbilisi State Medical University has implemented the American MD Program (USMD program), based on a joint project between Tbilisi State Medical University, and Emory University School of Medicine (ESOM, Atlanta, USA).

A new, innovative American MD program is based on modern requirements for developing a medical curriculum: the program is integrated - integration firstly implies fundamental knowledge of biomedical science, and a combination of research and clinical skills – and their implementation in medical practice as the final outcome.

Elaboration of solid research skills is the one of the key threads of 6-year curriculum, which serves training of students in the fundamental fields of the sphere, like: interpreting and applying the findings in the medical literature, carry out research of relevance to the population of Georgia and/or their countries of origin, etc.

"Discovery Phase" is the final step in the development of the idea – it is the course to teach students modern approaches and basic principles of different types of medical research, to understand ethical and governance issues of medical research, practical skills, to develop a research project, and to provide engagement with clinical research programs and research communities.

Herein, we present selected research articles and review papers produced by students within the framework of the Discovery Phase course. We thank authors, mainly Class of 2022 students, for their contribution in this issue and greatly value the involvement of study participants in the reported projects. We give a special acknowledgement to the professors of Tbilisi State Medical University: Rima Beriashvili, Gaiane Simonia, Tinatin Tkemaladze, Tamar Akhvlediani, Eka Chkonia, Nani Kavlashvili, Tamar Didbaridze and Tea Kochoradze-Margishvili for their valuable work on the guidance of research projects and involvement in article reviewing process.

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ექსპერიმენტული და კლინიკური მედიცინა

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№2

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის მედიცინის, სტომატოლოგიის, საზოგადოებრივი ჯანდაცვისა და ფარმაციის ფაკულტეტების სადისერტაციო საბჭოების მიერ ჟურნალი ჩართულია სამეცნიერო გამოცემების ნუსხაში, სადაც რეკომენდებულია სადისერტაციო ნაშრომების ფრაგმენტების გამოქვეყნება

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2022

სარჩევი / CONTENT

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STRESS LEVEL AMONG MEDICAL STUDENTS IN GEORGIA AND ITS CAUSATIVE FACTORS

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Abstract

The study is aimed to determine the prevalence and the stressors among medical students, as well as to observe an association between the level of stress and its causative agents. A questionnaire, consisting of 2 sections, one defining the stress level and the other - causation, was sent to the medical students from year 1 to year 6 of the Tbilisi State Medical University (USMD Program). Perceived stress varied among the academic years, with the mean of 22.4 (on the scale of 0-40), which indicates the moderate level of stress. The first four years had significantly higher stress levels compared to the last two. The most significant stressors were exams and the studying process, whereas least stressful turned out to be communicative stressors. The findings can be useful for medical school professors and administration to understand the students' well-being, which will be helpful to create a more comfortable study environment.

Introduction

Stress is defined as the body's non-specific response to demands made upon it, or to disturbing events in the environment. It is not just a stimulus or a response but rather, it is a process by which we perceive and cope with environmental threats and challenges [1].

Medicine is a highly competitive field, thus being a medical student requires a lot of effort and time. Medical students experience significant stress during medical school, which can lead to depression, burnout, and anxiety [2]. In fact, there are several studies in different countries indicating higher than average stress levels among undergraduate medical students [3]. High levels of stress may have a negative effect on cognitive functioning and learning [4]. The results of studies suggest that mental health worsens after students begin medical school and remain poor throughout the training [5]. Many researches have shown that there are more emotional disturbances in the medical student community than in the general population and identifying the main stressors is an important aspect. A study in a north Indian medical university showed that stress amongst medical students is a dynamic process as the stressors keep changing with the year of study and constantly changing expectations of the students and the system [6]. Thai medical school results showed that about 61.4% of students had some degree of stress and academic problems were found to be a major cause of stress among all students [7].

The local studies estimating the levels of stress in the medical students of Georgia could barely be located with the extensive internet-based search. The present study was conducted, with the goal of determining the levels of stress of the medical students doing the bachelors in the country. The aim further includes defining the specific causative agents and linking them to each year of the medical school.

The study results are beneficial for the students, to identify the upcoming difficulties each year can bring and they can implement some modifications to at least partly avoid the causative factors in the next academic year. The research results are also thought to be insightful for the university administration, they will have more perspective on the main stress-inducing factors for their students and subsequently will be able to improve their experience.

Methodology

The study was quantitative, using an online questionnaire as a tool, which was composed of 2 parts. First part was assessing general stress levels using a widely used "perceived stress questionnaire" by Cohen, which consists of 10 questions and is widely used. The questions asked about one's feelings and thoughts during the last month. The participants were asked to indicate by circling how often they felt or thought a certain way. For example, inability to control irritation or inability to cope with some things etc. Each question has a 4-point scale and individual scores range from 0 to 40. Higher score indicates higher perceived stress level. The calculated scores can be put in 3 categories: cumulative score 0-13 (low

stress); 14-26 (moderate stress); 27-40 (high stress). The results were divided by the academic year, thus mean, median and mode were calculated for each year.

The second part of the questionnaire consists of 28 questions, which are specifically designed for the study sample with the goal of determining the most stressful factors for the students of each academic year (1st, 2nd, 3rd, 4th, 5th and 6th). The above-mentioned 28 questions are themselves divided into 5 parts. The sections are titled accordingly: Evaluating stressors, Studying Process, Social Stressors, Communicative Stressors and Language Barriers. The scoring system for each question consists of 4 ranges: <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (extremely stressful). The answers are divided according to the academic years; the mean of the results is calculated and depicted on the charts.

The questionnaire was sent with Google Forms via Gmail and the results were analysed using Microsoft Excel.

Results and Discussion

Analyses of the perceived stress questionnaire are shown in **Table 1** and **Figure 1**.

Table 1. Perceived stress scale results; 0-13 (low stress); 14-26 (moderate stress); 27-40 (high stress).

Year	Mean	Median	Mode
1st	23	24	30
2nd	26	26.5	27
3rd	25.3	28	28
4th	24.8	27	27
5th	17.3	17	17
6th	18	19	25

Figure 1. 0-13 (low stress); 14-26 (moderate stress); 27-40 (high stress)

The second part of the questionnaire consists of 5 subgroups. The first subgroup of the questions was about evaluating stressors, emphasizing on homework and exams. The results showed that, the amount of homework given to students is more stressful on the first two academic years of medical school: 1st year (mean 2.208) peaking for 2nd year students (mean 2.214), followed by 4th (mean 2.13), 6th (mean 2.0), 3rd (mean 2.06), and the 5th year (mean 1.72). This could be explained by the transition from the school to the medical university, as adapting to the new environment and the different study process can be challenging.

Final exams turn out to be one of the biggest obstacles for each academic year. The 2nd year students have found the final exams most stressful (mean 2.857), followed by the 4th year (mean 2.666), then the 3rd year with (mean 2.647), 6th year (mean 2.5), 1st year with (mean 2.458), and at last the 5th year (mean 1.77). The fact that the first 3 academic years and 1st semester of the 4th year are more theory-based can play a role in students' perception of the exams.

The exam preparation time was most stressful for the 1st year students (mean 2.66), followed by the 3rd year (mean 2.411), 4th year (mean 2.4), 2nd year (mean 2.28), 6th year (mean 2.0) and lowest for

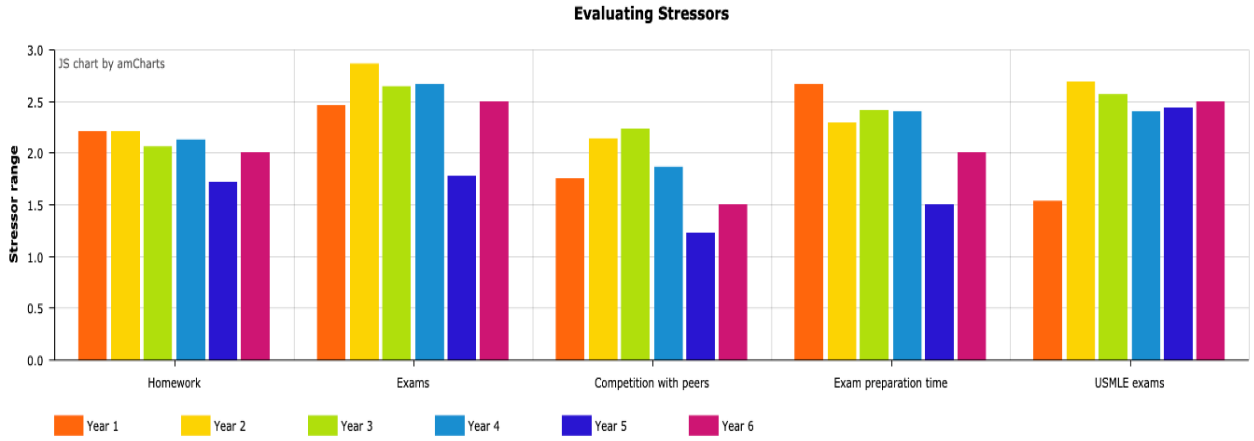
the 5th year (mean 1.5). This could be explained by the fact that students at the beginning of the medical school might find it difficult to overcome the huge study material compared to the senior students as it is significantly decreased in 5th year.

As many students are preparing for the United States Medical License Exam (USMLE) throughout the medical school, it is not surprising to see that it is one of the biggest stressing factors for each year. Especially for the 2nd year students (mean 2.69), as this is the time when the preparation for the USMLE begins, followed by the 3rd year (mean 2.56), 6th year (mean 2.5), 5th year (mean 2.44) and 1st year (mean 1.53).

Competition with peers is not a main stress inducing factor for most academic years except the 3rd (mean 2.23) and the 2nd year (mean 2.14). Followed by the lower mean scores for the 4th year (mean 1.866), 1st year (mean 1.75), 6th year (mean 1.5) and the 5th year (mean 1.22).

These results are demonstrated in **Figure 2**.

Figure 2. <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (extremely stressful).



Another subgroup of the questions was about social stressors as it can influence students' health and academic performance. Variable options were financial responsibilities, lack of infrastructure, sleep schedule, social and private life, living far from family, relationship with seniors.

The graph of financial responsibilities was designed to find out how stressful the financial side was for the students. As the results show, financial instability is the most stressful for the 4th year (mean 2.6), followed by 3rd year (mean 2.4), 2nd and 6th years with (means 2.5), 5th year (mean 2.0) and lastly year one with the (mean 1.8).

The lack of infrastructure, such as: student spaces, a campus, parking spaces or a cafeteria in the university, has the biggest impact on the 3rd year students (mean 2.8), 6th year (mean 2.66), followed by the 5th year (mean 2.6), 4th year (mean 2.3), 2nd year (mean 2.2) and 1st year (mean 2.08).

Sleep schedules have a huge influence on students' everyday performance. Balanced sleep schedule is a very good indicator of health. The students in the 1st, 2nd and 3rd years had the most problems with the sleep schedule (mean 2.6), followed by the 4th year (mean 2.24), 6th year (mean 2.25) and the 5th year (mean 2.1).

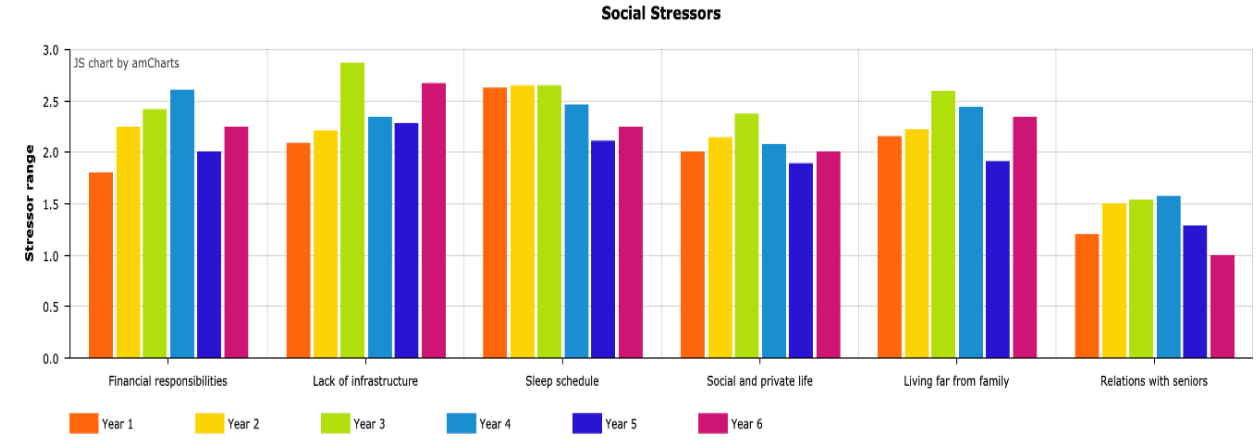
For a medical student, it is difficult to balance the social and academic life. According to the results it was the 3rd year students have the highest score (mean 2.3), followed by the 2nd year (mean 2.14), 4th year (mean 2.07) and the 1st and 6th years (mean 2.0), lastly the 5th year (mean 1.8).

Living apart from the family can contribute to the students' stress and can have an influence on many different aspects of life, including academic performance. Surprisingly, the 3rd year showed the highest scores (mean 2.6), followed by 4th year, (mean 2.4), 6th year (mean 2.33), 2nd year (mean 2.2), the 1st year (mean 2.15) and the 5th year (mean 1.90).

In the medical world, supporting each other is crucial. Relationships with seniors could be one the most effective ways to share experiences with each other and get valuable pieces of advice. Thus, difficulties with the seniors can also be a stressor. The 2nd, 3rd and the 4th years have the same mean score (mean 1.5), followed by the 1st and the 5th year (mean 1.2).

These results are demonstrated in **Figure 3**.

Figure 3. <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (extremely stressful).



Language barriers are the next subgroup of the questionnaire. The stressors include adapting to English, English among lecturers, language barriers at clinic, translating at clinic and communication with patients. Since at the Tbilisi State Medical University there are a lot of international students, having a language barrier could be significantly stressful for them. The questions were asked to find out how students were affected by these stressors.

The first question was about adapting to studying in English, since most of the student's first language is not English. It turned out to be the most stressful for the 1st year (mean 1.23), followed by the 3rd and the 6th years (mean 1.25), the 2nd and the 4th years (mean 1.08) and lastly year 5 (mean 1).

The next question was about the level of English among the lecturers, which was the biggest stressor for the 5th year students (mean 2), followed closely by the 1st year (mean 1.96), 4th year (mean 1.93) and the 3rd year (mean 1.81) and lastly 2nd year (mean 1.71) and the 6th year students (mean 1.5).

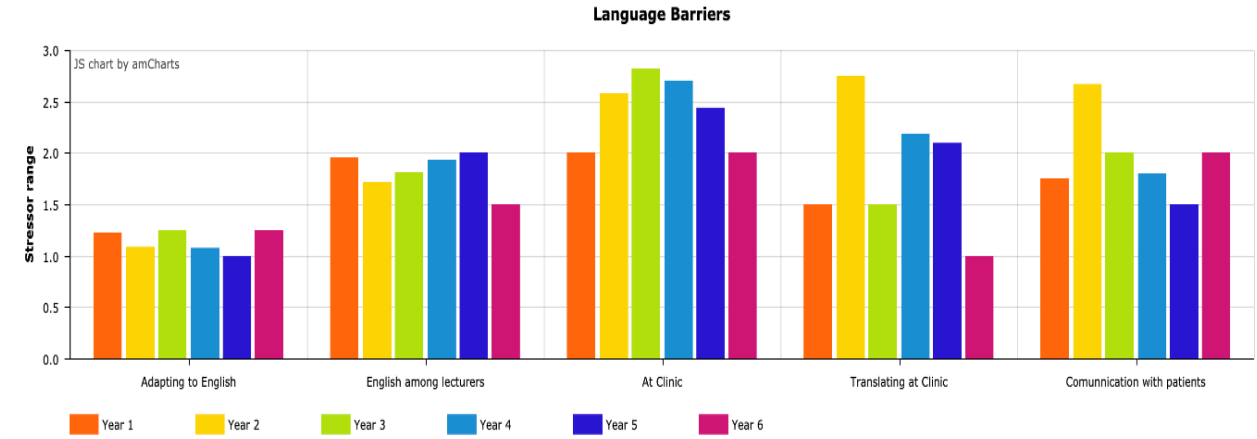
Language barriers at clinics could contribute to a very stressful situation between the student doctors and the Georgian patients. The next question was specifically asked about that. According to the questionnaire, it turned out to be the most stressful for the 3rd year students (mean 2.81), following the 4th year (mean 2.7), 2nd year (mean 2.57), 5th year (mean 2.44) and at last 6th and the 1st years (mean 2). This could be explained by the lack of clinical exposure for the first-year students and as for the 6th year students, years of practice.

As many medical students are not Georgian, translating from the Georgian to English during the clinical rotations and history taking is crucial, which turned out to be particularly stressful for the 2nd year students (mean 2.75), followed by the 4th year (mean 2.18), 5th year (mean 2.09), the 1st and the 3rd years (mean 1.5) and lastly the 6th year students (1.0).

The last section was about communication with patients, which once more was the biggest stressor for the 2nd year students (mean 2.67), followed by the 3rd and the 6th years (mean 2.0), the 4th year students (mean 1.8), the 1st year (mean 1.75) and finally the 5th year students (mean 1.5).

These results are demonstrated in **Figure 4**.

Figure 4. <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (extremely stressful).



The next section in the questionnaire is about communicative stressors. For example, getting feedback from the professors, receiving criticism about the performance, atmosphere created by the lecturers and generally the ability to communicate with administration. Good communication between students, lecturers and administration is extremely important for establishing a good and productive atmosphere.

Communication with lecturers turned out to be the most stressful for the 3rd year students (mean 1.94) followed by the 4th year (mean 1.8), 2nd year (mean 1.57), 6th year (mean 1.5) and lastly year 1 and 5 (mean 1.33).

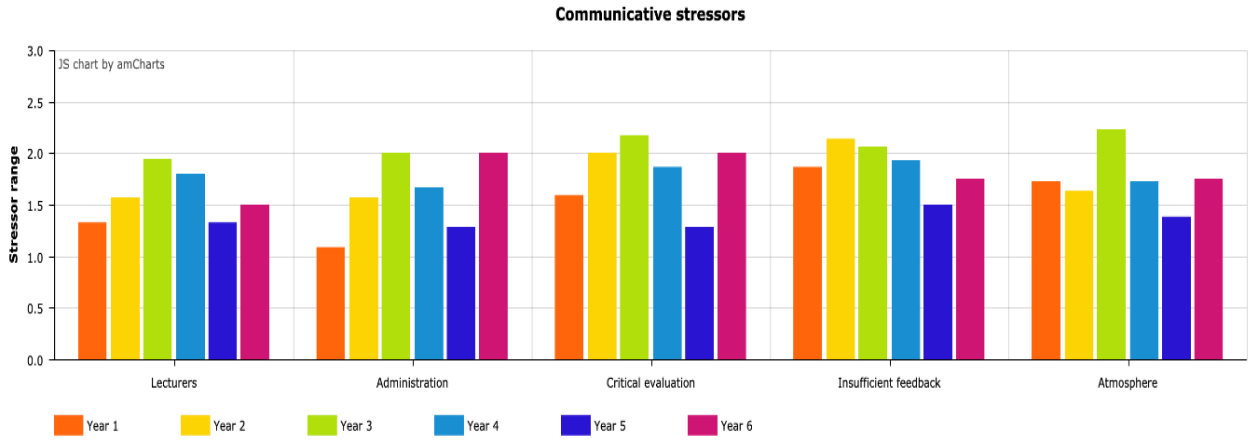
3rd year students also have the highest score for communication with the administration (mean 2.0) as well as the 6th year students (mean 2.0), followed by the 4th year (mean 1.67), 2nd year (mean 1.57), 5th year (mean 1.28) and lastly 1st year (mean 1.08).

Receiving critical evaluation from the lecturers was the most stressful again for the 3rd year students (mean 2.17). Followed by 2nd and 6th year (mean 2), 4th year (mean 1.86), 1st year (mean 1.59) and lastly 5th year (mean 1.27).

Getting insufficient feedback from the lecturers is the biggest stressor for the 2nd year students (mean 2.14). Followed by the 3rd year (mean 2.05), 4th year (mean 1.93), 1st year (mean 1.86), 6th year (mean 1.75) and lastly 5th year (mean 1.5).

The atmosphere created by the lecturers turned out to be the most stressful for the 3rd year students (mean 2.23), followed by the 6th year (1.75), 4th year (mean 1.73), 1st year (mean 1.72), 2nd year (mean 1.64) and finally 5th year (mean 1.38). These results are demonstrated in **Figure 5**.

Figure 5. <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (extremely stressful).



The next subgroup of questions is about the studying process and how the different factors, such as: the parents' expectations, patient reporting to the doctors, lack of workshops and the first aid training, studying non-medical subjects and the duration of seminars influence it. Overall, the duration of seminars and parents' expectations were least stressful for the students.

As parents play a major role in every student's life, the students were asked to assess if the parents' expectations play a role in their stress. The 2nd (mean 2.35) and the 3rd year students (mean 2.23) shared increased scores. Followed by the 1st year (mean 2.04), the 4th and the 5th (mean 1.7) and the 6th year students (mean 1.8).

The lack of first aid training was equally stressful for the 2nd, 3rd and 4th year students (mean 2.6), followed by the 5th year (mean 2.1) the 1st year (mean 1.9) and lastly the 6th year students (mean 1.75).

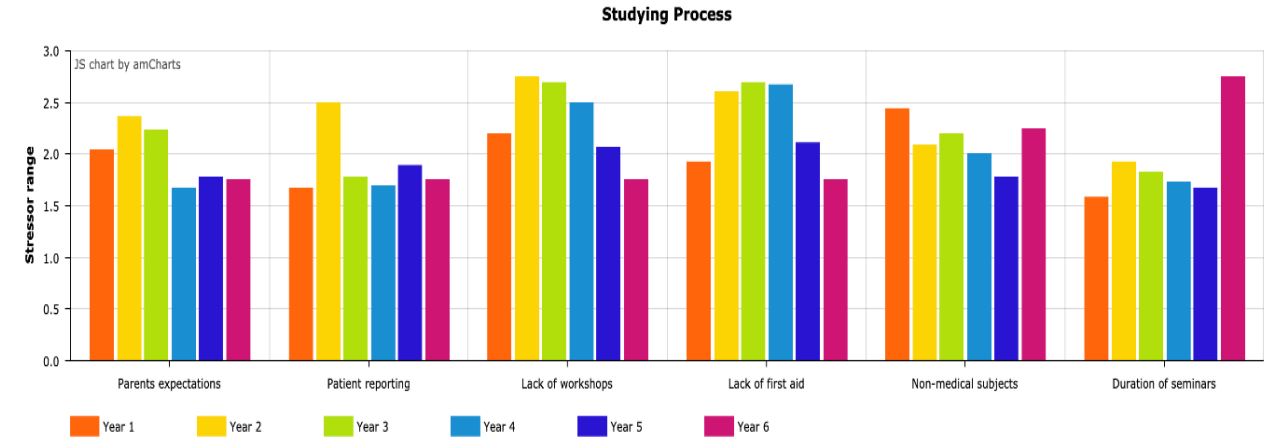
About the duration of seminars and its effect on students' stress level, surprisingly, the 6th year (mean 2.75) is followed by the 2nd and the 3rd years (mean 1.9), the 4th and the 5th year students (mean 1.7) and at last the 1st year (mean 1.5).

As practical workshops are important part of practicing medicine, the next question is about the intensity of workshops provided to the students, where with the 2nd year students (mean 2.7) expressed their concern, followed by the 3rd year students (mean 2.6), 4th year (mean 2.5), 1st year (mean 2.2), 5th year (mean 2.05) and lastly the 6th year students (mean 1.75).

Patient-doctor and doctor-doctor communications are without a doubt the most important parts of medicine, the students were asked to evaluate the effectiveness of communication with patients. The 2nd year students (mean 2.5) have the biggest problem with the interaction. Overall, with the low means, the 5th year (mean 1.8), followed by the 3rd year (mean 1.7), 6th year (mean 1.75) and the 1st and the 4th year students (mean 1.6).

Lastly, non-medical subjects have the biggest impact on the 1st year students (mean 2.4) as they are substituted by the medical ones from the 2nd academic year. The 1st year students are followed by the 3rd and the 6th years (mean 2.2), 2nd and the 4th years (mean 2) and the 5th year students (mean 1.7). These results are demonstrated in **Figure 6**.

Figure 6. <1.5 (low stress), 1.5- 2.0 (moderate stress), 2.0-2.5 (high stress), >2.5 (Extremely stressful).



Conclusion

Being a medical student is a difficult challenge as there are many factors causing stress during the studying years.

The research conducted showed that even though overall it gets easier to cope with the specific stress inducing factors as the students advance (4th, 5th and 6th years), there are some new things that become stress inducers at this point, such as the financial issues and the factors more connected to the clinical experiences.

As described, every year of medical school comes with a specific stressor, with the most difficult being the 1st, 2nd and 3rd years as the most important changes happen during this time. Moving from high school to the medical school is the toughest, as well as the transition from the first 2 years to the following ones, as the non-medical or basic subjects are substituted with the pathology and clinical rotations.

However, even though the research shows quite a few highly stress inducing factors varying throughout the different years of the medical school, on the overall perceived stress scale the moderate mean result was calculated.

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DO FAST/JUNK FOODS HAVE AN EFFECT ON ASTHMA IN CHILDREN

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Abstract

Certain types of food may increase or decrease the frequency of asthma exacerbations. The study is aimed at determining whether there is an association between consuming fast/junk foods and asthma. A well-adjusted, standardized questionnaire was used for the collection of information. This study is focused on finding a relationship between fast food intake and frequency and severity of asthma exacerbation rather than a causative relationship. This is a cross-sectional study done in the southern part of India in the state of Kerala. The correlation between fast food and asthma is established through a detailed analysis of the results. After adjusting for confounding variables like Environmental Tobacco Smoke (ETS) and allergens at home we were able to indicate that fast/junk food consumption is a risk factor for asthma symptom exacerbation in children.

Introduction

Asthma is one of the most commonly seen chronic diseases in childhood. Around 300 million people have asthma worldwide, and it is likely that by 2025 a further 100 million may be affected [1]. According to WHO 2016, 15–20 million people out of the global asthma population were from India alone [2]. It is a serious non-communicable disease with significant public health implications for both children and adults, including high mortality rates in severe cases. Despite knowing extensive details about asthma, its prevalence and severity increase every year. A recent study in the United States has shown that one in every 13 Americans lives with asthma, and similar global asthma reports have estimated that approximately 20-25 million Indians are affected by asthma, suggesting that almost every one in 10 asthma patients is an Indian. An epidemiological study conducted in India as a part of a global initiative showed that more than 6% of the child population is affected by asthma in India [3]. Over the past few decades, an increase in asthma prevalence has been noted to impact public health, and accordingly, several studies are conducted regularly to adequately assess the condition and its different aspects. According to current data, asthma is a complicated condition with interactions between genetic susceptibility, host factors, and environmental exposures playing a role in its etiology [4]. One of the growing problems that have been hypothesized for the rise in the incidence of asthma is the change in diet. Fast food restaurants and junk food items can be found in practically every city in the world. While most people are aware that fast or junk food is not the healthiest option, many are unaware of the extent to which it can harm the body. A poor-quality diet is believed to trigger more inflammation in the body, which can aggravate asthma symptoms. Many studies show consuming fruits and vegetables can prevent asthma while consuming fast or junk food increases the risk of contracting the disease. Several studies have been conducted regarding the effect of diet on asthma globally, but endemic to southern parts of India, there is a negligible amount of data regarding it [5]. This study aims to prove or show an association between increased fast and junk food consumption and an increase in exacerbation of asthma.

Methods

The study was initiated after every team member underwent a thorough review of the base topics to establish a solid foundation for the research and its state of the art. Data from previous studies were collected, explored, and then a proposal was drafted to initiate the research process. This study is a cross-sectional study conducted at the Amala Institute of Medical Science of Kerala between the period of January 2021 and June 2021. The consent forms were approved by the hospital administration and the consent was obtained from the parents in advance regarding the collection of data through a questionnaire-based survey. Children aged 6 to 14 years with the diagnosis of asthma and no exposure to smoke were determined as eligible for the study. The diagnosis of asthma was based on the GLOBAL INITIATIVE OF ASTHMA (GINA) guidelines. The diagnosis of asthma was met if the following criteria were met: (a) More than one of the following - wheeze, chest tightness, shortness of breath, and cough. (b) Symptoms occur variably over time and vary in intensity. (c) The symptoms occur or are worse at night or early morning.

(d) Symptoms are often triggered by exercise, laughter, allergens, or cold air. The severity of asthma was classified from intermittent to persistent asthma. The diagnosis was made by the pulmonology department based on GINA criteria and the participants were currently treated at the hospital. Inclusion criteria children with asthma flare-ups and informed consent from parents. Due to the limited number of patients during the covid pandemic, a sample size of 42 patients between the age of 6 to 14 years were taken. Of the 42 patients whose information was taken 6 were ruled out due to the exposure to smoke and the presence of other cardiopulmonary diseases. From the 36 study participants who survived the exclusion criteria 12 were classified into the main group based on their fast-food consumption (more than twice a week) and 24 as the controls who had not consumed any fast food or less fast food.

Data collection was done exclusively through interviews/surveys conducted with the parents/guardians or the physician of the participating patients with the help of a questionnaire. The questionnaire consisted of several questions regarding, the overall condition and the diet of the participant, course of asthma and its severity of the patient. Dietary habits were based on the consumption of specific food in the past 12 months. Specific food groups were included: meats, seafood, fruits, vegetables, pulses, cereals, rice, pasta, bread, nuts, eggs, and fast/junk food. The specific food was explained following the various regional food groups and fast/junk foods.

The asthma section of the questionnaire was derived from a pre-coded standardized questionnaire based on a phase 3 study conducted in New Zealand (ISAAC) [6]. The dependent variable was asthma severity and the independent variable was the amount of fast/junk food consumption. The severity was assessed with the different symptom presentations of asthma and was classified into intermittent, mild persistent, and moderate asthma. The symptoms and clinical presentations considered were wheeze, nighttime awakening, speech limitation, cough, and asthma exacerbation.

Results and Discussion

A total of 36 acceptable responses were collected and their data were recorded and compiled. From the 42 responses received, 6 were excluded due to responding to having been exposed to tobacco smoke at home. The majority of the responses were filled up by parents of the participant (80.6%, n = 29), the rest were filled by the guardian or the doctor of the child (n = 5, n = 2). According to the frequency of consumption, the study sample was divided into two groups - Main group consumed fast food more than two times a week (12 patients) and the control group consumed twice or less than two times a week (24 patients). 75% of the participants in the study were in the age group of adolescents between 10 and 14. 23 responses (63.18%) from the total were male children and held the majority in comparison to only 13 female children.

Table 1 shows the baseline characteristics of the study population. There was no age or sex-based predilection from our analyses of the data in general. 19.4% of our study population was at risk to get exposed to some kind of allergen at home. Dust, food i.e nuts, shellfish, pets, medication were considered as allergens in the study and all participants were asked if they had any exposure. The **figure 1** shows the trend in ages of starting consumption of fast/junk foods. In this figure, we can see that 50% of the study population who consumes fast/junk food starts to consume fast/junk food at the age of 8 to 9 years. The **figure 2** highlights the participants who consume fast/junk. This figure shows that a majority of our study population (77.8%) consume fast food albeit in different frequencies and quantities. The **figure 3** indicates the BMI statistics of the study population. As seen 52.78% of the study participants have a normal BMI, the remaining participants fall into the categories of obese (30.55%) and overweight (16.67%). The BMI was calculated with the CDC's calculator for children's BMI, and the children were categorized with the CDC classification [7]. **Table 2** displays the asthma symptom presentation of the participants in the past month. The largest group of the participants (30.6%) present with having episodes of wheezing at least 4 to 12 times a month, followed by 27.8% of the study participants presenting with wheeze 1 to 3 times a month. It can be seen that the majority (47.2%) of the patients have had no nighttime awakenings and 33.4% of the population are seen to have nighttime awakenings at least once a month. Finally, in **table 3** the participant's fast/junk food consumption and their asthma severity were tabulated. Only 8 of the participants did not consume fast/junk food and reported symptoms of asthma with intermittent severity. From the 12 study participants who consumed fast/junk food more than twice a week, 8 of them presented

with moderate persistent asthma, indicating 61.53% of them demonstrated more asthma symptom exacerbation. 8 of the 36 total participants who did not consume fast/junk foods had an intermittent asthma severity. Table 3 also showed that participants who consumed fast food more were classified with more severe form of asthma indicating they had increased presentations of asthma symptoms compared to other groups as none of the 12 more frequent consumers of fast/junk food were categorized as having intermittent asthma i.e. the least severe form among the 3. In general, the participants who consumed fast/junk foods more frequently presented with worse severity in comparison with those who didn't. However, there were an array of mixed responses with 32.14% of the fast/junk food consuming participants presented with intermittent asthma.

Table 1 - Patient/participant baseline characteristics

Characteristics	Frequency (n)	Percentage (%)
AGE GROUPS		
6-9 CHILDREN	*9*	*25*
10-14 ADOLESCENTS	*27*	*75*
SEX		
MALE	*23*	*63.9*
FEMALE	*13*	*36.1*
EXPOSURE TO ALLERGENS		
YES	*7*	*19.4*
NONE	*29*	*80.6*
HOW FREQUENTLY DID YOU CONSUME FAST FOODS?		
NEVER CONSUMED	*8*	*22.2*
OCCASIONALLY CONSUMED	*6*	*16.7*
ONCE OR TWICE PER WEEK	*10*	*27.8*
MORE THAN 2 TIMES A WEEK	*12*	*33.3*

Figure 1.

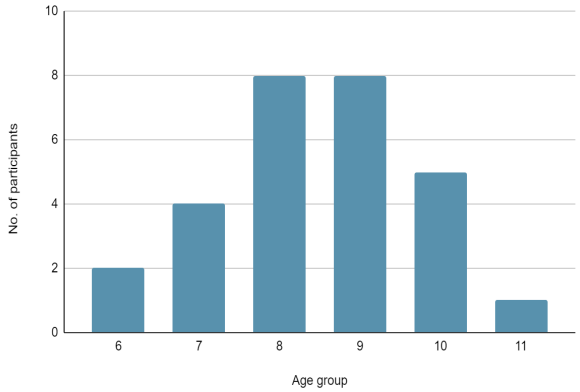

Figure 2.

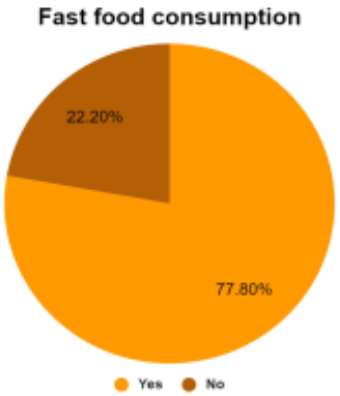

Figure 3.

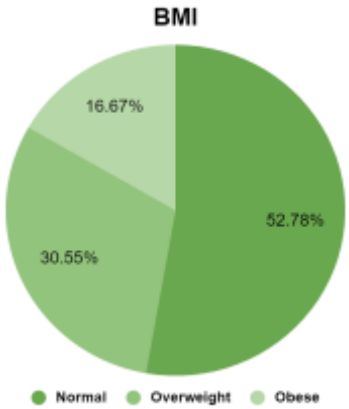

Table 2 - Asthma features in the participants

Symptom presentation	No. of individuals	Percentage (%)
WHEEZE		
none	*8*	*22.2*
1-3 times a month	*10*	*27.8*
4-12 times a month	*11*	*30.6*
More than 12 times a month	*7*	*19.4*
NIGHTTIME AWAKENING		
none	*17*	*47.2*
Once a week	*12*	*33.4*
More than once a week	*7*	*19.4*
ASTHMA FLARE-UPS/ EXACERBATION		
none	*8*	*22.2*
Less than twice a week	*14*	*38.9*
Twice or more a week	*14*	*38.9*

Table 3 - Fast/junk food consumption and asthma severity

Study groups		Asthma severity (abs. N/%)		
		Intermittent	Mild persistent	Moderate persistent
Main group	*More than twice a week (12)*	0	4	8
Control group (24)	*Non-Fast/junk food consuming participants (8)*	8	0	0
	Once or twice a month (6)	5	1	0
	Once or twice a week (10)	4	4	2

This cross-sectional study showed an association between the consumption of specific food groups, explicitly fast or junk food groups, and the severity of asthma in children aged 7 to 14 from Thrissur, Kerala. The main goal was to inspect any association between consuming fast/junk foods, frequency of consumption, and asthma symptom exacerbation.

The rate of fast and junk food consumption has been on the rise globally. The study setting is in a developing country and has accounted for an increase in fast and junk food consumption in the general population as well as the younger population especially the adolescents. From the responses of the study group, 77.8% of the participants were consumers of fast or junk foods and 50% of participants started consuming fast food at the age of 8 or 9. A study done to examine the relationship between BMI and junk food consumption previously has shown that although aware of the effects of consuming junk foods, adolescents are reluctant to change their dietary habits [8].

In this study, the frequency of consuming fast or junk foods more than twice a week was seen as correlating with an increased presentation of wheeze, and nighttime awakening, as more frequent asthmatic symptoms. Study participants with increased rate and quantity of fast or junk food consumption showed a worse presentation of asthma exacerbation and were categorized as moderate persistent in asthma severity. There have been similar studies conducted in various countries reporting both corroborating and contradicting conclusions. A cross-sectional study conducted in Brazil attributed the asthma symptom exacerbation towards obesity in children, in preference to their dietary patterns [9]. In contrast, a study done in Shanghai has illustrated the relationship between consuming fast foods and asthma and allergies and showed a correlation between consuming fast food, hamburgers, and asthma in an exposure-response form [10].

Studies have postulated fast and junk foods forward diets to be having lower levels of antioxidants & n-3 fatty acids and higher levels of n-6 polyunsaturated fatty acids and have generated possible mechanisms in which they affect asthma and other atopic medical conditions [11,12]. Fast and junk foods are predominantly higher in various unsaturated fatty acids, dietary sodium, and sugar, which suggests a connection between consumption of fast/junk food and asthma. Fatty acids and antioxidants involvement in immunomodulators, and dietary sodium predisposing to wheeze-like conditions [12]. The findings are

practically consistent with those of previous similar studies, and to the best of our awareness, this study is the first one in Kerala to examine the association between fast or junk food intake and asthma. An unexpected outcome was that 53% of the participants were classified as having a normal BMI and the rest of the participants were divided into overweight and obese, 30 and 17% respectively. Unlike a study previously conducted shows relatively less association between obesity and asthma symptom presentation [9].

Various limitations must be considered when interpreting this study. Through a cross-sectional study, a time-related association between consuming fast or junk foods and asthma can be confirmed. Compared with other studies in the same background, the small sample size makes this study inadequate for investigating and well-establishing the association between diet and asthma. Since a wider range in the age of the study participants may present as a confounding variable, a slight adjustment was made in the age groups (7-14) to accommodate a lesser margin of impact from the variable. Non-asthmatic children were excluded from the study because the goal of this study was to explore the link between dietary habits, specifical consumption of fast and junk foods, and asthma severity and symptom exacerbation rather than the risk of developing. The findings may not apply to the general population of asthmatic children in the region as the sample size is relatively small and the study is hospital-based. Despite the lack of statistical analysis in our study, a positive connection between disease severity and the consumption of fast or junk foods is seen. The positive link with severe disease shows that fast and junk foods are a predictor of disease severity rather than disease occurrence.

The ISAAC questionnaire used worldwide for asthmatic research was the strong foundation for the survey conducted. Although the questionnaire was inspired by a previously validated and recognized set of questions, it was filled by parents/guardians of the participants. A result of systematic bias is unlikely but there were relatively low responses that can indicate a non-response bias which can be attributed to the current ongoing pandemic situation. Additionally, there was only a basic explanation about the various food groups and did not include any names in particular which could cause a misclassification in the dietary section of the responses. Self-reporting also has a potential consequence of recall bias. Another plausible margin for an erroneous result is the socioeconomic factor which may confound the association found as it was not adjusted for. Statistical analysis was overlooked in the paper, owing to limited resources, and substantially low responses for the questionnaire. Further studies can be done in search of a causal relationship after large population-based cross-sectional studies have been conducted to show a statistical association between the consumption of fast/junk foods and asthma symptom exacerbations.

In conclusion, our study assessed the correlation between fast and junk food consumption and asthma symptom exacerbation in children. Our results have demonstrated that there is a positive association between fast or junk food intake and increased presentation of asthmatic symptoms. This is more prevalent in individuals who consume fast foods more than twice a week. More research is needed to validate the correlations found in this study and determine whether there are any possible causative links between fast and junk food intake and asthma symptom exacerbations.

Acknowledgement

The authors of this study are grateful to all the participants of the study and their parents/guardians. We would also like to thank our colleague, medical student Ms. Thrilochanan from the participating hospital for helping and coordinating with us throughout the data collection process. Lastly, the ISAAC PROGRAM was the basis of our investigation and their research work inspired the further endemic study we conducted.

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STRESS IN HEALTHCARE PROFESSIONALS DURING COVID 19: A COMPARISON BETWEEN GEORGIA AND UAE

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Abstract

The COVID-19 pandemic increased workload and stress for the healthcare workers leading to their burnout. To identify and compare the contributors and effects of increased stress between Georgia and the UAE. An e-questionnaire related to stress and its contributing factors was made on google forms and sent to HCP's throughout Georgia and the UAE. We received 75 responses, with 77% being HCP's from the UAE and 23% from Georgia. Most of the responders from Georgia stated that they felt hopeless. The increase in workload was more significant in HCP's of UAE, and the majority had chosen a score of 3 out of 5. This severe psychological impact was related to several stressors observed in this study. The lack of participants from Georgia curbed our results, and further investigations should be considered.

Abbreviations

UAE - United Arab Emirates; HCP - Healthcare Professionals; FHCW - Frontline healthcare workers; WHO - World Health Organization; RNA - Ribonucleic Acid; AKI - Acute Kidney Injury; ARDS - Acute Respiratory Distress Syndrome; SARS CoV- 2 - Severe Acute Respiratory Syndrome coronavirus – 2; COVID - 19 - Coronavirus disease of 2019; PPE - Personal Protective Equipment; USA - United States of America

Introduction

In the province of Wuhan, China, on 31st December 2019, there developed a case of pneumonia-like symptoms caused by a virus that started spreading like wildfire. This virus spread to other countries and was then declared a global pandemic on 11th March 2020 by the WHO. This RNA virus called the SARS- CoV2 or COVID 19 is transmitted through respiratory droplets. When acquired, it starts to show symptoms like fever, dry cough, malaise, fatigue, nausea, vomiting, diarrhea, etc., which means that it can affect any organ system [3]. People of all ages were affected by it, but the more severe manifestations were seen in those with other comorbidities like hypertension, diabetes, cardiovascular diseases, chronic lung diseases, etc. [15]. Of the fatal diseases, it also causes ARDS [6], pulmonary fibrosis [8], encephalitis, stroke, Guillain- Barre syndrome [4], AKI [1], etc. Hence the understanding of the disease is crucial at this point.

Due to its unfamiliarity, many countries took several measures to curb the spread of the virus, such as implementing masks, social distancing, routine sanitation regimens, use of hand sanitizers, etc. [10]. Developing countries like Georgia reported their first case of COVID infection on 26th February

2020. Since then, there have been 368,022 confirmed cases with 5,335 deaths reported [13]. The country handled the situation in four stages involving prevention, management, declaring a state of emergency, and placing movement restrictions [14]. In developed countries like UAE, the first case was diagnosed on 23rd January 2020, the total number of cases has been 632,907 confirmed cases of COVID-19 with 1,811 deaths, reported [12]. UAE implemented several initiatives to combat COVID-19, including surveillance and contact tracing, proper containment, mental health support, mass testing and treatment, government economic support, and national vaccination programs. However, despite these measures, the number of cases persistently rose due to noncompliance, increased testing, relapses, reopening, etc. [16] Healthcare workers faced the ultimate challenge of treating these patients with very little knowledge of the disease and its treatment.

As the workload for these frontline warriors started increasing, physician burnout became more and more imminent [2,7]. Burnout is a psychological syndrome characterized by emotional exhaustion, depersonalization, and a sense of reduced accomplishment in day-to-day work [10]. It meant that the frontiers were perishing and needed an immediate measure to combat this problem.

Therefore, this study aims to identify the contributors to increased stress and burnout in HCPs and suggest methods to alleviate the problem. We do so by in-depth comparing the stress levels experienced by HCPs in UAE and Georgia to find similarities and differences in how the situation affected them, the contributors to the problem, and how well the situation was and could be managed.

Methods

We have used an online questionnaire, which we shared among health care professionals (HCPs) in Georgia and the UAE via emails, Facebook, and WhatsApp groups.

An e-questionnaire was conscientiously prepared on google forms; intended to be brief to complete the survey within 2-3 mins. The participants were informed about the purpose of the study and data confidentiality priorly. Upon clicking on the link, all the participants were directed to the survey, and they had to answer several questions.

Participants were required to be 20 years and above, be capable of reading and writing English. They must also be currently working as health care professionals in Georgia or UAE with access to the internet.

The survey consists of several questions. The first section involves socio-demographic details such as country of practice, designation, current specialty, age, and gender.

The second section consists of the stress questionnaire: level of stress during the pandemic - scored from 1-10, where one is mildly stressed, and ten is highly stressed, symptoms experienced during the pandemic (for example, anxiety, depression, irritability, etc.), change in workload due to COVID-19 - scored 1-5 where one is no effect, and five is severely affected and what helped you during this pandemic (for example, friends and family, acceptance that you cannot control everything, etc.).

The third section involves a series of yes or no or maybe questions. The questions mainly relate to mental stress, lack of knowledge regarding prevention and protection against COVID-19, moments of hopelessness/pessimism, anxiety being infected during the pandemic, changes in motivation to work during this pandemic, and their opinion regarding the management of COVID-19 by their respective country.

The final section of the questionnaire was intentionally kept open-ended to gather a wide range of suggestions from the HCPs and to increase the depth of responses which would ultimately assist the health care community in coping with the stress of the pandemic.

Results

We received a total of 82 responses from both Georgia and the UAE. Out of 82, seven responses had to be excluded due to repetitions. The remaining 75 were considered for evaluation and comparison, of which 17 responses (22.7%) were from Georgia, and the remaining 58 (77.3%) were from UAE. The majority of those who responded were doctors - 35 (46.7%), closely followed by nurses - 34 (45.3%) (**Figure 1**). From Georgia, almost all of those who responded were doctors. On the other hand, there were mixed responses from UAE, predominantly from the nurses.

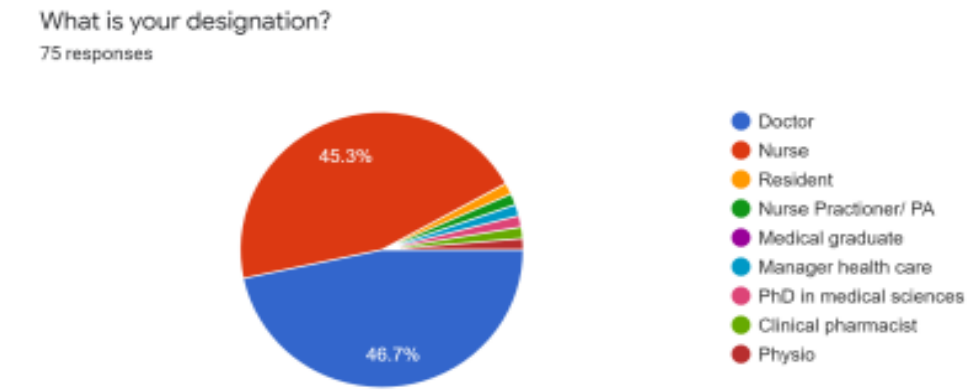

Figure 1: Designation of participants from both UAE and Georgia.

According to the age, 31 participants (41.3%) were between 30-39, which claimed the majority, 27 (36%) were between 40-49, 10 (13.3%) were 50-59, and 7 (9.3%) were 20-29.

In general, the stress level experienced by healthcare professionals during this time was similar in both countries. Participants were asked to score their stress levels from 1-10, and the prevailed level for both countries was seven. In Georgia, most doctors rated their stress >5 (**Figure 2**). In the UAE, most of them chose scores ≥ 5, with scores of 7 and 8 dominating (**Figure 3**). Concerning the stress, when asked to choose or describe some of the symptoms they experienced, the most common complaint was anxiety - 19 HCPs (25.3%). The other common complaints were irritability [N = 11 (14.7%)], insomnia [N = 10 (13.3%)], body pain [N = 7 (9.3%)] headaches and dizziness [N = 4 (5.3%)]. Additionally, 3 of the participants experienced symptoms of depression during this period.

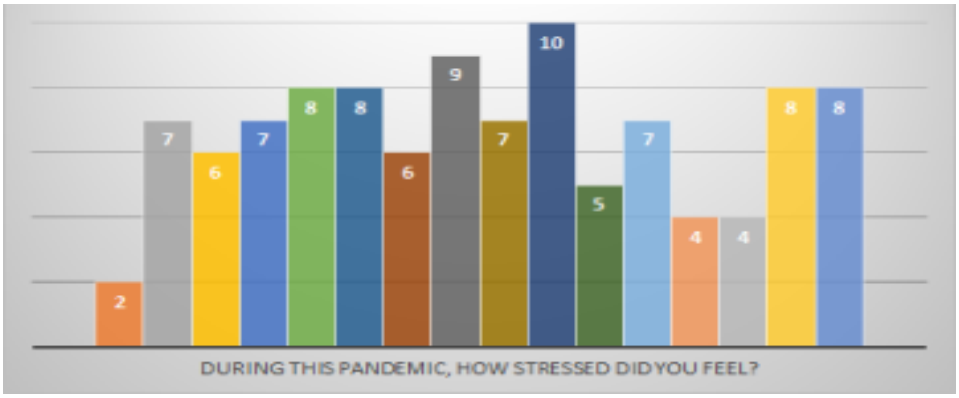

Figure 2: Stress scores in Georgia.
In this graph, each bar represents an individual and the score they chose for their stress levels (1 - 10). The most commonly chosen scores were 7 & 8.

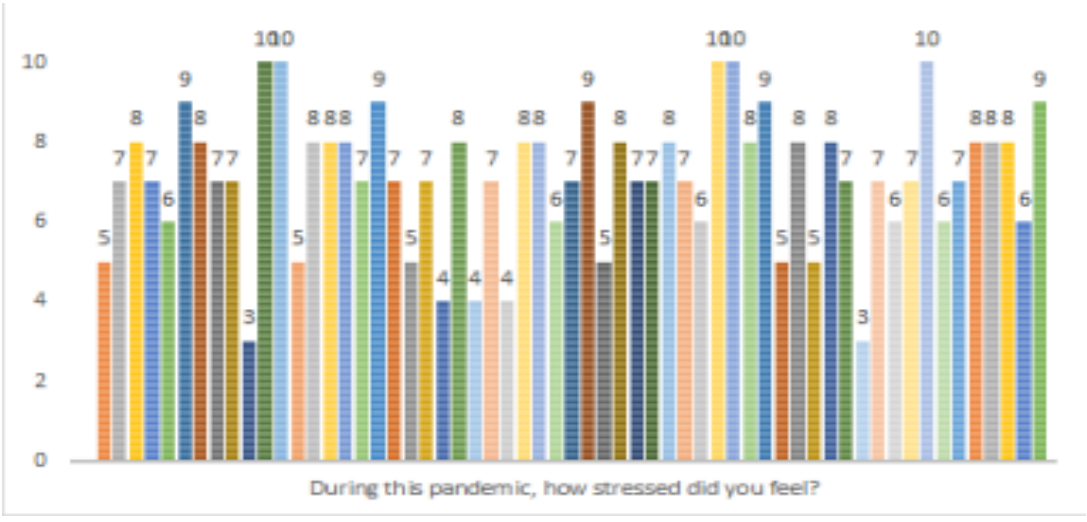

Figure 3: Stress scores in UAE.
Each bar represents an individual and the score stress levels (1 - 10) defined by an individual.
A score of 7 is the most predominating score selected.

The questionnaire revealed that a preponderance of health personnel was anxious about getting infected by COVID-19 (**Figure 4**) which comprised 66.7% (N = 50). However, 21.3% (N = 16) were unsure about their anxiousness. Of these numbers, most of the doctors from Georgia feared getting COVID-19. In the UAE, out of 58 responses, only 5 of them did not fear getting COVID-19. The remaining 53 responded 'yes' and 'maybe.' When asked about their change in motivation to work, the responses were almost equal. About 52% (N = 39) had responded 'No' and 40% (N = 30) responded 'Yes' and the remaining 8% (N = 6) were unsure.

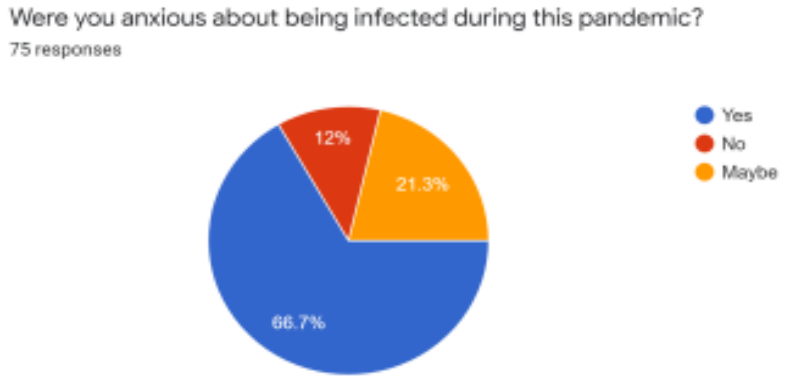

Figure 4: Anxiety about being infected by COVID-19.

Participants were asked to score how the change in workload affected them during COVID-19 from 1-5. Among the 75 participants, the median response rate from both countries was 31 (41.3%), with a score of 3 being the most chosen option. From Georgia and UAE most of the participants rated scores 3 (N = 6) and 4 (N = 7) and 3 (N = 25) and 4 (N = 21) respectively. It discloses that both countries have had mild to moderate increases in their workload due to COVID-19. When comparing results from the UAE & Georgia, the workload of the seven participants from the UAE (**Figure 5**) and only one participant from Georgia (**Figure 6**) were severely affected due to COVID-19.

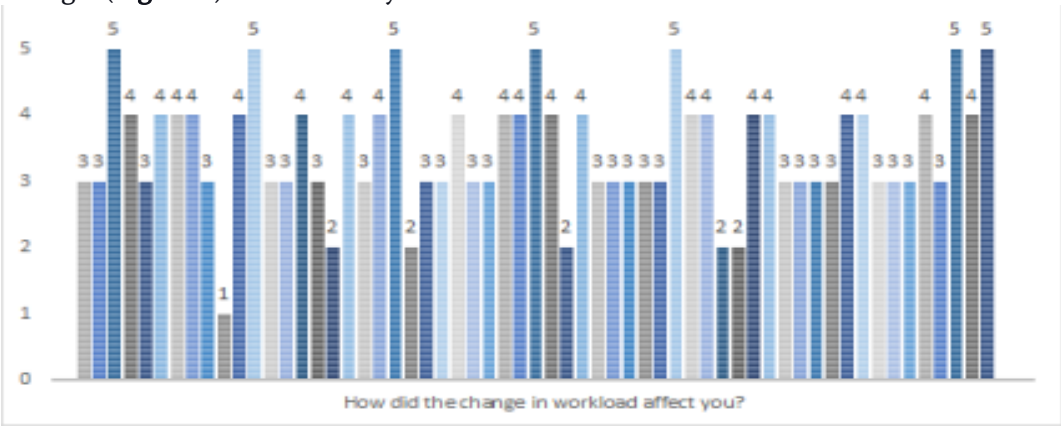

Figure 5: Change in workload for UAE.
Each bar represents an individual and the score their workload affected them (1 - 5) - defined by an individual. Scores 3 and 4 are most commonly chosen.

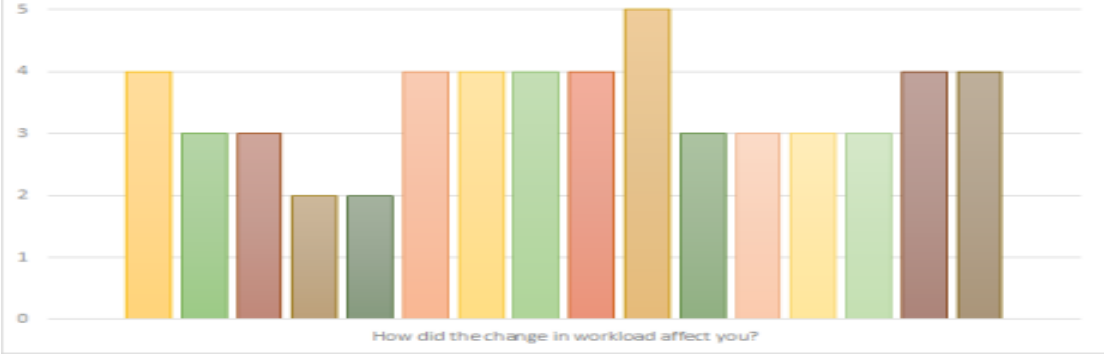

Figure 6: Change in workload for Georgia.
Each bar represents an individual and the score they chose for how their workload affected them (1-5) - defined by an individual. The most common score chosen was 4.

The questionnaire revealed that most participants (47.3%, N=35) did not feel hopeless and pessimistic (**Figure 7**), 31.1% (N=23) were uncertain of their feelings, and 21.6% (N=16) had hopelessness and pessimism during this period. Of these analyses, 7 HCPs from Georgia stated no, and six chose maybe. From the UAE, most participants chose that they did not feel hopeless/pessimistic during COVID-19. So, to compare, the feeling of hopelessness was more profound in Georgia than in UAE.

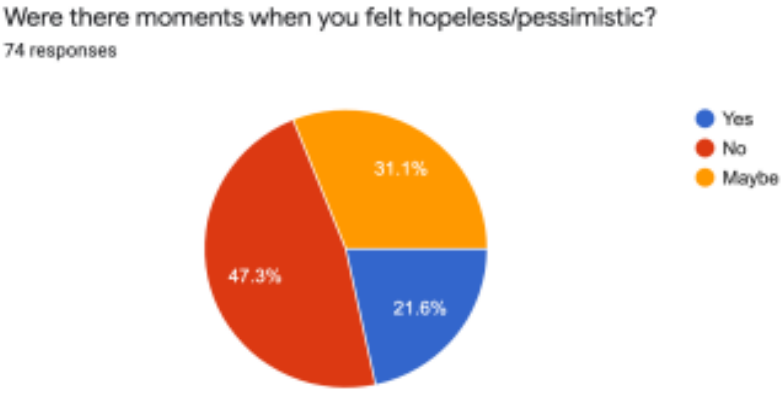

Figure 7: Moments of hopelessness/pessimism.

Concerning COVID-19, HCPs were asked if their country of practice handled the situation adequately (**Figure 8**), and most of them, i.e., 76%, responded positively (N=57) and 6.7% (N=5) responded negatively, while the other 17.3% (N=13) were unsure. While comparing this data between the two countries, the majority from Georgia voted for maybe (N=7) and that the situation was not handled aptly (N=5). From the UAE majority of participants agreed that the problem was addressed correctly (N= 54).

We asked our participants to choose what helped them relieve their stress during this period. Most of the participants, 49.3% (N=37) (**Figure 9**), selected 'acceptance that you can't control everything. The other popular options were 'friends and family,' with 40% (N=30) of the votes, and 'eating right,' with 6.7% (N=5) votes. Overall, while comparing this data between Georgia and UAE, they had an almost equally divided result.

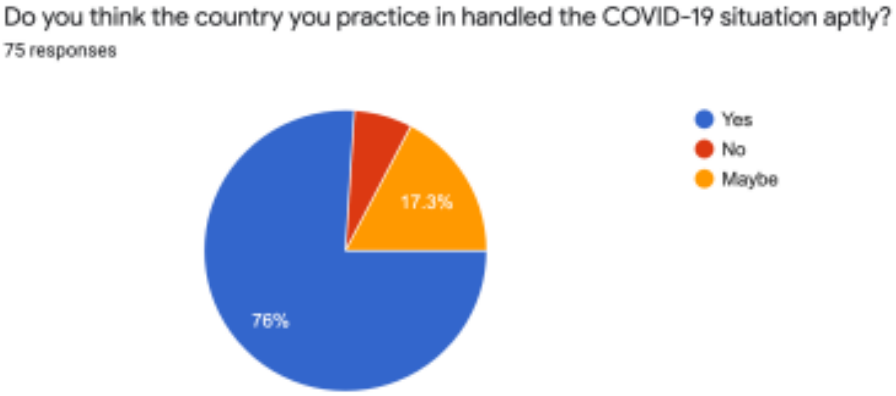

Figure 8: Country of practice handled the COVID-19 situation aptly?

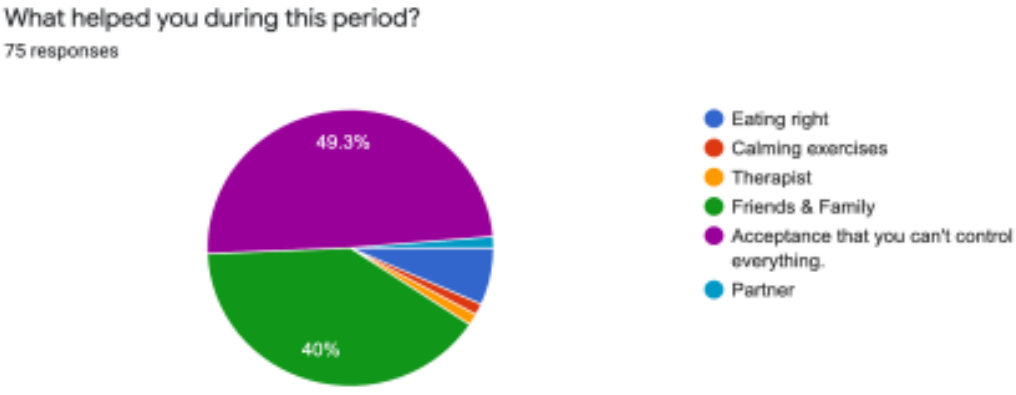

Figure 9: What helped you during this period?

In this questionnaire, we asked the HCPs if any suggestions could help colleagues cope with this situation. The most common recommendations received were "to stay calm and to maintain proper safety precautions such as wearing masks, PPEs, social distancing, and sanitization." Some participants also advised maintaining a healthy lifestyle by having a balanced diet, avoiding carbonated drinks, taking daily multivitamins, getting sufficient sleep, and exercising.

Overall, responders from the UAE believe that their country handled the pandemic adequately, although the responders from Georgia thought the government could have dealt with the situation better. In addition, the HCP's of UAE had a more significant increase in workload than the HCP's of Georgia. In general, both countries displayed similar stress levels among HCP's.

Discussion

The COVID-19 pandemic took the world by storm, and its most significant effect was seen on the frontline health workers, who dedicated all their time and effort to help control this critical situation. So, it goes without saying, it takes a toll on one's mental health. In this study, 75 FHWCs from the countries of Georgia and UAE participated, with most responses being from the UAE. Increased stress levels with a mean of 7 (scale 1-10) were observed in both countries, and most who responded were in the age group of 30-39. Doctors and nurses were affected almost equally, and the most common symptom was anxiety, followed by irritability and insomnia. Increased levels of stress were attributed to change in the workload and the fear of contracting the infection. A stress model questionnaire demonstrated the factors that contributed and assessed disparities between the countries of the UAE and Georgia in managing the situation.

Since the COVID pandemic, several studies have been performed to assess the increased psychological impact on the frontliners. Countries like the US [9] and India [5] have conducted studies showing mental health concerns due to burnout and stress. The US emphasized increased stress levels seen in women, especially those of color, and attributed the cause to increased workload and feeling less valued. In Kashmir, India, it was observed that feelings of pessimism, change in the workload, and anxiety of being exposed to the pathogen all contributed to the stress levels. Our conceptual model of the study also shows similar findings as those seen in the US and India. However, the differences observed were that the stress levels were identical in both doctors and nurses compared to the other countries.

An essential variable of 'feeling hopeless/pessimistic' during this pandemic helped us better assess the mental status of the FHWCs. Although the majority responded with no changes, the frontliners in Georgia felt more hopeless when compared to those in the UAE. Comparisons made between Georgia and the UAE showed differences in the way the country handled the situation. The majority of FHWCs from Georgia mentioned the government did not address the problem aptly. It can be considered a variable that resulted in the increased pessimism and hopelessness felt by healthcare workers in Georgia.

The study's main limitation was the lack of responses and an unequal number of participants from Georgia and the UAE, which affected the quality of the results.

Our study, which assessed COVID-19 related stress in healthcare workers, is essential. It identified anxiety, increased workload, and pessimism as attributable to the pressure overburdened healthcare workers face. Furthermore, the lack of resources provided by organizations lessens the efforts put in by these frontliners. So, interventions can be implemented, including better staffing, peer support groups [9], proper protocol implementation, and encouragement from colleagues and their organizations, with several studies owing to these strategies [11].

Conclusion

Although the world sees the FHWCs as the warriors in this war against COVID-19, it is vital to emphasize the psychological impact it has had on them. Our study identified change in workload, increased fear of being infected with the disease, anxiety, and pessimism as significant contributors to increased stress levels. The lack of resources and management of the situation by Georgia and the UAE respectively played a prominent role. Further studies should be conducted better to help our frontline workers in this situation of crisis.

Acknowledgments

The authors would like to thank all the HCPs who answered the questionnaire and made this study possible.

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MARIAM GIGILASHVILI, GURAM MATITASHVILI, RATI RAMISHVILI

ASSOCIATION BETWEEN HASHIMOTO'S THYROIDITIS AND DEPRESSION

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Abstract

Hashimoto's thyroiditis is a disease of the thyroid gland which affects the bodyin various ways leading to somatic complications. In addition to that, it affectsthe patient's mental health. The extent of psychiatric diseases in patients with Hashimoto's thyroiditis is not extensively explored. The primary objective of this study was to find the association between Hashimoto's disease and depression in female patients aged 36-50 from North America/Central America. Questionnaires were designed to assess the extent of possible depression in those patients. We compared 115 cases to 188 controls. After analyzing results, we found that relative risk for developing depression in patients with hashimoto's disease was 1.7202 (95% CI 1.4722- 2.0099, p value< 0.001). Thus, we concluded that Hashimoto's disease is associated with increased risk of development of depression in a given sample.

Introduction

Hashimoto's thyroiditis is a chronic autoimmune disease of the thyroid that destroys thyroid cells by cell and antibody-mediated immune processes[1]. It is the most common cause of primary hypothyroidism in North America with prevalence of 4-13% and it affects about 5% of Caucasian at some point in their lives. Hashimoto's disease is estimated to be 10-15 times higher in females than in men and mostly affects women aged from 30 to 50 years [1].

However, surprisingly little is known about its discoverer, Hakaru Hashimoto, who first described the disease in 1912. He presented four patients with a chronic thyroid disorder, which he termed struma lymphomatosa, characterized by diffuse lymphocytic infiltration with germinal centers, fibrosis, parenchymal atrophy and eosinophilic change in some thyroid follicular cells. He summarized the pathological findings in an article which was unrecognizedfor about two decades: the disease described by Hakaru Hashimoto was considered as the type of Riedel's Thyroiditis. In 1931, Graham and McCullagh used the term "Hashimoto" for the first time in the title of an article, strongly arguing that struma lymphomatosa was indeed distinct from Riedel's thyroiditis [2].

Hashimoto's thyroiditis is a disease which produces a wide variety of symptoms ranging from somatic symptoms such as cold intolerance, loss of energy, hypotension, etc. to psychiatric diseases. One of the mental diseaseswhich is common in patients with Hashimoto's disease is depression. It is a leading cause of disability worldwide affecting more than 264 million people [3]. Despite this, depression is a common condition that often remains undiagnosed and untreated; however, symptoms are more likely to be recognized today than in past decades. The characteristic symptoms of depression include loss of interest in activities that were pleasurable in the past, sadness, irritability, feelings of worthlessness, hopelessness, guilt or anxiety, concerns over death, or suicidal ideation. Associated symptoms mayinclude changes in appetite, weight loss or weight gain, sleep disturbances, psychomotor activity, decreased energy, indecisiveness, or distracted attention [4]. Like autoimmune thyroiditis, depression is a disease which is more common in women [3].

Even though depression is mentioned as the common psychiatric disease in patients with Hashimoto's thyroiditis, there is little evidence proving this association. Meta analysis conducted in 2019 reviewed nineteen studies comprising 21 independent samples with a total of 36 174 participants (35 168 for depression and 34 094 for anxiety). Patients with autoimmune thyroiditis (AIT), Hashimoto thyroiditis, or subclinical or overt hypothyroidism had significantly higher scores on standardized depression instruments, with an odds ratio of 3.56 (95% CI, 2.14-5.94; I2 = 92.1%). For anxiety disorders, patients with AIT, Hashimoto thyroiditis, or subclinical or overt hypothyroidism had an odds ratio of 2.32 (95% CI, 1.40-3.85; I2 = 89.8%). This meta-analysisestablishes the association between Hashimoto's disease and depression andanxiety disorders. It proved that patients with Hashimoto's disease exhibit an increased chance of developing symptoms of depression and anxiety or of receiving a diagnosis of depression and anxiety disorders [1].

Considering the high prevalence of these diseases they have been the major topic for research. There is a lot of literature about depression and Hashimoto's thyroiditis separately in recent years but only few of them studiesthe association between these 2 diseases. Finding the association between them has social, medical, scientific and economic importance. If association between these 2 diseases will be found and Hashimoto's will be identified as a risk factor for development of depression. Moreover, if awareness of these connections will rise, the patients with Hashimoto's thyroiditis will recognize symptoms of depression and try to seek help. Depression is one of the most commonly not treated and underdiagnosed diseases. Based on the data fromthe National Health and Nutrition Examination Survey, 2005 to 2008. 58.8% ofpeople with moderate depression (PHQ-9 10-14) have not received nor pharmacological nor mental health professional treatment, 54.9 % of peoplewith moderate severe depression and respectively 36.9 % of people with severe depression [5]. Screening for depressive symptoms in patients with autoimmune thyroiditis might help health care professionals to diagnose depression early and on the other hand will help patients to receive proper pharmacologic and mental health professional help.

Methodology

The study design we chose was retrospective cohort study. Hashimoto's disease was considered as a risk factor for developing depression. To conductit we decided to use a self- report - Patient Health Questionnaire (PHQ-9) (**Table 1**) which was an easy-to-use questionnaire. Filling these questionnaires took people several minutes. It consisted of 9 questions each having 4 possible answers: not at all, several days, more than half the days or nearly every day. Each of them had their individual score. Not at all - 0 score, Several days -1, more than half the days- 2, nearly every day 3. The sum of points was calculated and according to final results the severity of possible depression was assessed. 0 points- no depression, 1-4 minimal depression, 5-9 mild depression, 10-14 moderate depression, 15-19 moderately severe depression, 20-27 severe depression. Additionally, it involved 5 questions addressing patients' age, gender, ethnicity, geographic location and thyroid disorder. The identity of people who filled the questionnaire was kept anonymous. We posted this questionnaire in the global social online groupswhere people had various diseases of the thyroid. Next step was to create anew questionnaire for the unexposed cohort control group and compare it toour cases. The questionnaire for controls involved the same questions aboutage, ethnicity, gender, geographic location and PHQ-9 question. It didn't involve any questions about patient's diseases and was also posted in the global general population social online groups. After receiving results, we filtered them according to inclusion criteria and relative risk was calculated.

Inclusion Criteria

1- Age 36-50.
2- Gender- female
3- Ethnicity - Caucasian
4- Geographic location- North America/ Central America
5- Diagnosis of Hashimoto's Disease

Controls

Age matched females presenting with symptoms of depressive disorderbased on the PHQ-9 questionnaire.

Exclusion Criteria

1- Males
2- Age below 36 and above 50.
3- Geographic location except North America/Central America
4- Ethnicity other than Caucasian
5- Thyroid diseases other than Hashimoto's Disease

Table 1. PHQ-9 Questionnaire

		Not at all	Several days	More than half the days	Nearly every day
1.	Little interest or pleasure in doing things	0	1	2	3
2.	Feeling down, depressed, or hopeless	0	1	2	3
3.	Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4.	Feeling tired or having little energy	0	1	2	3
5.	Poor appetite or overeating	0	1	2	3
6.	Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7.	Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8.	Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9.	Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

Results

Data of 355 participants was analyzed. The age distribution of cases is the following: 0 to 19 - 1.7%, 20 to 35- 36%, 36 to 50 - 38% and finally 51 + was24.3%. 97.5 % of participants identified themselves as females, 2,3% as males and 0.3% preferred not to say (**Figure 1**). The most common thyroid diseases our cases had were- Hashimoto's Disease, Thyroid Nodules, Graves Disease, Goiter, Thyroid Cancer. Our aim was to analyze females aged 36-50 with Hashimoto's Disease. Out of 346, 114 of them had some degree of depression and were located in North America/Central America.

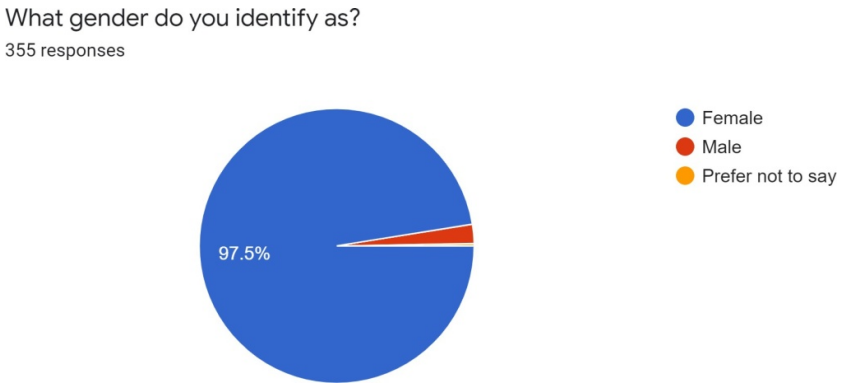

Figure 1. Gender Distribution among cases

More precisely, 1 had no depression, 12 had minimal depression, 39 had milddepression, 37- moderate depression, 14- moderate severe depression, 13- severe depression (**Figure 2**). On the other hand, a total number of 320 controls were studied and only 118 of them fit all inclusion criteria. Out of 118,50 controls had no depression, 40 had minimal depression, 10 had mild depression, 10- moderate depression, 4 moderate severe depression and 4 had severe depression (**Figure 3**). Relative risk for developing some degree of depression for the exposed group versus unexposed group was 17.20% (RR= 1.7202, 95% CI 1.4722- 2.0099, p value< 0.001). The study demonstrated that exposure to hashimoto's disease is associated withincreased disease occurrence.

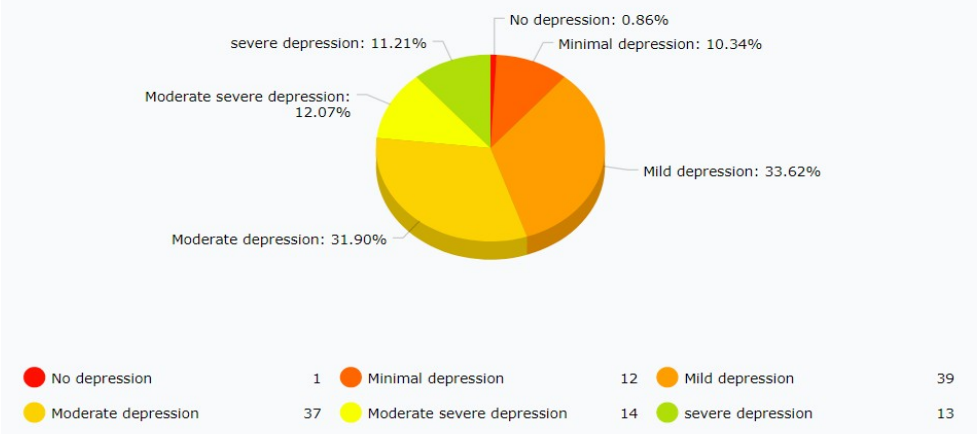

Figure 2. Distribution of severity of depression among cases

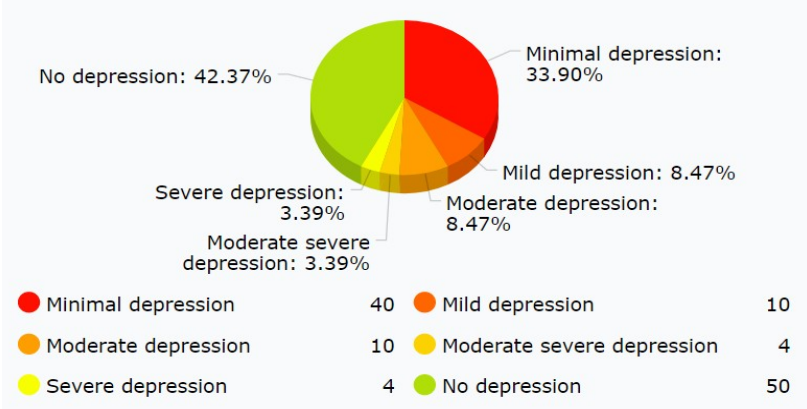

Figure 3. Distribution of severity of depression among controls

Discussion

Association between Hashimoto's disease and depression is an important topic for both patients and physicians, but because of lack of precise information it remains underexplored. Multiple studies have already aimed to explore the link between thyroid diseases and depression. Even though it's believed that there's a positive relationship between those two, some of thesestudies showed conflicting results further proving that more thorough researchabout this association is needed. Researches that took place in the Netherlands [6], Norway [7] and Korea [8], did not show the significant relationshipbetween thyroid diseases and depression, meanwhile studies held in Brazil [9] showed a significant association. Disparity of results should be attributed to regional, socioeconomic and lifestyle differences.

First studies which researched the association of thyroid disease and brain function were conducted by Marangell and Callahan using PET paradigms [10].Interestingly, Hendrick and colleagues noticed a high occurrence of hypothyroidism among patients with therapy-resistant depression [11]. Despite these indications, no direct association between particular alterations in brainregions because of thyroid disease and the development of a depressive disorder was found yet.

Considering the importance of this topic, our study aimed to explore theassociation of Hashimoto's disease and depression in 36-50 years old Caucasian females from North America/Central America. Results of our studyproved a significant relationship between Hashimoto's disease and developing a different degree of depressive disorders (RR= 1.7202, 95% CI1.4722- 2.0099, p value< 0.00) which once again demonstrates the significance of accessing mental health problems in the patients with Hashimoto's thyroiditis.

Different degrees of depression were assessed in these patients, most common being mild depression making up 33.9 % of all cases. Only 1 patienthad 0 score (no depression) on the PHQ-9 questionnaire while a total of 114patients had some degree of it (minimal depression- 10.41%, moderate- 32.17%, moderate severe- 12.17%, severe- 11.30%).

Our results might have various implications for physicians and patients. It is known that depression is one of the most common diseases and people affected with it seek for help without knowing

the possible root cause of their disease. Besides mental dysregulation, depression can be caused by various diseases including Hashimoto's disease. Depression associated with a thyroid disease requires different treatment than usual depression. Besides typical levothyroxine treatment, selenium supplementation can help to reduce the amount of thyroid antibodies and improve mood or well-being [12].

Additionally, patients with AIT and no symptoms of depression must be awareof the vulnerability to develop psychiatric issues. As a consequence, both a screening for psychiatric symptoms is advisable in patients with AIT and a testfor AIT is recommended in patients with depression. Recognizing this association can help with early identification of depressive disorders, help to educate these patients about the possible risks and provide an early treatment, which can further decrease the burden for the people with this condition.

Our study was conducted with a sample of sufficient size. We managed to investigate depression in a diverse group of patients with a variety of thyroid disorders, different age groups and ethnicities. We respected the privacy of our study participants and the answers and personal data of the cases and controls were kept private, givingthem maximum chance to give honest answers.

Even though we tried minimizing the limitations of our study, there are still some topics we have to address. Our study was held in a closed online group for people with thyroid problems. We believe there are more people in need ofhelp and compassion in these types of groups, which in itself might increase the incidence of mood disorders. Hence our research was likely affected by a sampling bias. Moreover, conducting online surveys has its disadvantages. Having face to face conversation with a patient while filling the questionnaireis more reliable and less prone to errors.

To conclude, there is known association between Hashimoto's disease and depression,but not enough evidence proving this association is available. Based on our research results we found that relative risk for developing depression in patients with hashimoto's disease was 1.7202 (95% CI 1.4722-2.0099, p value< 0.001). Thus, we concluded that patients with Hashimoto's disease are at increased risk for developing depression.

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ANA GOGOLASHVILI, MARIAM PESTVENIDZE, DIANA KERATISHVILI

ASSOCIATION OF LOW BIRTH WEIGHT AND AUTISM SPECTRUM DISORDER

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Abstract

Autism spectrum disorder (ASD) is a complex developmental condition resulting mainly in social and communication problems, the risk factors of which are still not substantially explored. The study aims to identify the probable association between ASD and low birth weight in the Georgian population, to promote early identification of disease in those patients.

A total of 100 patients aged 2-18 years with ASD diagnosed via ICD 10 were analyzed. Birth weight was obtained from medical records and the prevalence of low birth weight in ASD children was determined. Patients were stratified according to other possible risk factors. 11 out of 96 had low birth weight, out of which 73% were preterm, 55% were delivered with C-section, 45% had neonatal complications, and 55% had pregnancy complications. The findings showed an 11.5% prevalence of low birth weight in ASD patients that suggest a possible relationship between these two and warrant more extensive investigation.

Introduction

Autism spectrum disorder (ASD) is a complex developmental condition that involves persistent challenges in social interaction, speech and nonverbal communication, and restricted/repetitive behaviors. According to the World Health Organization [12], one in 160 children have ASD. In 2020, the CDC reported that approximately 1 in 54 children in the U.S. [4]. However, it is an estimated average, and the prevalence varies in different studies. The effects of ASD and the severity of symptoms differ in each person.

The diagnostic criteria vary in different countries [2]. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5), updated in 2013, is used in the United States [2]. According to DSM-5, ASD is characterized by deficits in social interaction and restrictive, repetitive interests and patterns of behavior [1]. In 2013, the American Psychiatric Association reclassified autistic disorder, Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS) as autism spectrum disorders. In contrast, ICD-10 classifies ASD in different subtypes: childhood autism, atypical autism, pervasive disorders and Asperger syndrome.

There has been ongoing research about the potential risk factors for the development of autism. ASD is considered to have a multifactorial origin, with both genetic and environmental factors playing a vital role in its development [5]. One potential risk factor can be the low birth weight which is categorized into3 types: low birth weight (<1500g -2499g), very low birth weight (1000g - 1499g), and extremely low birth weight (999 g. or less) [14].

Hisle-Gorman et al. tried to investigate prenatal, perinatal, and postnatal risk factors [6]. In adjusted analysis, they found a strong association between low birth weight and an autism spectrum disorder. A similar relationship was found in a case-control study conducted in Finland. According to it, there is a higher risk of ASD in an extremely low birth weight (<1000g) group with the adjusted odds ratio of3.05, 95% CI 1.4–6.5 [7]. They also found preterm birth to be an independent risk factor for ASD [7]. However, it is difficult to indicate only one risk factor for ASD [11]. It is unclear if these causes are primary or secondary risk factors for the disease and further investigation is needed [11].

While all the above-mentioned studies investigate singleton gestation, Losh et al. used a co-twin-control design to investigate low birth weight as a risk factor for ASD [9]. Although genetic effects are of major importance, a non-genetic influence associated with birth weight may contribute to the development of ASD [9]. Examining twins addressed the issue of genetic variants as a confounder [9]. Low birth weight infants were 3 times more likely to develop ASD [9]. However, as twins on average have lower birthweight than singleton births, it is controversial whether it can be generalized to every pregnancy and warrants further investigation [9].

Other possible correlations that should be taken into account are as follows: preterm birth, C-section, complications during the neonatal period, pregnancy and delivery, as well as medications used by mother during pregnancy [7]. Therefore, they should be considered as confounding factors [7]. Early screening in children with risk factors may lead to early diagnosis and intervention, which is crucial for a better outcome [8].

There have been studies determining an association between preterm birth and an autism spectrum disorder. However, the Georgian population has not been studied. This study allows us to research the situation in Georgia and have a more profound understanding of potential risk factors, in particular, low birth weight.

Methodology

This is a cross-sectional study conducted in Georgia that determines the prevalence of low birth weight (<2500g) in autistic spectrum disorder children. It also tries to determine the association of birth weight as a potential risk factor for autism spectrum disorder. Involved children were already diagnosed with ASD by ICD-10criteria (the international standard for defining and reporting diseases/health conditions which provide a diagnostic classification standard for all clinical and research purposes) or had features suggesting ASD (confirmatory test was not done yet). We gained access to the available medical records with the birth weight. A total of 96 patients aged 2-18 years with ASD were involved. We also stratified the risks to avoid confounders like neonatal complications (e.g. respiratory distress, infections, any need for neonatal intensive care) pregnancy complications (e.g. anemia, preeclampsia, amniotic fluid abnormalities), delivery method (vaginal or cesarean), delivery complications (eg. hemorrhage, asphyxia, dystocia, prolonged delivery), medication use (e.g. iron supplementations, aspirin, antihypertensive) and maternal psychiatric illness. Vaginal delivery included both physiologic and assisted methods. Inclusion criteria was children aged 2-18 diagnosed with ASD with ICD-10 criteria or having features of ASD. Exclusive criteria are neuropsychiatric developmental disorders concomitant with or without ASD, metabolic disorders like hypothyroidism or lysosomal storage diseases, congenital abnormalities, epilepsy, genetic disorders like down syndrome, or fragile X syndrome.

Results

This study investigated 96 patients aged 1.5-10 years old with confirmed or suspected ASD. Out of this 96, 11 (~11.5%) had low birth weight, 8 (~8.3%) were macrosomic, and77 (~80.2%) were born with normal weight (**Figure 1; Table 1**). Among the examined cases, 84 (87.5%) were term, 11 (~11.5%) were preterm, and 1 (~1%) was post-term (**Figure 2**). Majority (~69.8%) of them were delivered vaginally (**Figure 3**). Only 12(12.5%) patients developed neonatal complications (**Figure 4; Table 2**). 72 (75%) mothers of our cases did not have any complications during pregnancy (**Figure 5; Table 3**). The majority of deliveries (~96%) were uncomplicated (**Figure 6**).

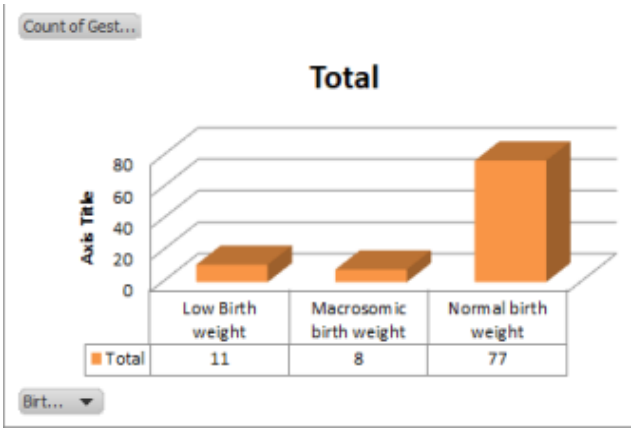

Figure 1. Distribution of birth weight among cases

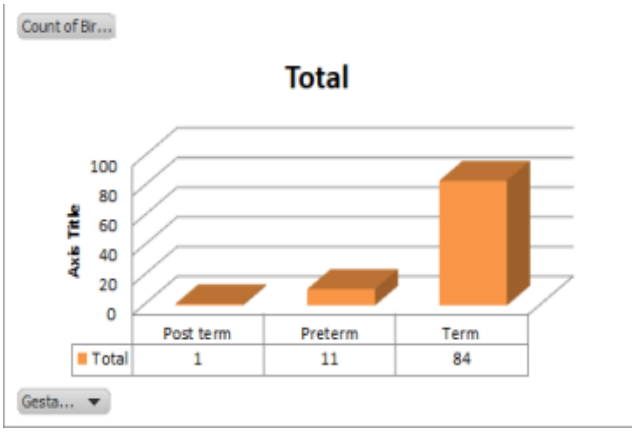

Figure 2. Distribution of gestational age among case

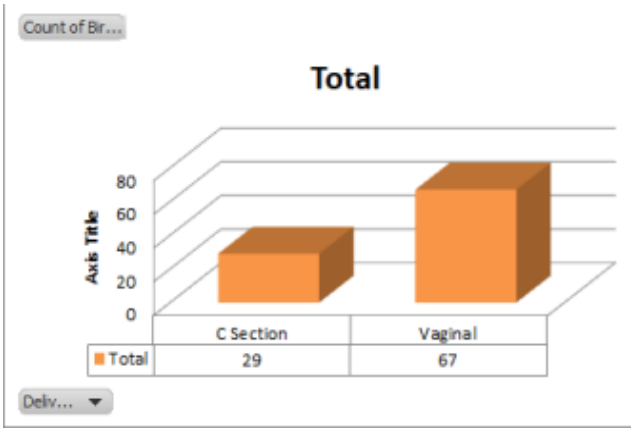

Figure 3. Distribution of delivery method among cases

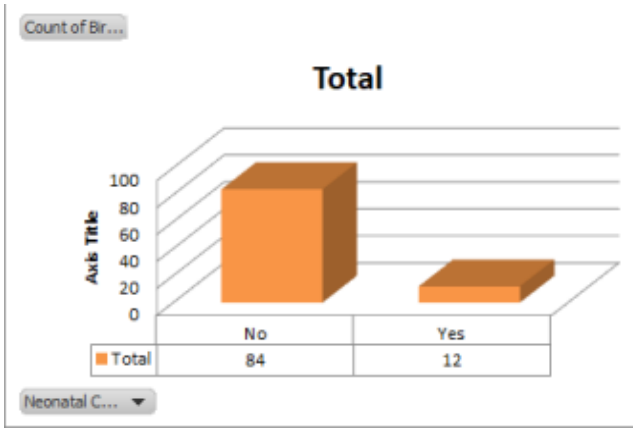

Figure 4. Neonatal Complications among cases

Table 1. Distribution of birth weight among cases

Count of Gestational age	Low Birth weight	Macrosomic birth weight	Normal birth weight	Grand Total
Post term	-	-	1	1
Preterm	8	-	3	11
Term	3	8	73	84
Grand Total	11	8	77	96

Table 2. Neonatal Complications among cases

Neonatal Complications	Low Birth weight	Macrosomic birth weight	Normal birth weight	Grand Total
No	6	8	70	84
Yes	5	-	7	12
Grand Total	11	8	77	96

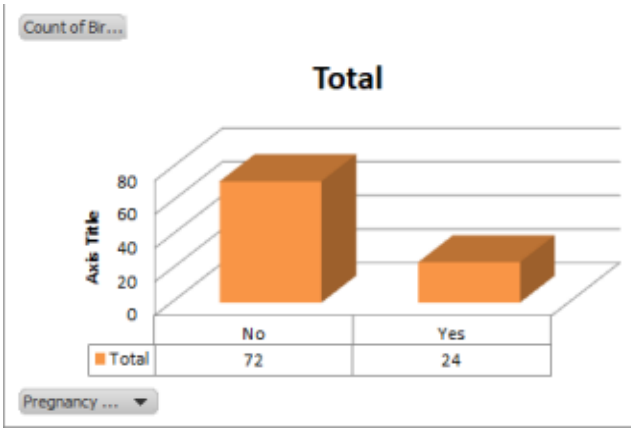

Figure 5. Pregnancy complications in cases

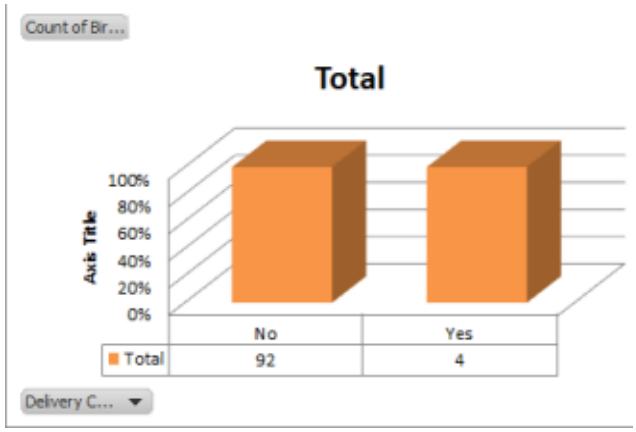

Figure 6. Delivery complication in our cases

Table 3. Pregnancy complications among cases

Pregnancy Complications	Low Birth weight	Macrosomic birth weight	Normal birth weight	Grand Total
No	5	7	60	72
Yes	6	1	17	24
Grand Total	11	8	77	96

Discussion

The study tried to find any correlation between low birth weights (as a potential risk factor). 96 children were included in the study. The results showed that the prevalence of low birth weight among ASD cases was 11.5%. According to WHO 2015 data, the prevalence of low birth weight in the Georgian population was 6.1% CI [5.6-6.6] [3]. Since our findings revealed a higher prevalence of low birth weight compared to the general population (11,5% versus 6,1% - p=0.0271) we can make an assumption that there is an association between low birth weight and ASD.

Birth weight is influenced by various maternal and pregnancy factors [7]. For example, uteroplacental insufficiency causes intrauterine growth restriction, smoking during pregnancy is also associated with low birth weight, maternal alcohol/illicit drug use likewise might be a reason for low birth weight, premature newborns have weight deficit, and etc. [7]. All of these factors by themself can be considered as risk factors for ASD [5,7].

In our study 8 (8.3%) out of 11 low birth weight infants were premature, but according to NCDC [10] overall prematurity rate is 8,7%, so almost the same, makes no difference. But our study results, revealing 11% of low birth weight more than overall low birth weight data (6,1%), raises a question: is low birth weight a confounder or is it independently associated with ASD? Answering this question is critical and requires a higher sample size, control group, and stratification of data. For example, Fezer et al. [5] conducted research that analyzed only the group of low-birth-weight infants who were not premature (18 patients) and obtained statistically significant differences compared to the general population (p=0.000).

Interestingly, there were 8 patients (~8%) with macrosomic birth weight. Several studies had inconclusive evidence about macrosomic birth weight and ASD association. Comparison of the prevalence of macrosomic birth weight in ASD patients should be compared to prevalence in the Georgian population. This will give us significant information but further emphasis should be paid to this. The purpose of this study is to question low birth weight as a possible risk factor for ASD development. As a result, more attention will be paid on low birthweight infants, including implementation of screening programs for ASD. This will aid in early diagnosis and initiation of interventions, which is crucial and has direct correlation with favorable outcomes.

Study limitations

The fact that our research had a small sample size (only 96 participants), and did not have a control group should lead to some limitations in our conclusions. The absence of a control group interferes with establishing the correlation between low birth weight and ASD and highlights the importance of having a control group in order to make a more precise interpretation.

Another potential limitation in our study is the lack of confirmatory assessment for ASD diagnosis in some patients and reliance on direct clinical assessment (evaluation of intellectual disability, deficits in social communications and interactions, presence of restricted, repetitive patterns of behavior). Individuals with only significant social communication deficits and lack of other characteristic features for ASD are usually evaluated for social (pragmatic) communication disorder. However, participants whose diagnosis was not confirmed yet had other clinical features of ASD making this diagnosis more probable.

Conclusion

Based on the results of the study there was a higher prevalence (11.5%) of low birth weight among ASD cases than in the general population of Georgia (6.1%) (p=0.0271). As low birth weight is a risk factor for development of ASD, conducting screening for ASD in lowbirth weight infants, would assist in early diagnosis and initiation of intervention leading to better outcomes. These results warrant further investigation with more extensive studies which will have a greater sample size and control group.

Acknowledgement

The completion of this study could not have been possible without the assistance of Dr. Nona Janikashvili, adviser and reviewer. We express our sincere appreciation to her for the support, suggestions, guidance, valuable comments, and provision. A debt of gratitude is also owed to Dr. Tamar Ediberidze, who helped us to access the medical records from the Institute of Neurology and Neuropsychology (INN). And we thank profusely the INN for permitting us to utilize this information. Their contributions are sincerely appreciated and gratefully acknowledged!

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AEROSPACE MEDICINE: FUTURE DOCTORS IN A NEW SPACE ERA

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Abstract

Currently there is a growing interest in Aerospace Medicine, a field focused on the physiology of the human body in outer-atmosphere and space environments. What interest exists in the specialty and whether medical students are capable of approaching common challenges in this area remains to be seen. A multiple-choice test along with a survey was structured and distributed to TSMU medical students. Year 1 and Year 2-6 students' scores were compiled and compared. Data collected from results revealed there is surprisingly great interest in Aerospace Medicine in the medical student community, as well as the lack of statistical significance between experienced students' performance and understanding of first-year students. Though the study confirms the existence of an interest in Aerospace Medicine, the lack of statistical significance when comparing students is most likely due to inadequate data and the ineffectiveness of the test.

Introduction

Aerospace medicine is a field focused on the health and physiology of humans in high altitudes and space, as well as how to overcome the challenges the human body tolerates while traversing these environments. Today there is a growing interest in this specialty, especially for space flight [1-3], as many are claiming the modern world is entering a 'new Space era' [4].

The possible future of a renewed space flight era is approaching at an alarming rate, garnering much interest from the scientific community as well as the international populace. Despite robust certification processes and medical guidelines [5,6], the ability and opportunity to fly suborbital is possible within the next decade [4]. In the following years, high-altitude flights and space travel will become more readily available to the general public. This will change the identity of the average patient subjected to the numerous consequences of high and outer atmosphere traversal- that of a fit individual, usually male and military, to patients with preexisting medical conditions and varying overall health [7,8]. Individualized preventive measures and treatment plans will need to be considered for each traveler. In order to ensure the well-being of individuals in this rising tide, collaboration and communication between medical personnel and aerospace specialists are warranted with a focus on teaching future generations [4].

The opportunities for medical students to receive exposure and an increased understanding of Aerospace Medicine are rare. What training does exist is limited to areas where such programs are offered as part of an elective or clerkship, therefore the true level of interest and clinical knowledge in medical institutions remains to be seen. Through gauging modern medical curricula's ability to train as well as identify future doctors and researchers skilled in the field of Aerospace medicine, one can better distinguish the state of ongoing educational programs and anticipate areas of improvement.

Though many researchers have explored the implementation of electives and programs amongst students, most medical students have yet to encounter the challenges faced in Aerospace Medicine or recognize it as a field of study [7]. Medical students, current healthcare professionals, and fellow researchers are often a subject in research projects because of their involvement in the community. The novelty of this experiment is based on its area of study during the time of newfound interest in spaceflight.

The purpose of this study was to observe how syllabi and modules in medical education are preparing students for such an esoteric specialty. Also, this study investigated the students' current level of interest in Aerospace Medicine and determined what factors influenced their opinions. Subsequently, the purpose of this study is twofold. Namely, are medical students capable of approaching common challenges familiar to Aerospace Medicine, and what level of interest exists for it presently amongst the medical student bodies.

In lieu of the former, evaluating how present-day medical curriculums are preparing medical students is always invaluable. The rigorous demands and challenges of the medical field are often scrutinized and analyzed in order to identify possible areas of correction, clarification, and/or

investigation. Though not alone, Aerospace Medicine is one of these highly specialized, uncommon fields and its complexity is only becoming more formidable from the constant updating of contemporary discovery and research. Awareness of the domain is likely to be heightened from greater attention and scrutiny as civilians take part in higher-altitude and spaceflight traversal, however enigmatic it may presently be. As such, discerning the true level of scientific knowledge amongst existing students could be enlightening. This can be achieved through a common standardized assessment to determine knowledge by presenting questions, scenarios, and/or cases in a logical and clinical manner. Students of various career paths are more than familiar with this type of examination as they often promote critical thinking and problem-solving skills. Utilizing the objective and targeted data from the study can then establish how thoroughly prepared potential candidates are from their studies and extrapolate if adaptation of these programs is necessary.

At the same time, the opinions and thoughts of a rising generation are integral in interpreting what interest already exists in the student bodies of medical universities. Several articles have hypothesized the potential effects of adapting qualifications for aerospace travelers, its impact, and the difficulties of Aerospace Medicine faced by experts and laymen. However, our investigation was unable to locate the prevalence of curiosity amongst the medical community. Each student understandably has a disparate background, perspective, outlook, and goals. Though subjective, distinguishing what motivates and drives a person can piece together observable patterns and trends. By collecting individual responses through a structured survey, this data will provide information about what kind of enthusiasm and passion for Aerospace Medicine exists and demonstrate a future in which the world and space travel communities would benefit.

This study hypothesizes that when presented with simulated cases and questions pertinent to Aerospace Medicine through a standardized test, students will be found insufficiently prepared by current medical school curricula. This study also hypothesizes that there is ample interest in Aerospace Medicine amongst the medical student community. If our hypothesis is correct, these findings will prove that enhancing current medical university education is paramount if indeed there is a future demand of clinicians and researchers for Aerospace Medicine.

Methods

This project relied on constructing scientific and clinical-based scenarios in order to assay a student's prior knowledge, problem-solving, and critical thinking in the sphere of Aerospace Medicine. Therefore, a thorough approach to the multidisciplinary aspects of Aerospace Medicine was necessary in order to identify key basics with which to test the student's knowledge. These may include an awareness of the challenges of high and outer atmosphere traversal, the physiological effects of gravity on the human body, diagnostic approaches to the common pathologies, modern management plans in isolated environments, and the real documentation of appropriate cases. These questions are the main device by which this study's objective data was collected, requiring each of the problems to be realistic enough to warrant scientific consideration or clinical approach while also being framed in a multiple-choice format familiar to students. Ergo, the goal is to present an applicable case to engage the subject and evaluate knowledge and understanding of the material. Constructed cases were to also vary from the sciences of human physiology, correct diagnosis of an ailing patient's condition, as well as correct 'next step' approaches for the treatment of the patient.

In order to fulfill this aspiration, questions were based on published medical journals and academia pertaining to Aerospace Medicine. Despite the abstruse nature of the field, a surprisingly great number of articles were found which provided notable cases and thought-provoking results [9-22]. Their topics ranged from inflight emergencies while aboard an airline vessel to the myriad of genetic, immune and physiological effects of microgravity and cosmic radiation upon the human body. What's more, resources like the International Space Station's 'Integrated Medical Group (IMG) Medical Checklist' provided insightful walkthroughs for emergency situations outside Earth's atmosphere. This plethora of reports and materials were distilled and implemented to form realistic questions that could potentially be canon to the field of Aerospace Medicine. All the relevant articles, in addition to resources associated with the topic, have been included in the bibliography of this study and deserve accreditation.

As previously mentioned, the test was enriched with a questionnaire that will allow students to express their thoughts and personal opinions on Aerospace Medicine. Simple questions were easy enough to include following the test portion for this very purpose in the hopes of gathering subjective information on each subject's own insights about the research's topic. Other information (student's nationality, prior knowledge on Aerospace Medicine, chosen career paths, personal experience with the test) were also enclosed in the hopes of revealing underlying influences and similarities.

Once finalized, the finished project was distributed with permission to the International and American Programs at TSMU. A test of 15 multiple-choice questions along with a survey about the individuals' general knowledge of Aerospace Medicine was compiled and structured in a Google form. A forward prefacing of the test and survey sections was also introduced, in order to ask for consent and verify students' intentions of taking the test with honesty and integrity. The Google Form was then attached to an email inviting medical students of both programs to participate as well as announcing the objective of the study. No time limit was incorporated for completing the survey in order to promote involvement, along with respecting and honoring students' time.

During the conduction period, multiple emails were sent in the ensuing weeks leading up to the deadline to politely remind willing students of the impending completion of the research. Responses were gathered and evaluated on a Google Sheet to filter out responses where consent was withheld, dishonesty was intended, and/or otherwise was ladened with errors. Test results and answers of the survey were analyzed in order to format the data for patterns between the objective test scores and subjective data.

At the end of the study, all scores were accumulated and averaged in order to address the central aim of this study and evaluate the beginning hypothesis. This was done by first establishing the mean test scores of Year 1 students from both programs as a baseline, which was then compared to the averages of the following class Years 2 through 6 in an effort to see if there is a significant difference between both groups. If the average score of Years 2-6 was higher than that of those in Year 1 student's average score, the null hypothesis would be rejected and the study would conclude that current medical curricula are suitable in preparing medical students for a greater, future incidence of common challenges seen in Aerospace Medicine. However, if the average score of students from Years 2-6 was not statistically significant, the null hypothesis would be accepted and conclude that current medical education programs are not preparing students for a greater incidence of common challenges seen in Aerospace Medicine.

Results

A total of 138 responses were received from both the International and American Programs of TSMU, with 98 and 28 responses from each. Of those, 12 subjects were excluded from the study due to either not consenting to be included in the research or stating they would use outside resources in order to complete the test. The remaining responses of 126 stated they were from a variety of countries (**Figure 1**).

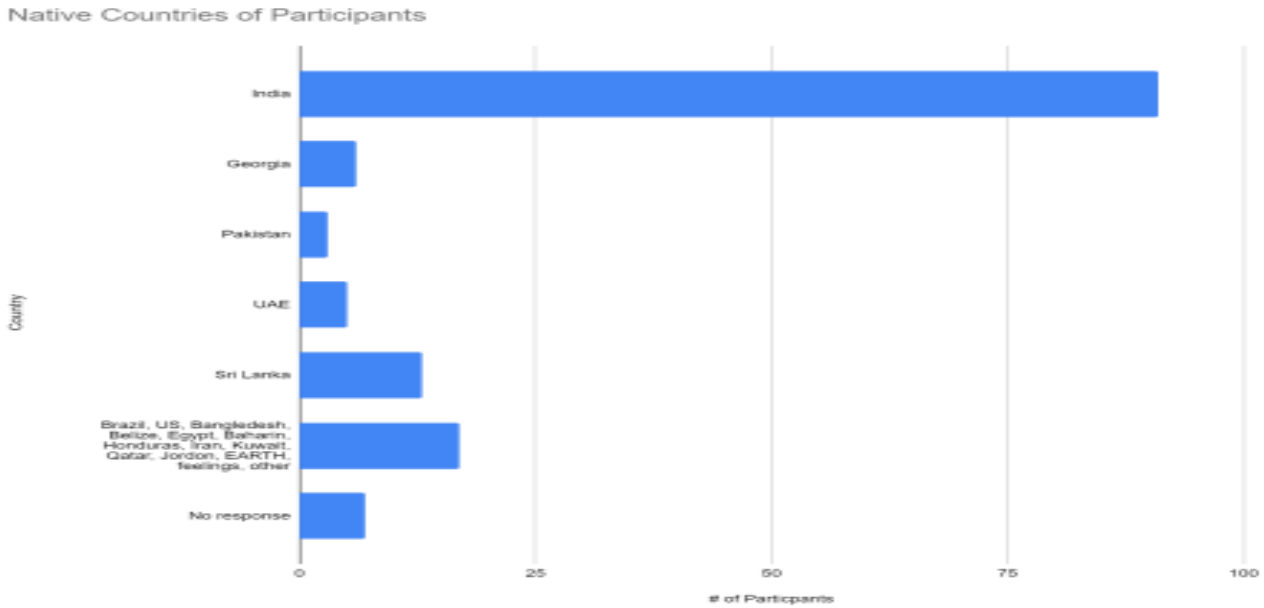

Figure 1. Native Countries of Participant

64 of these students have never heard of Aerospace Medicine before participating in this research (**Figure 2**). Despite this, 53 candidates expressed great interest in Aerospace topics (**Figure 3**), and 118 responses agreed with the statement 'We are entering a new Space era.' (**Figure 4**). Though 76 participants considered the test portion of the study to be very interesting (**Figure 5**) and 96 responses expressed they would like to learn more about the field in the future (**Figure 6**), a similar number of 69 students considered the quiz to be quite challenging (**Figure 7**).

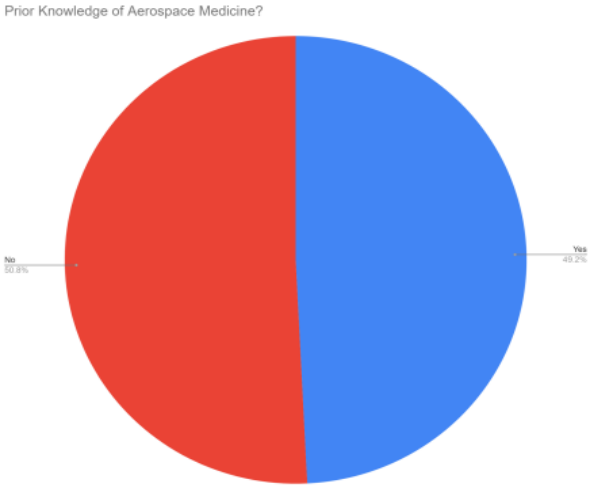

Figure 2. Prior Awareness of Aerospace Medicine.

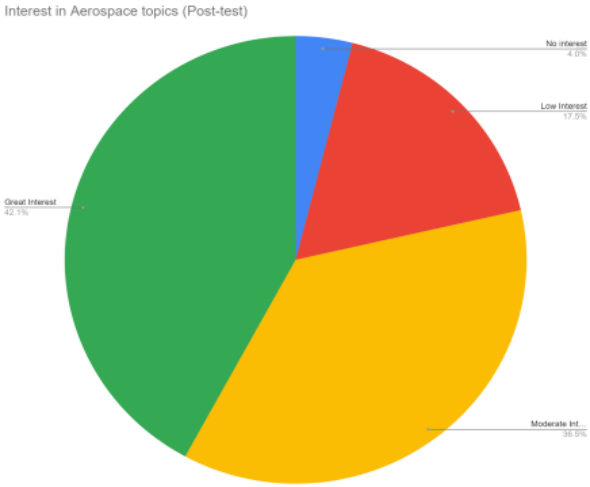

Figure 3. Interest in Aerospace topics (Post-test).

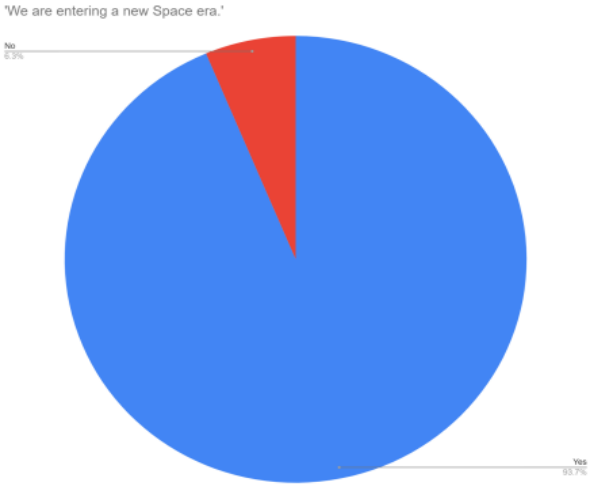

Figure 4. 'We are entering a new Space era.'

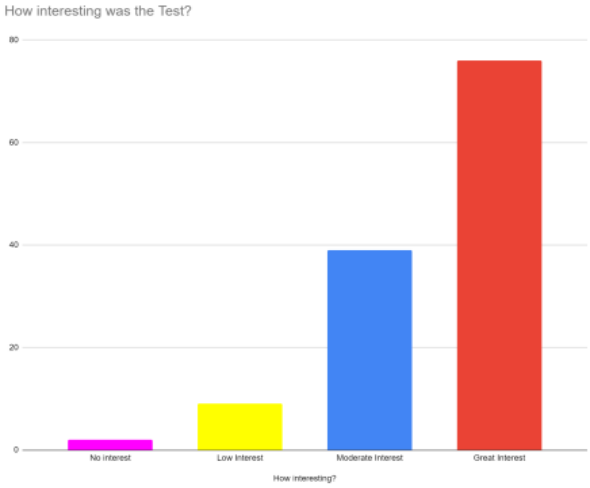

Figure 5. How interesting was the test?

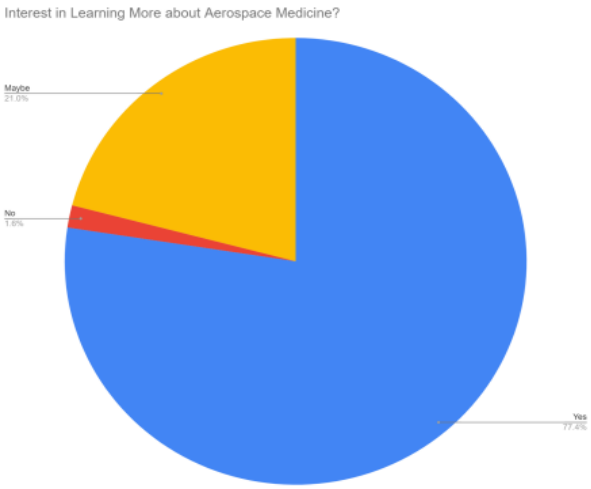

Figure 6. Interest in learning more about aerospace medicine?

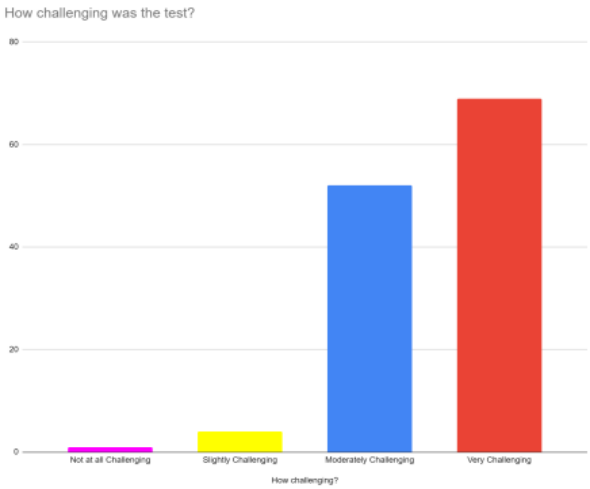

Figure 7. How challenging was the test?

From the survey data, it can be inferred that there was a large number of students who were aware of Aerospace Medicine, were interested in topics related to the subject, and wanted to learn more about it in the future. This was greater than expected, seeing as how obscure Aerospace Medicine appears to be and how few medical professionals are involved in the field today. Also, surprising was the near-total confirmation from participants of the statement, 'We are entering a new Space era.'. This statement was coined from a 2019 article about how different regulations in the process of becoming an astronaut have eased somewhat, to the point of allowing civilians to qualify for space travel [4]. Most likely being unaware of this published article, similar influences and events would have had to impact these medical students in order for them to agree with the statement. If nothing else, this hallmark as well as the other aforementioned data provides evidence for the current existence of an interest in Aerospace Medicine amongst medical students.

From the test portion of the included 126 responses (**Figure 8**), there was an overall average score of 5.91 from all participants, a median of 6, and a range of 0-12. When separating by class year, 21 and 105 responses were from Year 1 and Years 2-6 students respectively. Year 1 student scores' mean was 5.86 (SD 2.29), while the average of the Years 2-6 students was 5.92 (SD 2.41). Using these values, a right-tailed T-score of equal variances for the study was calculated to be .454 (pooled SD 2.39), from which a p-value was derived to be 0.325. At a 95% CI, the study demonstrated a lack of statistical significance and summarily fails to reject the null hypothesis. This outcome indicates medical students are not adequately prepared by modern medical curricula for challenges in Aerospace Medicine, establishing the original hypothesis is correct with the current data and power of this study. The study's analysis ventures to conclude that improvements and adaptations will need to be integrated in order to prepare future doctors and researchers for challenges that may arise in the future.

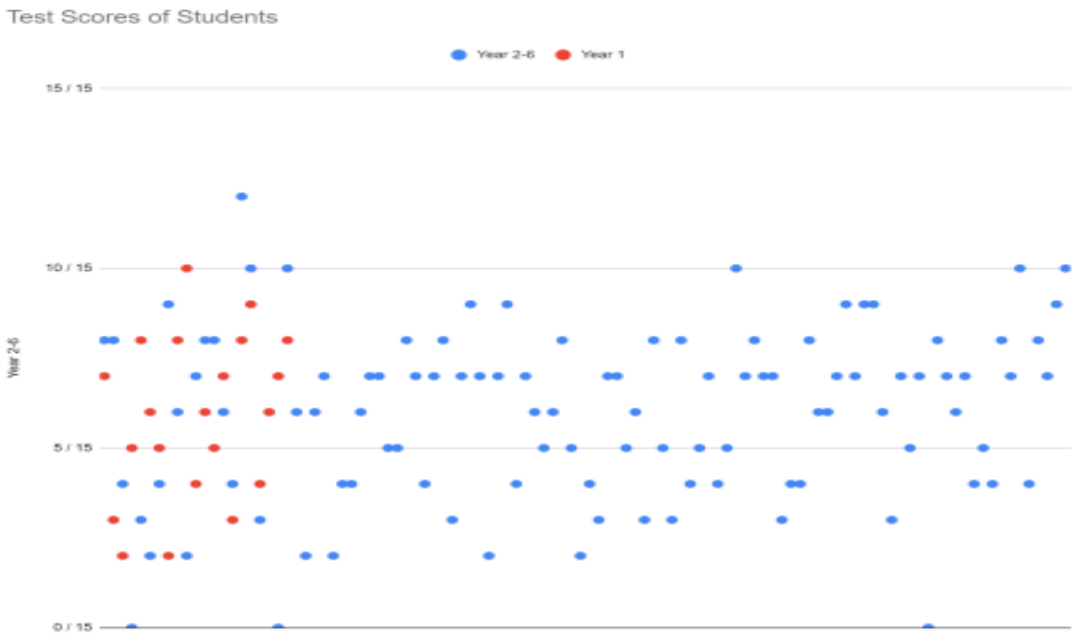

Figure 8. Test scores of students.

Discussion

According to survey results, approximately half of the participants were aware or had heard of the field of Aerospace Medicine, with a surprising percentage agreeing the world is entering a new space era. Also, 77.3% expressed interest in learning more about the subject. Further extrapolation from these results validates the theory that a more substantial number of candidates are interested in furthering their knowledge and understanding of Aerospace medicine. They are also aware of events related to aerospace which may carry over to future achievements and developments in the field. Nevertheless, the supposition from this study assumes some potential preference of causing a Pygmalion effect or observer-expectancy

bias, thereby impacting these findings. Therefore, the findings of this study acknowledge the existence of an interest in Aerospace Medicine amongst medical students and its subsequent future effects.

The test portion of this study concludes the lack of statistical significance when comparing the average scores of Year 1 students to Years 2-6 students with a 95% CI. This outcome fails to reject the null hypothesis, establishing medical students are not being prepared by modern medical curricula for challenges in Aerospace Medicine. However, due to the recognizing inherent shortcomings of this study, there is the possibility that inadequate power handicapped the true conclusion of this study. Were this power increased, the resulting data may have demonstrated a suspected different outcome from the study.

Reasons of Insufficient Power must be acknowledged. These ensuant flaws may have restricted the study's capability to reject the null hypothesis, which warrants careful consideration for future similar research: 1. There is the possibility that the number of participants was not numberable enough to produce a reliable study. Although a greater than expected number of students from both programs of TSMU were involved, 126 responses are not likely to be reflective of the rising medical community that competes globally. Furthermore, programs from only one medical school can do little to properly represent the variety of educational institutions and their unique programs' capacities in preparing medical students. For a future study, an exceedingly greater number of medical students from diverse universities around the world would increase the data, and therefore the power, in determining the results of our study. 2. The 15 questions test, which was essential in gathering informational data, is somewhat lacking in amount and quality. The final values emerged in stark proximity to each other with their individual standard deviations completely overlapping each other. Perhaps a greater number of questions could have been included, notwithstanding the complexity of Aerospace Medicine or the respect for students' time in order to advocate participation. 3. The test relied heavily on the honesty and integrity of the student to participate without searching the internet and/or confiding in a friend. Although versatile and potent as a survey, Google Form is limited when being implemented as a testing medium in that the ability to tell if the student was answering questions based on their own knowledge or through internet assistance was inconclusive. In order to mitigate this factor, preliminary questions included a direct interrogative as to a student's honorable intentions. Those who answered in the negative were excluded from the final study. However, this measure can be easily circumvented. For a future study, competent means of delivering and recording student responses while ensuring veracity will immeasurably affect performance and results.

Besides the possible insufficient power, there were also biases identified within the study. One of these was the bias of a willing subject. By announcing the research area of focus, those previously interested would be more likely to participate in the study compared to those who have never heard of it before or were otherwise uninterested. This could have conflated the results and skewed the outcome of the study. Questions were implemented to mollify this effect; however, it is a bias nonetheless and hard to avoid in consenting participants. Another was confirmation bias; the hypothesis depended on the assumption that medical universities weren't properly outfitted with the means to teach students on this material. This may have led to more difficult than necessary topics and scenarios for question creation, again misrepresenting the reality of medical education programs. To overcome this bias, the study constituted a familiar test apparatus with multiple choice answers, relying solely upon the basics and common elements of Aerospace Medicine, and allowed subjective answers for the survey. Alas, despite best efforts, there isn't the possibility of fully removing confirmation bias from the study in consideration of individual performances and the intricacy of the field.

If the present study can be considered reliable despite these flaws, there are a couple of inferences of the future to be made. As technological advancements improve and a lower threshold of qualifications allows a greater number of citizens to span upper and outer atmospheres, this will undoubtedly increase the expansion and value of the Aerospace Sciences (including engineering, physics, medicine, etc). This will increase the demand for researchers skilled in this area of medicine, predictably happening in the short term and possibly within the decade. Years later, as this situation accelerates at an exponential rate, general physicians will also need to be aware and trained to diagnose as well as manage patients' ailments commonly seen in Aerospace Medicine. Implementations will have to be considered in medical universities in order to expedite a student's education and skill, beckoning institutions to include this specialty in their curricula. If this possibility has been accentuated by this study, planning and starting

early to enhance modern education before this necessity is likely to alleviate the hassle of performing under stress and streamline the process.

In conclusion, this study confirms the existence of an interest in Aerospace Medicine amongst medical students and its future possible effects. Additionally, the conclusion demonstrates the lack of statistical significance when comparing the average scores of Year 1 students to Years 2-6 students with a 95% CI. This outcome fails to reject the null hypothesis, establishing medical students are not being prepared by modern medical curricula for challenges in Aerospace Medicine.

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PAPILLARY THYROID CARCINOMA CONCOMITANT WITH THYROTOXICOSIS

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Abstract

Contrary to the preconceived belief, the occurrence of hyperthyroidism in patients with thyroid cancer is gaining in incidence. The research team retrospectively analysed studies such as serum TSH and T4 levels, thyroid ultrasound results, and the histopathology reports of 9 patients (age range 20-67) from Mardaleishvili Medical Center. All patients were diagnosed with PTC. 7 patients had microcarcinoma (mean 6.1mm) and 2 – large tumors (mean 14mm). Graves' disease was found in 5 patients, toxic nodular goiter in 3 and one patient's record was lacking comorbid disease information. One patient had confirmed lymph node involvement. All patients underwent total thyroidectomy. Overall, all 9 patients with hyperthyroidism were subsequently diagnosed with thyroid cancer. Management of such patients has questioned the validity of the guidelines published by the ATA and emphasizes the fact that malignancy should not be excluded in patients with thyrotoxicosis.

Introduction

The incidence of well-differentiated thyroid cancer saw a great increase in the first decade of the 21st century [1]. Recent cancer research studies have projected that thyroid cancer will overtake colorectal cancer as the fourth leading cancer diagnosis by 2030 [4]. Patients with thyroid cancer rarely present with a palpable thyroid nodule [2] and hence depend on ultrasound and cytologic findings. The guidelines released by American Thyroid Association in 2015 recommended against the cytologic evaluation of hyperfunctioning nodules as they are deemed to be rarely malignant [3]. This case series presents patients who presented with symptoms of thyrotoxicosis and were eventually diagnosed with papillary thyroid cancer thus underlining the importance of keeping thyroid malignancy in the differential diagnosis even with hyperfunctioning nodules. Thyrotoxicosis usually presents with symptoms of heat intolerance and palpitations which can prove to be detrimental if not treated. The treatment usually involves surgery. The underlying etiology is often due to Graves' disease or lymphocytic thyroiditis with concurrent papillary thyroid cancer. The ramifications of these findings will influence the management guidelines of patients presenting with hyperthyroid symptoms.

Methods

This case series study was established on the comprehensive data about nine patients collected from Mardaleishvili Medical Center. Obtained information included patient history, complete blood count, serum TSH and T4 levels (the method used immunochemiluminometric assay [ICMA], thyroid ultrasound results (Device - Philips Affinity 50), histopathology report, and the surgery protocol. Other non-relevant tests were filtered out. The research team retrospectively analysed obtained patient data. Each member of the research team was responsible for analysing the particular aspects of each patient's portfolio (eg, tumor size, histopathology report, surgery protocol, etc.). Tables were created based on the comparison of the different patient variables.

Results

From 9 patients we found with papillary thyroid carcinoma and thyrotoxicosis, 5 patients (55.5%) were from 20 to 29 years old, 3 patients (33.3%) were more than40, and just 1 (11.1%) was in the 30 to 39-year range. All of them were females. The mean age of patients with PTC and toxicosis was 34.7 (range 20-67). 5 patients (62.5%) had Graves' disease and 3 patients (37.5%) had a toxic nodular goiter. There was no data about 1 patient regarding their comorbidity. Tumor size ranged from 3mm to 15mm with a mean of 7.8mm. 77.8% of patients (7 patients) had microcarcinoma (≤10 mm) with a mean size of 6.1mm and 22.2% (2 patients) had large size tumors (>10 mm) with a mean size of 14mm.

Table 1 shows the mean tumor size in different age groups. In the 20-29 age group we had 5 patients and the mean tumor size was 9.8 mm. Only 1 patient was in the 30-39 age group with a tumor

size of 3 mm. 3 patients were in the 40+ group with a mean tumor size of 6.17 mm. We compared tumor size and comorbidities (**Table 2**) and found that the mean size of the tumor in patients with toxic nodular goiter was 6.3 mm and in Graves' disease - 8.7 mm.

Table 1. Mean tumor size in age groups

Age Group	Number of Patients	Mean size (mm)
20-29	5	9.8
30-39	1	3
40+	3	6.17

Table 2. Correlation of patient comorbidity and tumor size

Patient N	Comorbidity	Size (mm)	Mean size (mm)
1	Unknown	8	8
2	Chronic autoimmune thyroiditis	7	6.3
5	Toxic nodular goiter	8	
6	Toxic nodular goiter	4	
3	Graves' disease	7.5	8.7
4	Graves' disease	13	
7	Graves' disease	15	
8	Graves' disease	3	
9	Graves' disease	5	

As for staging, only 1 patient (11.1%) had confirmed regional lymph node metastasis, 4 (44.4%) had confirmed localized tumor and lymph nodes could not be assessed in 4 (44.4%) patients. Comparing age and stage (**Table 3**), we found that the mean age in the pT1aN0Mx group was 28.3 years and in pT1aNxMx - 42.5 years (mean age in combined in T1a group was 36.4 years). We had 1 (one) 29- year-old patient in the pT1bN1aMx group and 1 (one) 28-year-old patient in pT1bN0Mxwith a mean age of 28.5 (combined T1b group).

Table 3. Correlation of staging and age

Patient N	Age	Staging	Mean age	Mean age in the same "T" group
1	20			
2	42			
5	23	pT1aN0Mx	28.33	
3	41			
6	67			
8	35			
9	27	pT1aNxMx	42.5	36.43
4	29	pT1bN1aMx	29	
7	28	pT1bN0Mx	28	28.5

T stands for tumor size, N for lymph nodes (LNs), M for metastasis.

TX: primary tumor cannot be assessed, T0: no evidence of primary tumor, T1: tumor 20 mm or smaller (T1a: 10 mm or smaller, T1b >10 mm but not more than 20 mm), T2: >20 mm but not bigger than 40 mm, T3: larger than 40 mm or has begun to grow outside the thyroid, T4a: any size that has grown extensively beyond the thyroid, T4b: any size that has grown towards spine or into nearby large blood vessels.

NX: regional LNs cannot be assessed, N0: has not spread, N1: has spread to regional LNs (N1a: spread to pretracheal, paratracheal or prelaryngeal LNs, N1b: cervical, retropharyngeal or superior mediastinal. MX: distant metastasis cannot be assessed, M0: no metastasis, M1: has spread to distant LNs, internal organs, bones, etc.

All (100%) of patients had a total thyroidectomy. In addition, 4 (44.4%) patients had extended sparing excision of cervical lymph nodes. Pre-euthyroid TSH andFT4 levels were not available for all patients, and we decided to exclude this data. **Table 4** depicts all the data we gathered in a single table.

Table 4. Depicting all patients' data

Patient N	Age/Sex	Presenting Symptoms	Comorbidities	Size (mm)	Stage
1	20/F	Insomnia	--	8	pT1aN0Mx
2	42/F	Tachycardia, tremor	Chronic autoimmune thyroiditis	7	pT1aN0Mx
3	23/F	Tachycardia	Toxic diffuse goiter	7.5	pT1aNxMx
4	41/F	Tachycardia, muscle pain	Toxic diffuse goiter	13	pT1bN1aMx
5	67/F	Tachycardia	Toxic nodular goiter	8	pT1aN0Mx
6	35	Tachycardia	Toxic nodular goiter	4	pT1aNxMx
7	27	Tachycardia, tremor	Toxic diffuse goiter	15	pT1bN0Mx
8	29	Tachycardia, dry mouth	Toxic diffuse goiter	3	pT1aNxMx
9	28	Tachycardia, sweating	Toxic diffuse goiter	5	pT1aNxMx

Discussion

The foundation for the thought that cold nodules are more likely to be malignant was laid by studies conducted in the 1960s when the prime method of investigating thyroid nodules was by radio-iodine scintigraphy [1]. The increased use of imaging by ultrasound has led to the discovery of many more microcarcinomas which now constitute 32.1% of all papillary thyroid cancer cases [2]. The case series showcases patients with Graves' disease, autonomous functioning thyroid nodules (AFTN), and chronic autoimmune thyroiditis presenting with histologically proven papillary thyroid cancer. The presentation of thyrotoxicosis in such patients can either be due to concurrent non-functional thyroid cancer with thyrotoxicosis due to Graves' disease or other autonomous hot nodules, or it could be due to hyperfunctioning thyroid cancer nodules. This can be differentiated with the help of radio-iodine intake which was not done on our patients.

Just as in studies done previously, papillary thyroid cancer is the predominant type of cancer seen in graves and AFTN patients .100% of our patients were diagnosed with PTC compared to 88% in a study conducted by Joy U L Staniforth et al [3]. This study also reaffirms the notion that most cases of Graves diseases presenting with thyroid cancer are seen in young females [4]. All the cases seen in this study are female with most of them between the ages of 20-29. 7 out of the 9 cases were below 1 cm and hence are classified as microcarcinomas which is consistent with the findings conducted by Pzaitou-Pannayiotou et al [5]. When comparing the sizes of the nodules, the mean size of PTC nodules in Graves'disease was larger than in AFTN. This is in contrast to a study by Sunil Dutt Sharma et al. that showed cancer in AFTN patients had larger tumor sizes and were more aggressive [6]. The potential for malignant spread cannot be determined because all our patients presented before the tumor could metastasize.

The management of patients with hyperthyroidism due to Graves usually entails medical treatment which does not prevent the progression of cancer if present. This highlights the importance of cytologic studies with FNA to further analyse the thyroid nodules which may help the physician to uncover cancerous nodules and proceed with total thyroidectomy as was seen with the patients in our studies. The prognosis for patients with papillary microcarcinoma in conjugation with Graves or AFTN is excellent when treated with total thyroidectomy.

It has also been suggested that occurrence of thyroid cancer in patients with hyperthyroidism is because of the fact that increasing number of total thyroidectomies are performed in patients with refractory hyperthyroidism and subsequent investigation of the whole gland is associated with discovering microcarcinomas incidentally [1].

In our study all 9 patients underwent total thyroidectomies, however only one patient had

thyroidectomy because of refractory hyperthyroidism. In this case no nodule was discovered on ultrasound and microcarcinoma was detected on histological investigation incidentally. 7 patients had suspicious nodules discovered on routine ultrasound and only one had palpable thyroid nodule on physical examination.

Conclusion

Even though there is no clear association between thyrotoxicosis and thyroid cancer, patients with a history of hyperthyroidism have an increasing incidence of detecting malignancy. In this study 9 Patients with history of hyperthyroidism due Graves disease, toxic multinodular goiter and chronic autoimmune thyroiditis were diagnosed with papillary thyroid cancer during their disease course. In all cases malignancy was detected before metastasis and patients were managed with total thyroidectomy. Increasing incidence could be explained by the fact that patients with hyperthyroidism undergo more extensive investigations and routine screenings with ultrasound that increases the probability of detecting microcarcinomas. However, previous belief stating that thyroid cancer should be suspected in euthyroid patients with non-functional nodules is largely questioned.

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ZURA KATSITADZE [1], SOPIKO GOGIA [1], KHATUNA LOMAURI [2]

UPTAKE OF INFLUENZA VACCINE IN PREGNANT WOMEN IN GEORGIA IN 2020-2021

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Abstract

Utilization of influenza vaccine among pregnant women in Georgia remains suboptimal. To uncover some of the contributing factors to low uptake of influenza vaccine among pregnant women in Georgia. A cross-sectional survey was conducted in Spring-Summer 2021 on the postpartum women as the focus population. Females >18 years old were asked to complete the survey. The survey contained 14 items. The questions were categorized into 5 main groups. A total of 200 surveys were delivered to the hospitals. Survey results reveal that regnant women's awareness and attitudes regarding the Influenza vaccination were subpar and not conductive to reliable efforts in optimal vaccine uptake. While the absolute majority of the study subjects confirmed that they had heard about the Influenza vaccine, less than a quarter of them accepted to be immunized. Importantly, half of the responders discussed the subject of immunization with their healthcare provider, however, had not made the final decision for vaccination. There is meaningful space to encourage pregnant women's awareness and education on benefits and safety of influenza vaccination during pregnancy. This is preferable to be performed through the education and information campaigning conducted by health care providers working in perinatal care facilities.

Introduction

Influenza viruses cause annual seasonal epidemics worldwide. Increased rates of infection are associated with the 2nd, 3rd trimesters of pregnancy [1] and the hospitalization rate due to Influenza complications is 4 times higher than that of the general population [2]. Official data from the USA[2], Canada [3], and Australia [4] suggest that 7-9% of patients in intensive care units (ICU) are pregnant women. Among these reports, the USA and Australian studies show viral pneumonia (confirmed with bilateral infiltrations on chest x-ray) in 40-49% of hospitalized patients; while in Australia, 20% of cases were further complicated by secondary bacterial pneumonia.

Influenza vaccine is estimated to prevent 40-50% of influenza hospitalizations in pregnant women per year [5]. Additionally, preterm birth and fetal growth restriction are also positively affected by the antenatal vaccine [5]. Since 2004, the Advisory Committee on Immunization Practices (ACIP) and the American College of Obstetricians and Gynecologists (ACOG) recommends influenza vaccination in women regardless of the trimester of pregnancy [6,7]. Nowadays, most countries' health authorities have issued recommendations for Influenza vaccination in pregnant women, with many low and middle income countries providing the vaccine for free and on-demand. Still, the coverage with Influenza vaccination remains very lower in this population, even in high income countries [8]. The reasons for the negligible level of influenza vaccine uptake during pregnancy are not well understood, although concerns about vaccine safety and efficacy are often cited by pregnant women as two of the determining barriers to vaccination [9-11].

In Georgia, Influenza vaccine popularity and uptake among pregnant women remains miserable; so are studies uncovering grounds for this problem in the country. We have approached this issue from the standpoint of knowledge of perceived benefits or harms of the influenza vaccine by pregnant women. The special questioner covering the topics of attitude and knowledge about the influenza vaccine, its benefits, and risks has been created and distributed among women who were pregnant in the 2020 flu season, or would have probability to be pregnant during the 2021 flu season.

Like the similar studies carried out in other countries [12,13], a clear pattern has been defined: lack of knowledge about vaccine's benefits and risks plays the main role in declining to be immunized.

Our findings define a clear framework for future efforts and are conductive to specific strategies which would be helpful in increasing the rate of influenza vaccine uptake in pregnant women in Georgia.

Materials and methods

Study design and participants

This cross-sectional study was developed and performed in Georgia to understand current and future influenza vaccination-related attitudes, practices, and beliefs in the pregnant Georgian population. The survey was conducted during May 17-June 30, 2021, in 3 private maternity care hospitals. Considering the novelty of the survey in Georgia, no established sample size was determined in advance. Females >18 years old were asked to complete the survey. It was conducted in accordance with all applicable laws of the Republic of Georgia.

Survey Instrument

The survey contained 14 items. The questions were categorized into 5 main groups: 1. Knowledge the influenza vaccine; 2. History of influenza vaccine experience; 3. The readiness of women to receive the influenza vaccine during the pregnancy; 4. The main reasons for rejecting the vaccine. 5. Socioeconomic characteristics of women, including age, educational level, and the type of the health insurance. The additional items asked about the presence of chronic diseases and whether the influenza vaccination was offered to the patient by obstetrician or no.

Results

A total of 200 surveys were delivered to the hospitals. Of these, 150 were given to Ob/Gyn healthcare providers and distributed to their patients during healthcare visits; 50 were distributed to the pregnant women in the reception area of one of the participating maternity clinics. The response rate was low for the former (32%) and even lower for the latter (<20%).

The mean age of the subjects was 26.4 years. A total of 29.4% of the responders have more than 1 higher education and 58.8% have at least 1 academic degree. All but 1 responder have heard about the influenza vaccination. A total of 18.31% (N=13) of the responders reported at least one influenza vaccination in the past. 81.95% of the subjects have never received an influenza vaccine.

Only 23.66% (N=17) of subjects have received or are planning to receive influenza vaccine during the pregnancy. 29.16% (N=21) of the responders have not decided whether they will receive the vaccine. 45.83% (N=33) of the subjects are not going to receive the influenza vaccine (**Fig. 1a**).

A higher proportion (37.5%) of the women in the 18-28 years' age range were compliant with the recommendation than in the other, older, age groups (**Fig. 1b**).

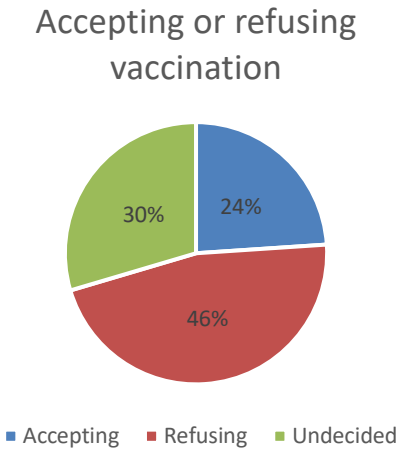

Figure 1a

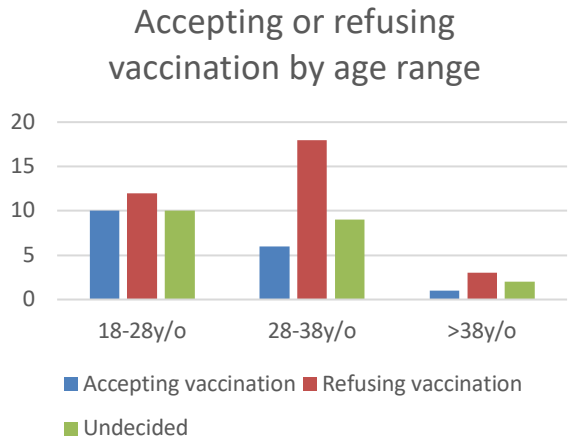

Figure 1b

Women with more than 1 academic degree had a high rate of vaccine acceptance of 46.66%, while any other formal education achievement fell under 25%; there was no positive correlation with having acquired 1 academic degree (**Fig. 1c**). Interestingly, the interviewees who disclosed having 1 or more academic degrees had a higher likelihood of having decided either for or against the flu vaccine rather than being undecided (70% and 80% respectively for 1 and >1 academic degrees); however, this did not necessarily indicate similarly higher odds of having the accurate knowledge (specifically in the 1 academic degree subgroup).

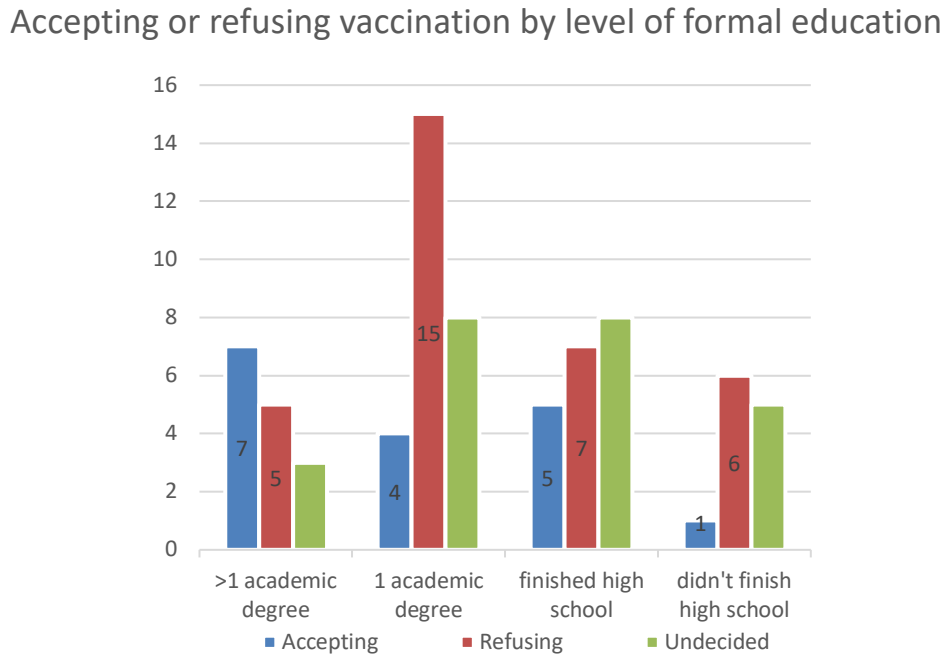

Figure 1c

A total of 35.9% of interviewees refusing the vaccine are avoiding the vaccination due to pregnancy. 30.30%did not define the reason for not receiving the vaccine. 18.18% are avoiding the vaccination due to the possible side effects to the fetus. 6.06% (N=2) think that the vaccine may negatively affect their health and 33.33% (N=11) think that the vaccine may not have a protective effect (**Fig 2a**). In contrast, the women who agreed to be vaccinated most commonly stated protective effects on the fetus as their motivating factor (**Fig. 2b**).

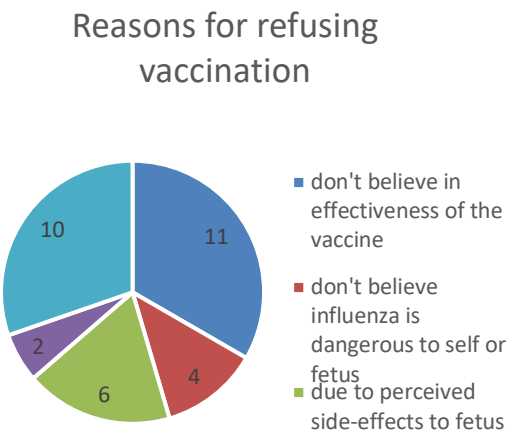

Figure 2a

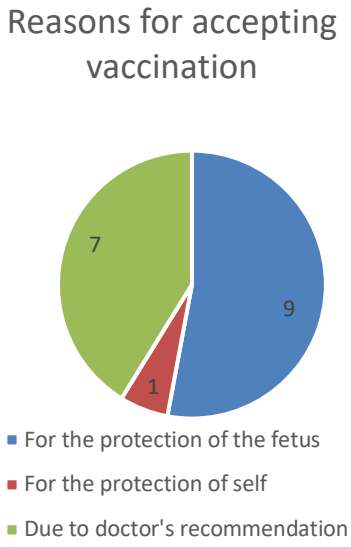

Figure 2b

23.61% (N=17) of the respondents have heard about the vaccine from more than one source. 48.61 (N=35) patients had not had the conversation about influenza vaccination with their healthcare provider, but this number could be due to the study carried out during spring and summer, non-influenza season.

A promising (however slightly) fact of the matter was the positive correlation between having discussed influenza vaccination with the healthcare provider and agreeing to influenza vaccination, with the NNT=7.66 (**Fig. 3a**). Another potential cue for deciding upon a flu vaccine was a previous history of getting one (**Fig. 3b**); those who accepted vaccination during current pregnancy were much more likely to have had a history of flu vaccine sometime in the past (OR=24.28), although this did not inquire into the timing of previous flu vaccines (eg. during previous pregnancy or outside any pregnancy).

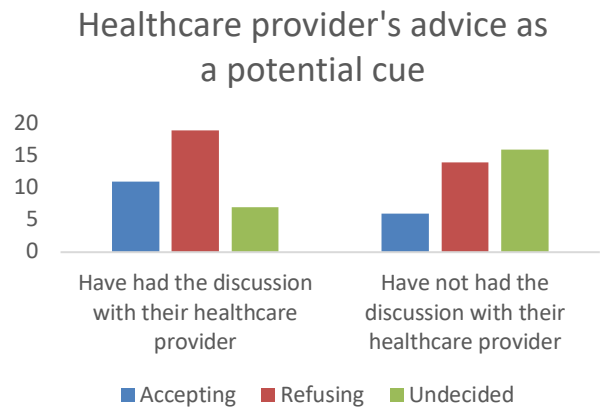

Figure 3a

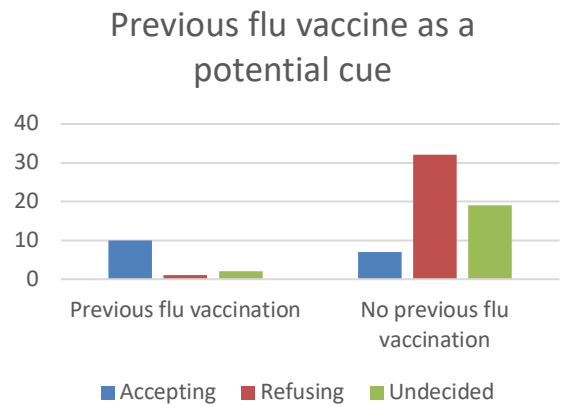

Figure 3b

Discussion

Prevention is the pinnacle of the public health response to Influenza, and vaccination is the most reliable means to this end. This holds that much truer for pregnant women who stand a higher risk of complications to them, their fetuses, and afterwards infants. Hence, pregnant women are classified as a high priority group, to be vaccinated in all trimesters and during breastfeeding during the influenza seasons. Immunization of women during pregnancy may be advantageous for the mother and the fetus; regarding the fetus, this is achieved in two ways: the passage of antibodies from the mother to the fetus during pregnancy, and by preventing infection in the mother and therefore decreasing the infant's risk of exposure [12]. Currently, the flu vaccine is not approved for use in children under the age of six months, making the latter point all the more significant. A randomized study in Bangladesh [14] showed that inactivated influenza vaccine given during pregnancy reduced laboratory-diagnosed influenza incidence by up to 63% in infants aged 6 months or younger. Still, vaccine utilization in pregnancy remains lower than in most other risk groups.

In Georgia during the 2018-2019 seasons, 465 cases of Influenza were laboratory-confirmed, of which 462 were type A and 3 were type B; out of these cases, 35 lethal outcomes were Influenza type A [15,16]. In 2009, 33 pregnant women passed away in Georgia, 5 of them due to confirmed Influenza infection. The incidence of upper respiratory tract diseases in infants was estimated 831.4 per 1000 in 2018 [15,16] in Georgia; in the same age group, 3635 hospitalizations were attributed to all infectious diseases.

Our study revealed vaccination coverage is much lower than that of some European and American nations – for example, 37% in the United States [17], 40-42% in England [18]; while France estimated a low rate of 7.4% in a national representative survey carried out during 2015-2016 [19]. Such discrepancies could partly be explained by the fact that methods and timing of data collection varied between these studies. In this line, our study is limited by the fact that the survey was conducted during a non-influenza season (late spring and early summer) and in a single year. In addition, our sample size was small, and the response rate was fairly low too. Therefore, these results may not be generalized to all populations of pregnant women in Georgia. Moreover, we have no information on the women who refused to participate in the survey, and they may be in some significant ways, different from the overall sample.

Of the surveyed patients, 23.66% reported receiving or planning to receive the influenza vaccine during the 2020/2021 season. This was in spite of the fact that almost all responders knew about the existence of the "flu shot", and 51.38% recalled a discussion of the topic with their obstetrician during their prenatal course. However, according to the survey carried out among Georgian obstetrician-gynecologists in 2015, only 43% of physicians reported recommending influenza vaccination during pregnancy [20]. In our study, women who had discussed the issue of influenza vaccination with their physicians were more likely to get immunized (OR=2.12). This number, although appreciated, is in no way a cause for self-contentment. According to the previous study, it seems like the obstetrician-gynecologists currently are not up to par, when it comes to Influenza vaccination, with their ability to convince their patients of its necessity [20]. Indeed, if the vaccination gap is to be closed, not only would the other half of the patients have to have the discussion with their providers, but also the providers must be better trained to deliver discussion. Finally, a history of influenza vaccination was positively correlated

with chances of getting a vaccine during the current pregnancy. This on one hand is good news in that it alleviates the pressure from obstetrician-gynecologists and evens it out to all primary care practitioners. However, on another hand, it brings to the spotlight the dire state of flu vaccination efforts in the general population of Georgia. Regardless, the takeaway point from this is to emphasize the benefits of vaccination in all women of childbearing age, knowing what palpable difference it might make down the line during a future pregnancy.

The high rate of declining to be vaccinated, even after the suggestion of their care providers, underlines the need to provide reasoning and education along with the vaccine. The majority of women in our study incorrectly believed that pregnant women have the same risk of complications from influenza as non-pregnant women; 33.33% of the vaccine refusers also did not believe that vaccination had a proven efficiency in preventing the seasonal flu (especially the complicated) in mothers and infants. A greater educational effort in pregnant women is important not only for vaccination promotion, but also so that the pregnant women who do become infected seek medical attention early on; current statistics in the USA [2], Canada [3], Australia [4], revealed that all pregnant women hospitalized for influenza sought help after more than 2 days had passed, and not in the optimal timeframe for the pharmacological therapy to be most effective.

The need for better education efforts for pregnant women becomes obvious when exploring women's thoughts on safety. The vaccine is considered to be safe during all stages of pregnancy and breastfeeding; no serious adverse effects or undesirable outcomes have been identified in either women or infants [21,22]. Nonetheless, 18.18% of the vaccine refusers in our study identified doubts about vaccine safety during pregnancy and breastfeeding, as the primary rationale for their decision.

Several factors were identified as the potential positive cues for influenza vaccination. The younger age group (18-28y/o) had a higher likelihood of complying with the recommendation, and so did the women who had earned more than one academic degree.

Noteworthy, in Georgia, the very low vaccine coverage among pregnant women (and the general public) could be explained in part by the fact that national recommendations were relatively recent; countries that have implanted similar policies earlier have been showing a positive trend in immunization coverage over many years [23,24].

In summary, this is the first study surveying pregnant women in Georgia to uncover their knowledge and attitude towards influenza vaccination.

While high income countries of Europe and the USA have a long history of Influenza vaccination campaigns and investigating the barriers to their vaccination efforts, such studies have been scarce in the developing world. As such, both the physicians' possible reluctance to recommend vaccination and the reasons for pregnant women's hesitancy are of great interest. Overall, in our study the most commonly cited barriers for vaccination were doubts about safety and uncertainty about its necessity which highlights the importance of education programs for Georgian woman in reproductive ages and their physicians. Finally, additional research is needed to better define the barriers and the prompts currently present; the long-term goal of increasing vaccine uptake could only then be addressed appropriately.

Acknowledgments

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IMPACT OF THE COVID-19 PANDEMIC ON THE REGULARITY OF CHECK-UPS IN PEOPLE WITH CHRONIC CONDITIONS

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Abstract

The COVID–19 Outbreak has put the healthcare system under duress and disrupted the flow of care provided to chronic patients. The study aimed to observe if there was a decrease in the regularity of check-ups and identify the factors that played a role in this downfall such as healthcare access, monetary funds, fear of contracting the virus, and other lifestyle factors. The results showed that there was a decrease in the consistency of check-ups due to influence by the aforementioned factors. A cross-sectional study was done to do the same with the geography of the United Arab Emirates. To conclude, the healthcare system needs to find approaches to combat the shortcoming as that were faced during this pandemic in regards to the regularity of check-ups for chronic patients.

Introduction

The SARS-CoV-19 coronavirus (COVID) Outbreak began in December of 2019 in Wuhan, China. It was reported as a flu-like condition that eventually turned fatal. Currently, the amount affected is about 10% of the global population, or 780 million people (WHO estimate as of early October 2020). As the pandemic took over the globe many countries declared a state of emergency and imposed safety restrictions. One of the many countries that were affected quite severely was The United Arab Emirates [1].

The 1st case in UAE was reported on January 29th, 2020 in a family from Wuhan. By the end of February, UAE had 27 patients who contracted the virus. This rise in cases forced them to shut down schools and universities beginning in March and even reschedule an international event, Expo2020 which was predicted to bring 33 billion dollars in revenue. The first death was then reported on March 20th. Despite a sterilization campaign in mid-March to contain the virus, it had infected a total of 241 people. This led to the government implementing a lockdown which reduced movement for 10 hours. By April 24th, the government took a further initiative to impose a 24-hour lockdown for 2 weeks where only one family member was allowed to leave for essential amenities. Malls were shut down by the end of March along with places of worship. On March 22nd, UAE suspended all flights for 2 weeks and continued to restrict flights with high-risk countries such as the United States. The drastic rise in cases pushed the country to increase COVID testing for free. Currently, the UAE has had 620,309 cases till June 2021 with 1775 deaths [2,3].

As the pandemic worsened, the healthcare system took an indirect hit in delivering appropriate care. When diagnosed with a chronic condition, it becomes essential to visit a primary physician regularly to monitor the progress of the condition and prevent any complications. However, during the ongoing COVID-19 pandemic, patients have cancelled and postponed many out-patient visits due to fear of getting infected and to decrease the number of contacts with healthcare professionals [4]. Many resorted to telemedicine due to a shortage in healthcare resources. The decline in monitoring may not seem significant, but the cumulative risk over time can prove to be consequential. Under normal circumstances, chronic diseases can be controlled with timely intervention and a decrease in the regularity of check-ups can result in more visits to the ER with exacerbations.

This research observes “How the COVID-19 pandemic affects regular check-ups in Patients with chronic conditions”. Routine care for chronic disease is an ongoing major challenge. Currently, global healthcare pivots around providing relief for those affected most by the pandemic and this disrupts the continuous stable care received by chronic patients. This research aimed to observe how COVID-19 downplays health via aspects other than being infected. Moreover, it is to identify areas that impacted the regular care and allow this to be the foundation for future research. It hopes to provide data that will help

the healthcare system to better adapt and improve during a crisis, and identify the areas in healthcare that were affected most by the pandemic.

The hypothesis states that the frequency of regular check-ups has declined since the outbreak and an observational study was conducted to discover what aspects contributed to it. Some of the factors considered are the fear of infection, lack of healthcare access like transportation, difficulty obtaining medications, reallocation of hospitals to COVID facilities, and other personal aspects including adherence to medications, physical activity, financial issues, and insurance.

Methods

A total of 60 patients with various chronic diseases were asked to participate in the research. The inclusion criteria were any patients over the age of 30. It was also mandatory for them to be residents of the UAE. The exclusion criteria consisted of any patient who was diagnosed during the period of the pandemic and those who had CKD requiring hemodialysis.

A survey was created through Google Docs consisting of 19 questions (**Table 1**). It was then posted and shared on various social media groups. The survey provided the participants with detailed instructions of the criteria as mentioned above.

Table 1

1	Gender	Male Female
2	Age	30+
3	What disease(s) do you have?	-
4	Do you have insurance?	Yes No
5	Did you go on regular checks up before the pandemic?	Yes No
6	Did you go on regular check-ups during the pandemic?	Yes No
7	Did your health become worse during the pandemic?	Yes No
8	Were you physically active before the pandemic?	Yes No
9	Were you physically active during the pandemic?	Yes No
10	Did you notice a change in quality in the clinic? If so, was it good or bad? (More than 2 can be picked)	Yes No Good Bad
11	Did you have to change your doctor because of a clinic/hospital shut down?	Yes No
12	Did fear of getting Covid make you go for fewer checkups?	Yes No
13	Did you go for fewer checkups due to financial reasons?	Yes No
14	Did you go for fewer checkups due to problems like transport? (Note: There were periods when buses and metro were shut down)	Yes No
15	Did you take medications regularly during Covid?	Yes No Sometimes
16	Did you have problems getting medications during Covid?	Yes No
17	Did Covid cause you mental stress?	Yes No
18	If yes, did you talk to your doctor about it?	Yes No
19	During covid, did you get hospitalized because of your disease?	Yes No

The questionnaire was designed as a cross-sectional study to assess the risk factors affecting the regular check-ups of those diagnosed with chronic conditions. There were two components in the survey. The initial part required patients to identify optimal information such as gender, age, and the chronic diseases they suffer from. The second component consisted of observing the influence of the risk factors such as insurance, monetary funds, transportation, lifestyle modifications (physical activity, medication adherence, reallocation of primary doctor), and mental stress. It also analyzed the fear of COVID in patients and whether it deterred them from regular check-ups.

The survey was created in May 2021, and implemented throughout May-June 2021. After the survey was completed, data was collected in an organized manner.

Results

Age and Gender - 60 participants took part in this survey, out of which 44 (73.3%) were males and 16 (26.7%) were females. Although our inclusion criteria involve anyone between the ages of 30-70, most of the patients are between the ages of 54-57. In the participants ranging from the age of 30-70, the mode is 54, 57, with a mean of 52.7, and a median of 54 (**Figure 1**).

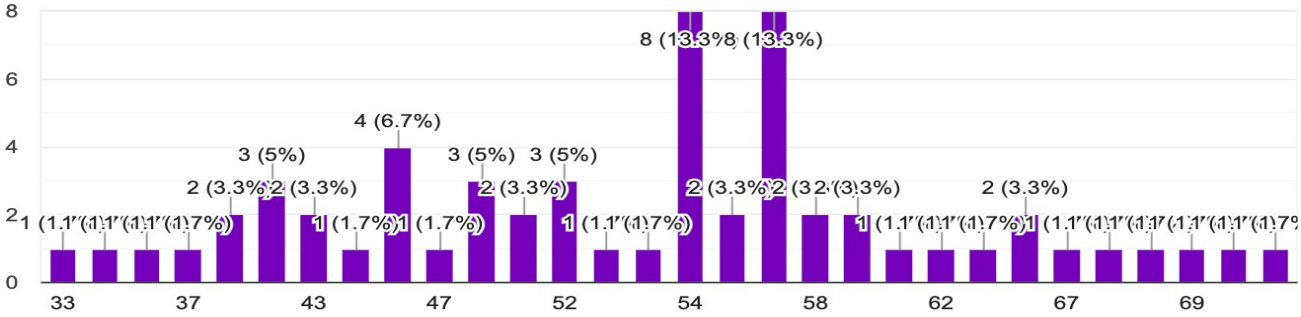

Figure 1

Distribution of Disease - The study consists of various diseases where 35(58.3%) have hypertension, 21(35%) - diabetes, 10(16.6%) - hypercholesterolemia, 5(8.3%) - thyroid diseases, 3(5%) - cardiovascular diseases, 2(3%) - gout and there were 2(3%) with other chronic illnesses like Parkinson's disease and hemorrhoids (**Figure 2**).

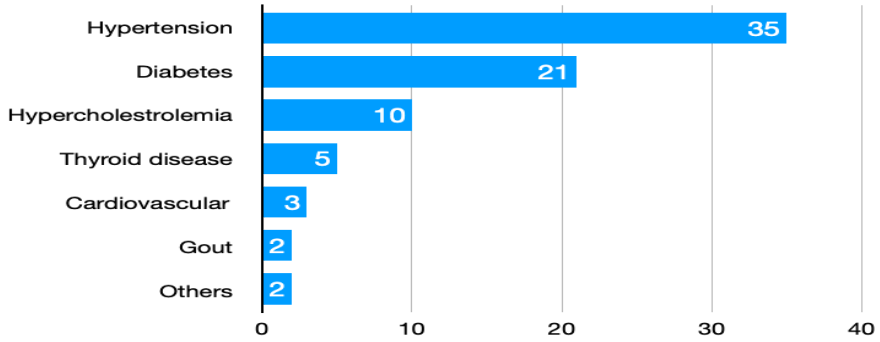

Figure 2

Impact on the regularity of check-ups - Before the pandemic started, 54 (90%) of our participants were compliant with regular check-ups. However, since the pandemic began, there was a decline of 42.5% in regular check-ups, where 23 people stopped going for check-ups (r=0.425), and 31 continued to attend (r= 0.57). 6 participants were not visiting clinics regularly amongst whom 1(16.6%) switched to regular check-ups while the rest continued to neglect regular care (**Figure 3**).

Physical activity levels -The survey also observes the changes in the lifestyle habits before and during the pandemic, i.e., medical adherence and level of physical activity. 88.3% claim they were active before the pandemic and 11.3% were not. Amongst the physically active participants, there is a decline of 20% activity whereas 79% continue to exercise. This shows the lockdown had a considerable impact on the ease of exercising (**Figure 4**).

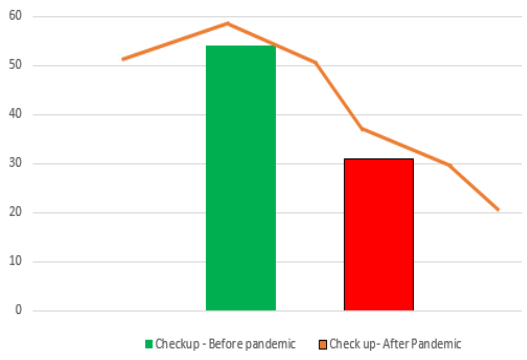

Figure 3

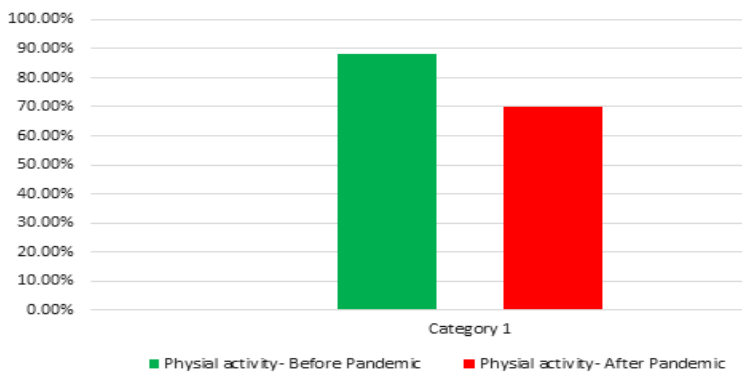

Figure 4

Adherence to Medications - When medication adherence is assessed, 85% claimed to take medications regularly, 13.3% said sometimes and 1.7% said they didn't. It is also important to note that 18.3% faced difficulty in getting medications which could be due to various reasons, for example delay in import of foreign medications. However, to further understand the association between regular check-up and medical adherence, it can be seen that amongst the 23 people who stopped regular check-ups, 73.9 % are adherent to medication and the rest 26.1% are adherent to medications sometimes. In the same manner, when physical activity and regular check-ups are assessed, only 56.5% of 23 participants are physically active (**Figure 5**).

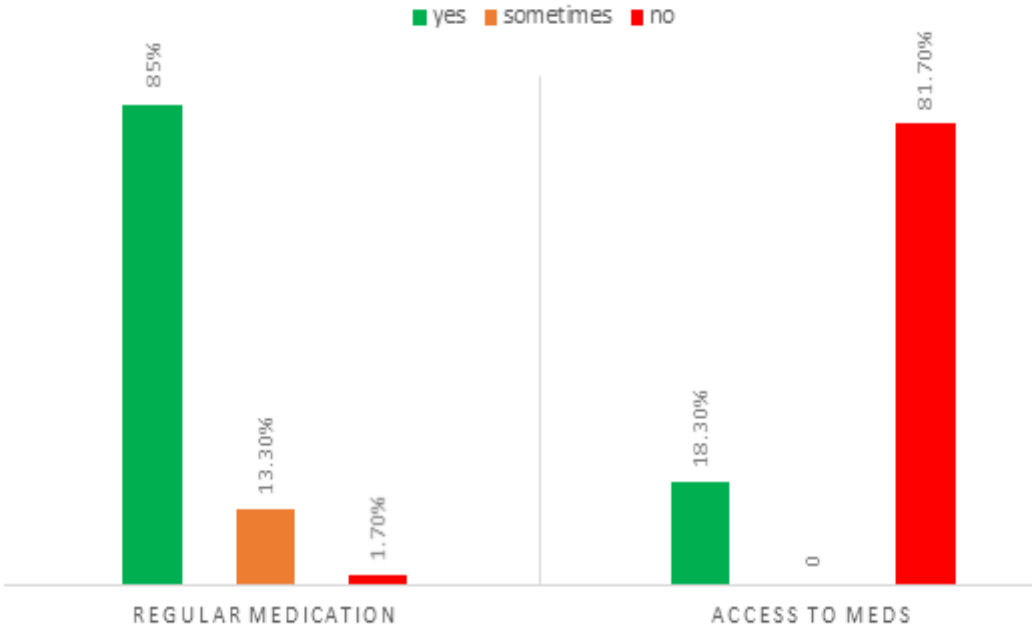

Figure 5

These results can be further combined to show that 13 participants amongst the 23 practice both lifestyle habits. (Ratio of 0.56).

Access to medication was also assessed, and no significant data was found.

Finance and insurance - 78.3% of the participants have medical insurance and 21.7% don't, but when inquired about financial problems only 15% stated that they did face an issue in getting appropriate care. Of the 23 participants who did not go for regular check-ups, 8 had financial issues, among which 5 participants had monetary issues due to factors other than insurance, and 3 of them faced financial difficulties due to lack of insurance. From this, we infer that although finance was a considerable factor that affected the regularity of check-ups, insurance in itself did not play a great role.

Fear of Infection - Fear is a major factor that is associated with the decline in check-ups. In general, a ratio of 0.55 of participants admitted to having fear of being infected with COVID-19. However, specifically, there is a ratio of 0.65 that stops regular check-ups due to fear of the virus.

Transport - Transport can be a major barrier to accessing healthcare services. About 85% of participants in the study did not have these issues. In the 15% of people with transport issues, it was noticed that 56% stopped regular visits. This value holds a great significance in associating lack of transport with fewer check-ups.

General effect on health - 86.7% said their health did not deteriorate and 13.3% said that their health did, but contrary to our expectation only 1.7% were hospitalized for their condition. The worsening of health in this situation can be attributed to lack of healthcare since 6 out of the 7 patients that had exacerbation of their condition were not attending regular check-ups. The minimal deterioration of health in comparison can go to confer that although the regular check-ups were lacking, the participants managed to overcome the risk of increased mortality of their disease by adhering to their medications timely among other healthy practices (**Figure 6**).

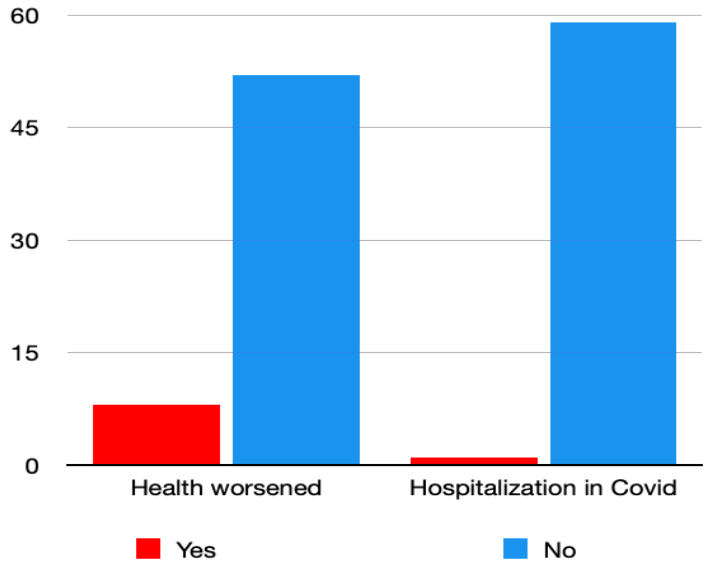

Figure 6

Clinic Quality - Upon asking about changes they might have noticed in the healthcare system 58.3% noticed a good change in the quality of the clinic and additionally 13.3% said they've always had a clinic of good quality which shows that the health care system took early precautions against the virus and improved the experience of the general public. However, out of the 23 people who stopped going for healthcare 4 claimed that the experience was bad. This infers that one of the factors that provoked delay in healthcare was a decline in its efficacy (**Figure 7**).

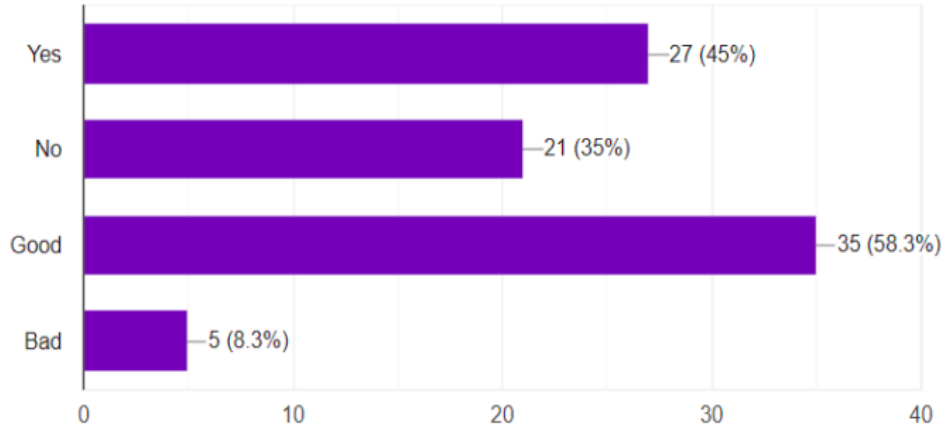

Figure 7

Hospital Shut down - One of the major outcomes of the pandemic is the lack of resources due to the shutdown of hospitals. The patients in the survey were asked if they had to switch their primary health care clinics and 81.7% deny any such changes which go to show that most clinics in UAE had a separate unit for Covid patients. However, amongst 11 people who did have to change, there is a decline of check-ups in 8 patients (ratio of 0.34). The information shows that although most did not change clinics, this dilemma may play a significant role in reducing check-ups as most of the medical personnel reallocated into Covid departments.

Mental Stress - Mental stress, in general, can affect your overall health and this has majorly been impacted in the pandemic. 53.7% of the patients faced mental stress but only 16.7% talked to their doctors about it. The decline truly can be attributed to whether they had an opportunity to talk to their physicians. It is noted that among the 23 patients (16.7%) who claim a lack of conversation with their doctor, 12 had stopped going for regular check-ups. Hence, it is seen that among the 32 that faced mental stress, only 11 truly lacked a mental assessment during check-ups. This percentage, although small, shows that mental health is not prioritized during the ongoing pandemic and the health care providers must actively monitor the mental health of the patients as it can affect morbidity and mortality (**Figure 8**).

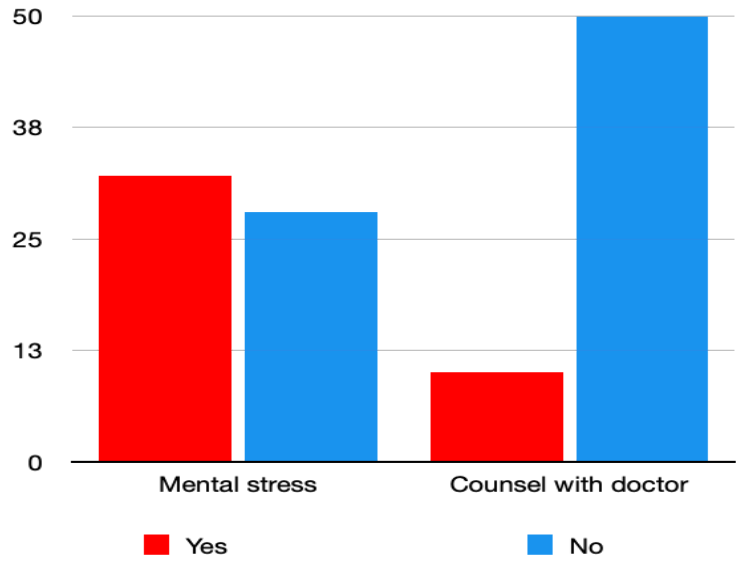

Figure 8

Risk factors are summarized in **Table 2**.

Table 2

Risk factors	Yes	No	Sometimes
Insurance	47	13	
Transport	9	51	
Med.adherence	51	1	8
Mental stress	32	28	
Finance	9	51	
Change in hospital	11	49	
Fear of Covid	27	33	

Discussion

Numerous studies have been conducted on account of chronic patients who delayed regular check-ups during the pandemic. Many countries such as the USA, India, and Germany have participated in these surveys and portrayed significant results. However, our research is one of the first to be conducted in the United Arab Emirates and has assessed the delay quotient and the risk factors that provoked the outcome.

An article from India that assesses check-ups in patients with diabetes during the pandemic noted that 80% of the study population were regular with their exercise. In compliance with this, our research also averaged that 70% of the population were consistent with the same. It was also noted that 40% of the Indian population expressed some form of anxiety, and 53.7% of our population also experienced considerable mental stress [1].

In a research-based study in the United States, an estimated 41% of US adults had delayed or avoided medical care. Our research shows that 23 out of the 60 participants (38.3%) who were compliant with check-ups have postponed their visit due to the pandemic [6]. Therefore, it can be presumed that there was an overall decline in the regularity of check-ups in various countries due to the pandemic (US> UAE).

The same source states that among 8% of the population that does not have insurance, 81.6% continue regular check-ups [5].

On the contrary, in our research 21.6 percent of the population did not have insurance yet out of these, 69.2% received healthcare on a normal basis from which we can infer that the effect of lack of insurance on chronic check-ups is more severe in the UAE than in the US. In addition, the article from the US stated that 69.7% of their patients with chronic conditions delayed routine healthcare check-ups,

however, the sample population of UAE showed that only 36.7% delayed their check-ups. This infers the factors provoking delayed check-ups were more severe in the US and less problematic in the UAE [5].

In terms of future perspectives, the research can expand its sample size to factor in a larger population's opinion. It can take into account more varied risk factors such as diet, contracting the virus, etc, to have a broader spectrum of results. Our research can be the basis to lead appropriate changes in the healthcare system to increase its efficacy and pattern of care.

Health education and promotion are important components of disease prevention in general but during a pandemic or healthcare emergencies they play a key role in active response by offering well-established tools (in case of absence of specific drug therapies or vaccines) in communicating and effectively engaging with the public. The usually long-lasting and trusted relationship between primary care physicians and patients ensures an open line of communication and may even prevent misinformation and misperceptions.

Well-developed primary health care systems can help identify high-risk groups i.e. chronic patients, and focus on preventing the infection more aggressively. It also improves patient satisfaction, hospitalization rates, and clinical outcomes.

Telemedicine has played a remarkable role during the pandemic due to benefits such as time efficiency (no waiting period in clinics, no commuting) and a decrease in direct contact with physicians. It helps reassure patients that they can reach out to their providers without putting their health at risk. Furthermore, it can help with decreasing the frequency of onsite visits by encouraging at-home monitoring of certain health parameters [6].

We have inferred that lack of insurance has deterred people from going for their regular check-ups to be more cost-effective, as many face obstacles regarding their financial situations. Here, in addition to the fact that telemedicine is the safest option in such situations, it also proves to be a better alternative option for those who lack healthcare insurance as it is relatively inexpensive [5].

The pandemic has affected the health of many by either inducing a certain degree of worry, anxiety and fear or worsening existing mental illness. According to our survey results, although several patients faced these issues, only a fraction of them had a conversation about it with their physician. This goes on to show that there isn't enough importance put on mental health which can be combated by physicians running screening tests and enquiring about the patients' stress levels at every check-up [5-7].

By studying the facts highlighted by our study we can better strategize how to maintain quality healthcare provided to chronically ill patients in adverse conditions such as the CoVID-19 pandemic.

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FEMALE PREDOMINANCE OF ANOSMIA IN COVID-19 INFECTION

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Abstract

Covid-19 is a pandemic that has changed the very structure of the world in the past year. Anosmia was not mentioned as a symptom during the first wave of the pandemic. Still, recent literature from both Western and Chinese started describing anosmia as an early symptom along with other flu-like symptoms. This paper discusses whether anosmia can be used as an early diagnostic symptom and if there is a female predominance of anosmia in Covid-19 patients. We used 290 Covid-19 positive patients from Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamil Nadu, India. The results of our study demonstrated that there is a female predominance of anosmia in Covid-19 patients. Anosmia can be used as an initial diagnostic tool for the infection.

Introduction

Covid-19, an emerging pandemic, has caused a vast disaster throughout the world and substantially has a massive impact on our global healthcare and economy. There has been newer evidence that Covid-19 has frequently been reported with neurologic symptoms such as anosmia and ageusia. Although Covid-19 initially has not been reported with anosmia alongside typical symptoms such as fever, cough, fatigue, and myalgia, Coronavirus, in general, is known to cause neurologic, gastrointestinal, and hepatic dysfunction in humans and animals. Evidence from newer research [7] has suggested that olfactory dysfunctions are widely reported throughout the world. Detection of mild olfactory dysfunction before severe dysfunction such as respiratory distress, cardiac arrest, and thrombosis can be helpful for timely treatment and management of the patient.

Additionally, Anosmia during Covid-19 infection has a higher predisposition towards the female gender than male Covid-19 patients. This paper focuses on the early detection of Covid-19 disease to help avoid severe untreated complications and establish that female patients are more likely to present with anosmia during their infection period. However, the exact pathologic mechanism or differences in inclination towards the female gender has not been addressed in this paper.

Methods

This is a retrospective observational study of 290 patients infected with Covid-19 between June 1, 2020, to July 1, 2020, in GMKMC, Salem, India. All patients described as having anosmia had a complete loss of smell during the period as per the medical records. The patients underwent a Sniffin-sticks smell test to identify anosmia.

Due to the pandemic restrictions, not all patients underwent smell testing during their stay in the hospital. Patients included in this study are aged between 18 to 80 years, with complete medical records, and the genders are female and male. Patients excluded were with prior neurologic diseases and patients previously diagnosed with anosmia.

Results

Two hundred and ninety patients were included in this study. Most of the patients were males; 65% (186 patients) and 35% (104 patients) were females.

Based on **table 1**, Out of a total number of 290 patients, 102(35.2%) reported having anosmia before getting admitted to the hospital - 37 (36.3%) of the 102 patients recovered from anosmia in a few days after admission. Additionally, 89 (30.7%) patients developed anosmia during their stay in the hospital.

Table 1. Distribution of patients with anosmia before admission and during their stay in the hospital.

	Present	Not Present
Before Admission	102	188
During Hospital Stay	154	136

A chi-square test of independence was performed to examine whether anosmia could be used as an early diagnostic symptom. The relation between these variables was significant, X2 (1, N = 290) = 18.9082, p = .000014. Anosmia can be used as an early diagnostic symptom.

Based on **table 2**, 66% (69) of the female and 46% (85) of the male population associated anosmia as a symptom during their Covid-19 infection, identified by the Sniffin-sticks smell test. The difference signifies that female patients predominantly present with anosmia than male patients during their infection period.

Table 2. Percentage distribution of Covid-19 infected females and males with anosmia during their stay in the hospital.

	Not Present	Presented with anosmia
Males	101	85
Females	35	69

A chi-square test of independence was performed to examine the relationship between gender and anosmia during Covid-19 infection. The relation between these variables was significant, X2(1, N = 290) = 11.4185, p = .000727. Female gender predisposes to have anosmia during Covid-19 infection.

Discussion

Seven known coronavirus variants cause infections in humans, including SARS-COV 2 (Covid - 19). SARS COV-2 enters through the ACE-2 receptor by binding its spike protein S1 [2]. The expression and distribution of ACE-2 receptors throughout the nervous system serve as an entry point for SARS-COV-2 to cause numerous neurological dysfunctions. Evidence from studies has shown olfactory dysfunction as the most common sign of Covid -19 infection [6]. The olfactory dysfunction in Covid - 19 has caught the attention of otolaryngologists all over the world. Anosmia has been reported as the first symptom or following mild symptoms such as cough, fever, fatigue, or developed gradually accompanying pulmonary dysfunction during their hospital stay.

The mean age of our patients included in this study population was 48 years, 65% were males, and 35% were females. The prevalence of anosmia was significantly higher in female patients (66%) than male patients (46%). The patients included in anosmia predominance in the female gender were chosen with impaired olfactory dysfunction during their inpatient stay with moderate to severe infection. The study is similar to Klopfenstein et al. [5]; A retrospective study showed 47% presented with anosmia. The study states that 67% of patients reported with anosmia were female patients [5]. The study also found that olfactory dysfunction is often accompanied by dysgeusia in Covid -19 patients [5].

A systematic review and meta-analysis conducted by Agyeman et al. [1] describe a high prevalence of olfactory and gustatory dysfunction in patients infected with Covid -19, and 41% of 8438 patients had anosmia. Our study also found out that 65.9% of the patients have had anosmia either before or during the hospital stay. Another survey by Printz and Constanidis [9], concludes that anosmia is more prevalent in Covid -19 patients than in patients suffering from other respiratory infections. 95% of the taste disorders are disorders of the olfactory system [7]. A study by Vinayachandran and Balasubramanian [10] states that the loss of taste could be secondary to anosmia rather than a problem in the gustatory system. So patients presenting with ageusia should also be tested for anosmia. A study by Hornuss et al. [4] states that anosmia was the sole symptom present for many patients, so primary care physicians and otolaryngologists should be aware of this putative presentation.

Thus, it should be mandatory to make anosmia a tool for initial diagnosis or to create a suspicion of Covid -19 infection and isolate the particular patient. Giacomelli et al. [10] also agree with our study that more research should be done in non hospitalized infected patients if anosmia can be used as a clinical screening tool. Since Covid-19 is highly transmissible, isolation of the patient results in the most significant curb of the spread of disease. In developing countries and underdeveloped countries, the amount of RT-PCR tests are limited; Anosmia can be used as a tool by patients to isolate themselves and contact the respective Covid-center or a Covid-clinic for further support.

The female patients should be prioritized for neurologic care while dealing with Covid-19 infection. The female gender potentially is linked to developing other neurologic dysfunctions since ACE-

2 receptor expression is throughout the nervous system. Female patients with previously diagnosed neurologic impairment should be cautious since serious complications such as acute encephalopathy are reported in Covid -19 infection [11]. Future studies should potentially study the morphologic differences for this prevalence of anosmia in the female gender.

We were only able to get the data of 290 patients. Since the number of infections is still increasing, the values of the results might change for a different sample size. The entire data was taken from a single hospital because of the limited resources. If the sample taken was much more extensive and diverse, there might be some changes in the results. The amount of research previously done on this topic is also significantly less than other areas of interest related to Covid-19 infection. We believe that many more researchers should take up this topic and try to find the answers on a larger scale so that it could help in a better understanding of anosmia in Covid-19 patients and maybe help decide the treatment plan and prognosis of the infection.

Conclusion

Anosmia in Covid-19 is a crucial symptom to be considered in Covid-19 infection. Covid-19 infection accompanies olfactory dysfunction in more than half of the cases. Potentially an initial symptom for clinical diagnosis. Additionally, Women with Covid-19 infection have a higher degree of developing anosmia.

Acknowledgement

We would like to express our special thanks to the General Medicine and General Surgery faculty from GMKMC, Salem, Tamilnadu, India, for providing the patient data for the research.

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POST-COVID SYMPTOMS AND ITS RELATIONSHIP BETWEEN PATIENTS AGE AND GENDER

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Abstract

With the advent of the Coronavirus in 2020, our economic and social situations have drastically changed. As a result, rather than finding a way to eradicate the virus, we have made several societal and behavioural changes that dictate us to live along with the virus. Nevertheless, the world is making strides toward creating a cure for Covid. Furthermore, to completely eradicate the virus, we must monitor the patients' symptoms during infection and after they recover (post-covid). Again, forming links connecting human components such as ages and gender with post-covid symptoms help us understand the virus to a much greater degree. This survey aims to scrupulously determine the relationship between people of distinct age groups, gender, and the post-covid symptoms they are exhibiting.

Introduction

With the announcement of the pandemic, the societal, behavioural, and economic changes that have occurred in our lives are of no ordinary magnitude. Moreover, with the elevation in the number of cases, the world is forced to take steps to eradicate this virus and restore society to what it was before covid. Though monitoring the patients' symptoms during covid is vital, it is also essential to monitor their symptoms after recuperating from covid. It is crucial to find empirical shreds of evidence linking the post-covid symptoms to human factors such as age and gender since it will deepen our understanding of the virus [1]. It will also give a basic idea of the various functionalities and dependencies required by the virus to manifest specific symptoms within patients. Consequently, we can construct an effective healthcare plan for a person combating severe post-covid symptoms based on age and gender. Social factors in correlation with age and gender also play an integral role in determining the types of post-covid symptoms experienced by a person [5]. For instance, the age group and the gender that had the most exposure to the outdoor environment due to social obligations such as work can be concluded to be the most affected.

The common variants of post-covid symptoms affect various systems, ranging from the respiratory to the excretory. Also, the post-covid symptoms differ in magnitude ranging from a simple fever to something complex like persistent anosmia. Some well-known post-covid symptoms are fever and chills, body aches, sore throat, shortness of breath, headaches, nausea, vomiting, diarrhoea, constipation, etc. In contrast, some obsolete Post covid symptoms are persistent anosmia, persistent ageusia, skin discolouration, hair loss, etc [4]. Apart from this, there are also speculations about the Coronavirus amplifying the underlying health conditions of a person. Not to mention, assumptions about coronavirus affecting the fatigue levels one feels after covid compared to before covid [4].

Ergo, this study aimed to assess the presence of a connection between age, gender, and the post covid symptoms exhibited by various people within the geographical confines of India. Respectively, to speculate and determine the most prevalent and the minor post-covid symptoms according to age group and gender was the focus of this research [6]. Subsequently, we can achieve a much extensive understanding of the virus through constructing links between the human factors and the pathogen.

Methods

This survey was conducted entirely online using google-forms. In addition, the survey was delivered to the respondents using a wide array of social media platforms. Furthermore, the survey was constructed under the cross-sectional researching model to record the various post-covid symptoms carefully. The survey established the respondents' consent. The survey had 20 questions, separated into three distinct parts. The survey recorded the credentials of the person in the first part. Additionally, the first part also confirmed whether the person had contracted covid or not. The survey further explored if that person had recovered from corona in the last six months or not. Then, the second part of the survey encapsulated the questions intended to record the post-covid symptoms and the possibility of the person having an underlying condition like hypertension, diabetes, heart failure, etc. Finally, the third part of the

survey contained the question that evaluated if there were any changes in the intensity of the underlying condition.

The prevalent question type in the survey was the yes / no model. Almost all the questions except the credential questions followed this pattern. The survey contained three conditional questions (one at the end of each part). Depending on the respondent's answer, the survey would progress into the next section or automatically end by submitting the response. Furthermore, this method was employed to ensure the recording of the responses in a more orderly function.

The data analysis was done with the aid of google sheets, soscistatics.com and quantpsy.org. Percentages were calculated for all the relevant and critical questions. Two chi-square analyses were conducted. One was to examine the relationship between the people who had post covid symptoms and the biological gender of the participants. Another one was to investigate the integrity of the relationship between the people who had post covid symptoms and different age groups of the respondents. In both cases, the results were significant as the two-tailed p-values were <0.05.

Results and Discussion

A total of 305 responses were received with the consent of the respondents. And, the majority of the respondents were males, 63.6% (n-194).

Respondents' gender: **Figure 1a** shows that most of the respondents who had post-covid symptoms were male, with 63.6% (n - 194). Female and the other gender respondents are responsible for 36.1% (n-110) and 0.3% (n-1) of the total recorded responses.

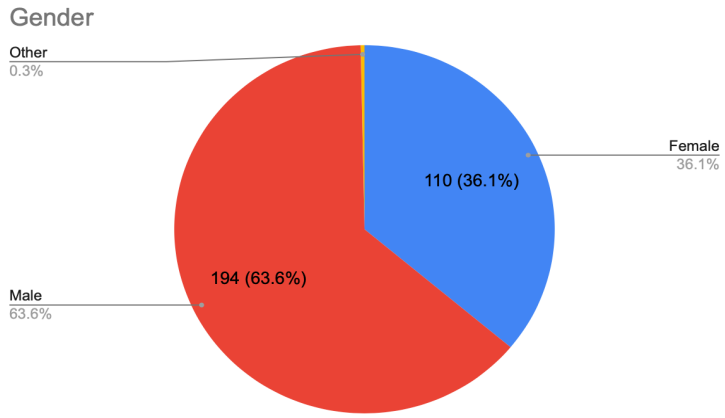


Figure 1a. Illustration of the respondents' gender (out of the people who had POST Covid symptoms).

Respondents' age: **Figure 1b** expresses that predominantly, the 11 to 20 age group has responded more than any other age group with 32.8% (n-100) of the total responses. The age groups between 21-30 with 28.2% (n-86) and 41-50 with 16.1% (n-49) hold the second and third positions, respectively. The age group with the least number of respondents happens to be 0 - 10 with 0.9% (n-3).

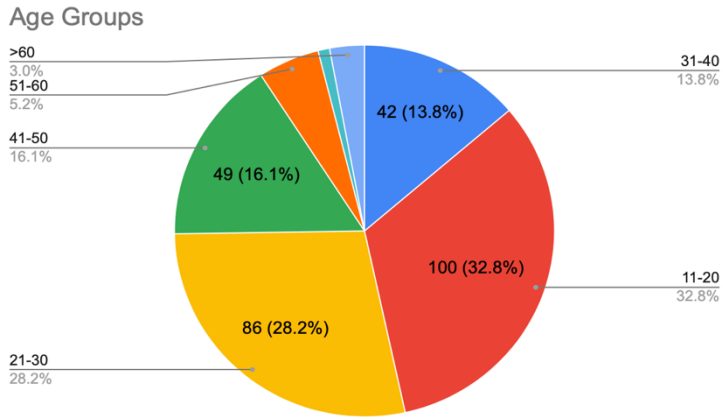


Figure 1b. Illustration of the Respondents' age (out of the respondent who had POST covid symptoms).

Respondents who had post covid symptom: **Figure 1c** illustrates that 65.2% (n- 305) exhibited POST covid symptoms out of the total responses. Alternatively, almost 34.8% (n-163) of the respondents did not show any post-covid symptoms.

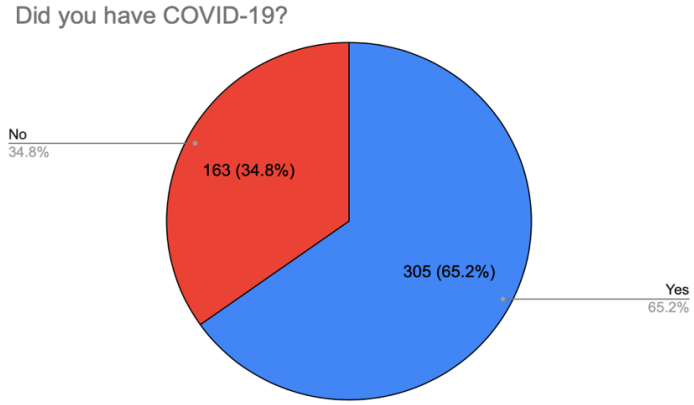


Figure 1c. Illustrates the Respondents who had POST covid symptoms.

Various symptoms in general and also based on different age groups and gender: Our data in **figure 2** delineates the various post-covid symptoms experienced by the respondents. Upon close evaluation, it is evident that the most common symptom experienced by the respondent is fever and chills, with 14.1% (n-216). The predominant symptom next to fever and chills is headaches, with 11.3% (n-173) of responses. It is also worth noting that despite headaches being the second most prevalent symptom, only a minute difference exists between headaches and the number of responses for anosmia, with 11.2% (n-171). Additionally, by a small margin, the third most common symptom among the respondents are persistent ageusia and sore throat, both having 11.1% (n-170). The least common symptom reported by the respondents is rash or discolouration, with 3.1% (n-47). Next to rash or discolouration, hair loss with 5.5% (n-84) of responses is also a lesser common symptom.

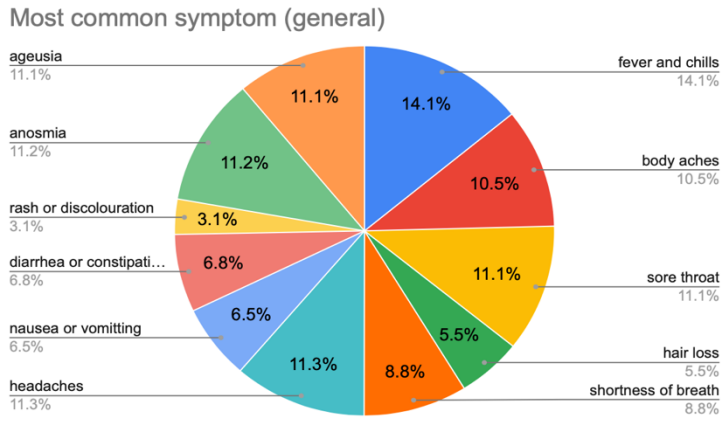


Figure 2. The various POST covid symptoms in general.

Various POST covid symptoms based on different genders: Figure 3 expresses the various POST covid symptoms based on different genders. From evaluating the data on **figures 3a**, fever and chills, which procured 14.3% (n-138) of the responses, are the most common post covid symptoms among the male respondents. Persistent ageusia with 12.3% (n-119) follows fever and chills as the second most common symptom among the male respondents. Following persistent ageusia, persistent anosmia with 11.7 % (n-113) is the third most common symptom experienced by the male respondents of this study. On the other hand, rash and discolouration with 3.4% (n - 33) is the least experienced symptom, followed by hair loss with 3.9% (n-38).

According to the given data on **figures 3b**, fever and chills with 13.9% (n-77) are the most experienced symptom out of all the other post-covid symptoms. Though it is a small margin between the second and the third most exhibited symptoms, body aches procure the second place with a response rate of 10.8% (n-60) followed by shortness of breath and heavy chest, both having 10.7% (n-59) of the responses. However, the least common symptom exhibited by the students appears to be rash or

discoloration, which holds 2.4% (n-13) of the reactions followed by diarrhoea or constipation with 5.8% (n-32) of the female responses.

Figures 3. The various POST covid symptoms among male (3a) and female (3b) responders.

Figure 3a.

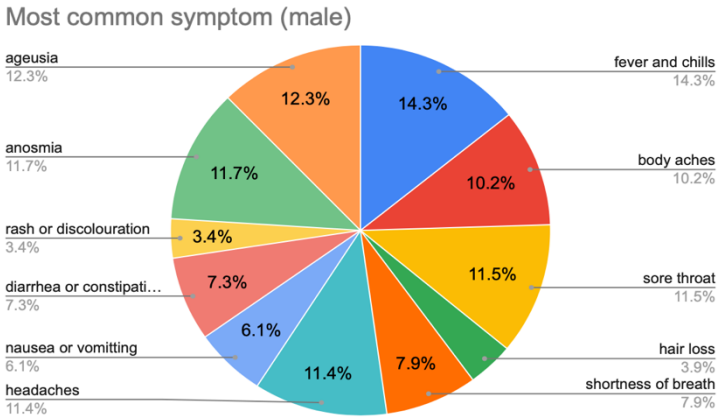
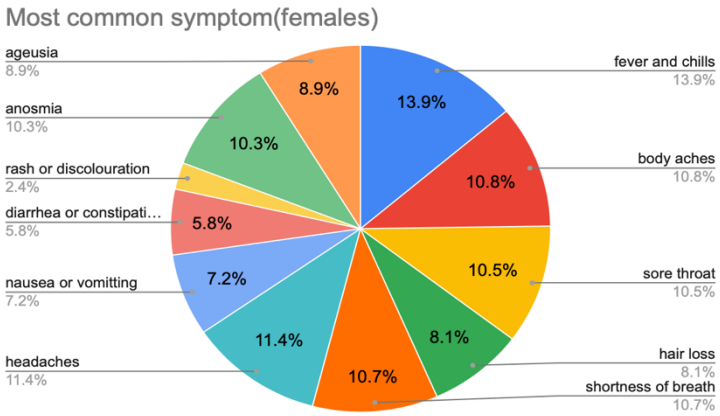


Figure 3b.



The various POST covid symptoms on different age groups: **Figure 4a** illustrates the various Post covid symptoms experienced by respondents between 11-20 years of age. It is apparent that fever and chills with 14.3% (n-78) are the most widespread symptom exhibited by the respondents of the 11-20 age group. Subsequently, sore throat with 12.1% (n-66) is the second most experienced post covid symptom next to fever and chills. Also, persistent ageusia and persistent anosmia with 11.3% (n-62) is the third widespread symptom reported by the respondents of this age group. Rash and discolouration with 2.6% (n-14) is the least experienced symptom in this age group, followed by hair loss with a response rate of 5.3% (n-29).

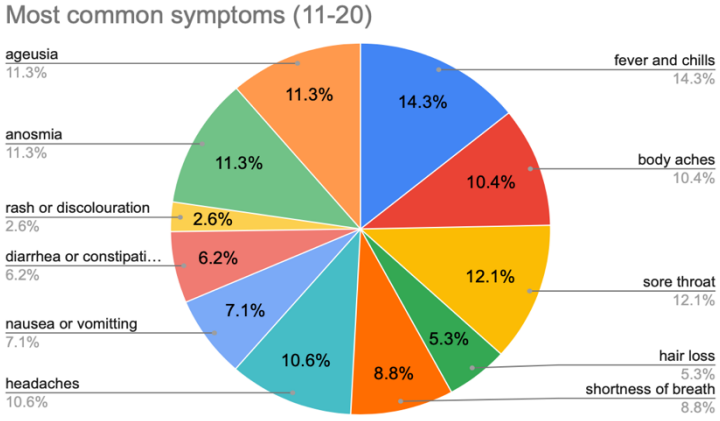


Figure 4a. The various Post covid symptoms experienced by respondents between 11-20 years of age.

Figure 4b illustrates the various Post covid symptoms experienced by respondents between 21-30 years of age. The three symptoms with the highest response rate among the respondents of this age group

are fever and chills with 15.1% (n-58), headaches with 12.8 % (n- 49), and persistent anosmia with 11.7% (n-45). In this age group, out of all the other post-covid symptoms, rash and discoloration possess the lowest response rate with 2.6% (n-10). Nausea or vomiting with 5.7% (n-22) is the least experienced symptom next to fever and chills.

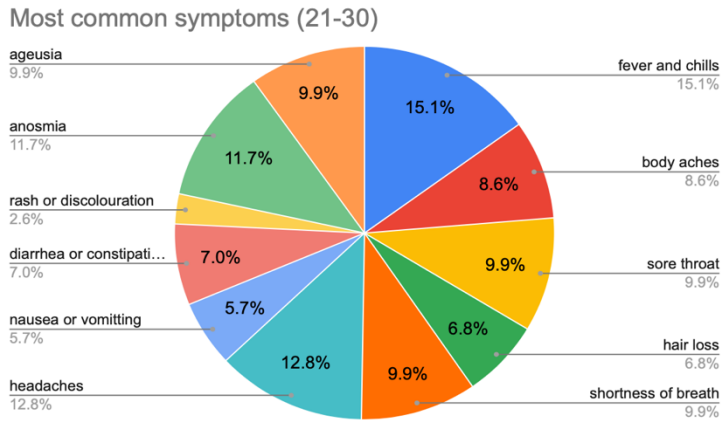


Figure 4b. The various Post covid symptoms experienced by respondents between 21-30 years of age.

Figures 4c illustrates the various Post covid symptoms experienced by respondents between 31-40 years of age. Among the respondents of this age group, fever and chills are the most prevailing symptom, with 12%. Persistent ageusia and sore throat hold the second and the third position for the most experienced symptom with a response rate of 11.2% (n-28) and 10.8% (n-27), respectively. On the other hand, rash and discolouration with 4.4% (n-11) is the symptom with the least amount of responses, followed by hair loss with 6.0% (n-15).

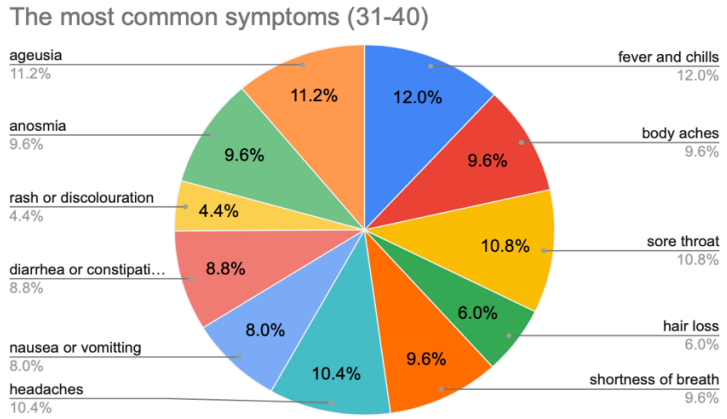


Figure 4c. The various Post covid symptoms experienced by respondents between 31-40 years of age.

Figures 4d illustrates the various Post covid symptoms experienced by respondents between 41-50 years of age. This data gives information about the symptoms shown by the respondents between the ages of 41 and 50. Among the respondents of this age group, with 14.8% (n-34), fever and chills are the Post covid symptoms with the most traction. In this aspect, both ageusia and body ache with 13% (n-30) and headaches with 12.6% (n-29) hold the second and the third position for the most ubiquitous symptoms, respectively. Holding 4.3% (n-10), hair loss contains the smallest number of responses next to rash or discolouration with 2.2% (n-5).

Figure 4e illustrates the various Post covid symptoms experienced by respondents between 51-60 years of age. The respondents aged between 51 and 60 have predominantly contracted body aches and persistent anosmia with 14.3% (n-11). The recorded responses show that next to persistent anosmia and body ache, the respondents have prevalently experienced headaches and fever with a proportion of 11.7% (n-9). Alternatively, the least exhibited symptom in this age group is rash or discolouration with 3.9% (n-3), followed by hair loss and nausea or vomiting with a response rate of 5.2% (n-4).

Figure 4f illustrates the various Post covid symptoms experienced by respondents above 60 years of age. It is apparent that among the respondents above 60 years of age, sore throat is the most commonly

exhibited symptom with 15.1% (n-8). Following sore throat, body aches procure the second place with 13.2% (n-7). And, shortness of breath and fever hold the third position regarding the number of respondents with 11.3% (n-6). In this age group, it is patent that the least experienced symptom is hair loss with 1.9% (n-1) followed by headaches and diarrhoea or constipation, with both having a response percentage of 5.7% (n-3).

Figure 4d. The various Post covid symptoms experienced by respondents between 41-50 years of age.

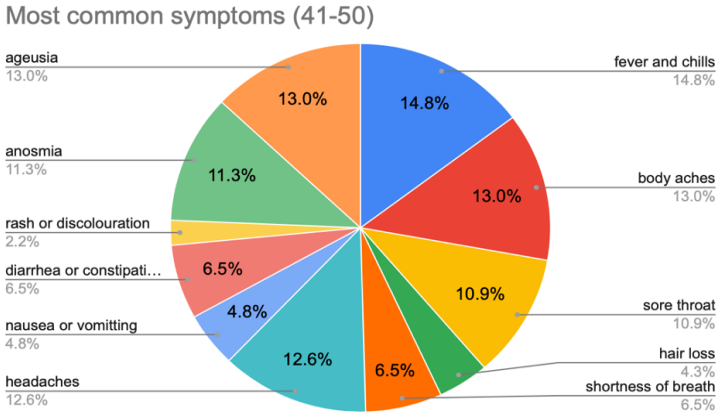


Figure 4e. The various Post covid symptoms experienced by respondents between 51-60 yearsof age.

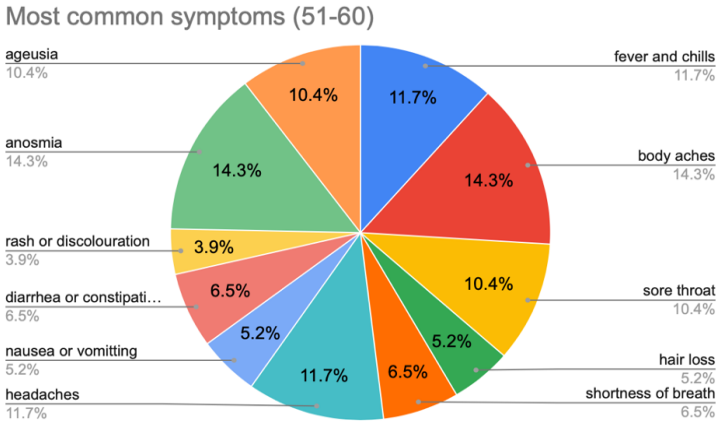
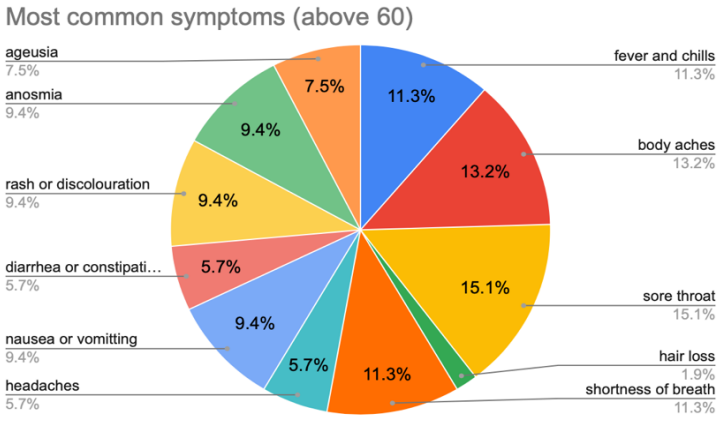


Figure 4f. The various Post covid symptoms experienced by respondents above 60 years of age.



The magnitudes of underlying conditions over different age groups and gender: **Figure 5** shows magnitudes of underlying conditions over different age groups. When considering the magnitude of underlying conditions over different age groups, the peak of the line graph is at the 41-50 age group. There is an upward spike from the 21-30 age group to 41-50 in the number of people who have a pre-existing condition. Still, there is a moderate downward slope in the numbers from 41-50 to above 60. The age group with the least amount of responses is 21-30. To summarise, the frequency of the underlying diseases is constantly fluctuating, with the highest point at the 41-50 age group and the lowest point at the 21-30

age groups. Thus, we can conclude that the number of respondents with underlying health conditions is relatively identical in adults and teens.

Figure 5. Magnitudes of underlying conditions over different age groups.

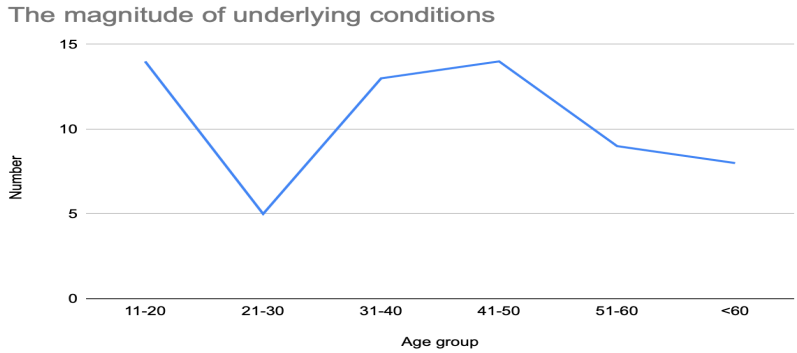
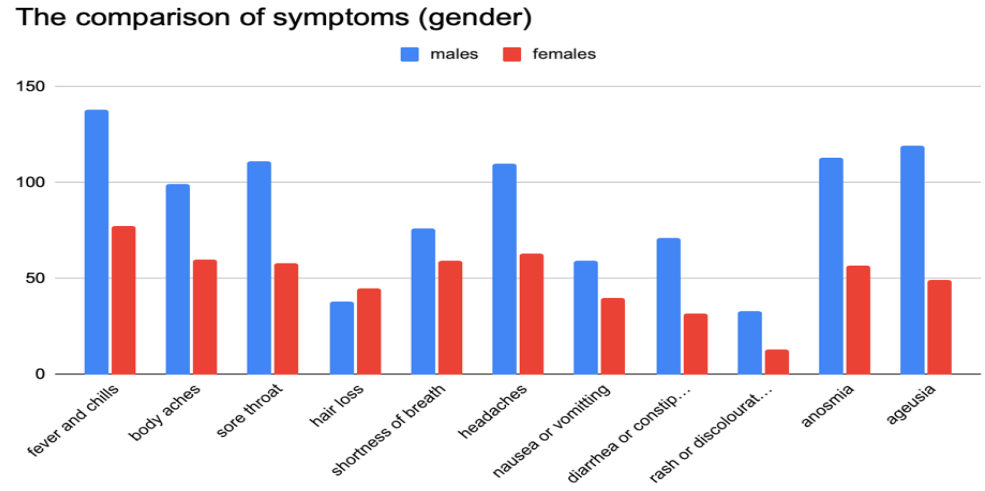


Figure 6 shows magnitudes of underlying conditions over different gender. The comparison of symptoms between males and females clearly depicts that fever and chills are the most common symptom, in both genders. However, the symptoms with the most responses next to fever and chills are different for each of the genders. In the case of males, the second most common symptom is ageusia, whereas, in females, it is headaches. Also, anosmia in males and body aches as well as shortness of breath in females can be conferred to be the third most ubiquitous symptom respectively. When comparing the symptoms based on genders, there are a lot of aspects to consider. For instance, the external and internal environmental factors are meant to be considered when speculating the relationships among post covid symptoms. Although for almost all of the symptoms, the number of positive responses of males outnumbers that of females by a wide margin, hair loss is the only symptom where the number of positive reactions for females outnumbers that of males. The symptom with the most minor traction for both males and females is rash or discolouration.

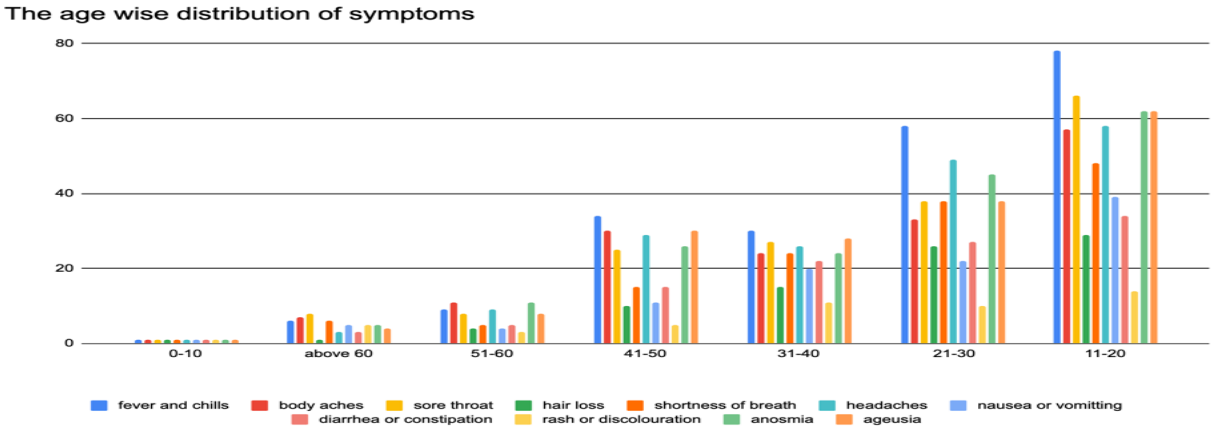
Figure 6. Magnitudes of underlying conditions over different gender.



The age-wise distribution of Post Covid symptoms: **Figure 7** shows the age-wise distribution of Post Covid symptoms. The respondents of this study can be categorised into six distinct age groups. Out of all the other listed symptoms, fever and chills are the most common symptoms in four out of the six age groups. In other words, among the respondents of the age 11-50, fever and chills appear to be the most predominant Post Covid symptom. The recorded results for Post Covid symptoms for age groups between 11-50 are relatively similar with minor exceptions. However, the recorded answers of the respondents above 50 are quite different from the answers of the respondents below 50. Above 50 years, the response count for fever and chills declines significantly to the point where it is no longer the most predominant post covid symptom. For the respondents between 51-60, body aches are the most common symptom. Persistent anosmia and persistent ageusia are considerably higher in the respondents above 50 than below 50. The sudden drop in the prominence of specific symptoms like fever and chills in the groups above 50

may be due to the deterioration of the immune system that accompanies ageing [6]. This trend provides us with discernable and empirical proof of a relationship between the post-covid symptoms exhibited in people and their subsequent age [3].

Figure 7. The age-wise distribution of Post Covid symptoms.



In summary, the data collected from this study shows that the connection between the gender and the age groups of the participants who had covid and exhibited post-covid symptoms are significant. This study also gave emphatic answers to several speculation subjects, such as amplifying the underlying health conditions the respondent might have had and amplifying the base fatigue level after recuperating from covid compared to before covid [7]. Apart from this, the study established the most ubiquitous symptom and the least experienced symptom.

This study's main agenda was to determine the precise relationship between age groups, gender, and the post-covid symptoms respondents exhibit, respectively. Nevertheless, the real intent of this study was to further our understanding of this pandemic from a societal and scientific viewpoint. Furthermore, aiding future generations prepare mitigations for a pandemic of a similar magnitude in the future [1]. Thus, this study functions not only as an esoteric piece of research but also assist us to evolve and adapt ourselves for similar situations in the future.

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ZAHEER AALIF, RAVI NARENDRANATH, MOHAMMED BILAL MUNEER, BASHEER SAFAR

RESISTANT HYPERTENSION IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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Abstract

Obstructive sleep apnea (OSA) is a sleep disorder characterised as complete or partial upper airflow cessation during sleep. Although it has been widely accepted that OSA is a risk factor for the development of hypertension, the studies focusing on this topic revealed inconsistent results. We aimed to clarify the association between OSA and resistant hypertension among the population of Calicut district in the state of Kerala. The study was conducted using the STOPBANG questionnaire. All individuals included were between the ages of 35-65 and had previously documented resistant hypertension. The responses from 49 people that fit the inclusion criteria have been analysed. 16/49 of the participants were placed in scores of 0-2, 15/49 were placed in scores of 3-4 and 18/49 were placed in the scores of 5-8. Based on our study, we can conclude that OSA is related to an increased risk of resistant hypertension among the population studied.

Introduction

Worldwide, hypertension is the leading preventable cause of mortality. Hypertension is the most frequent medical condition in the United States, impacting 75 million adults. Treatment-resistant hypertension, defined as an elevated blood pressure despite the use of three antihypertensive drugs from different drug classes (including a long-acting calcium channel blocker, a renin-angiotensin system blocker, and a diuretic) or a controlled blood pressure with four or more medications, affects 10.3% of adults worldwide and 19.7% of adolescents[3,4,15,24]. All types of treatment-resistant hypertension harm target organs such as the brain, kidneys and heart, resulting in myocardial infarction, stroke, chronic kidney disease, and heart failure.

According to data from 1950 to 2014, India's total prevalence of hypertension is 29.8% (95 percent CI 26-7-33.0) [1]. A meta-analysis of prior Indian prevalence studies reveals a considerable rise in hypertension prevalence from 3% to 4.5 percent in the 1960s to 11%–15.5 percent in the mid-1990s [20]. Hypertension prevalence studies in urban and rural populations from the mid-1990s to the present reveal an upward trend, with a bigger increase in urban (33.8%) than rural (27.6%) populations [1,5]. Nonetheless, people in urban India have better blood pressure control (20.2 percent) than those in rural India (10.7 percent) [1]. The data on resistant hypertension in India are lacking, as compared to the statistics available of hypertension in the general population.

Despite the fact that pharmacologic control is the cornerstone of hypertension therapy, lifestyle changes are just as important. Obesity, which underpins many occurrences of hypertension and influences its treatment, is immediately addressed by health behaviours that promote weight loss, such as physical activity and hypocaloric diets. Other lifestyle behaviours such as sodium restriction, smoking cessation, moderate alcohol consumption, and treatment of obstructive sleep apnea (OSA) are also recommended as blood pressure control techniques [5,11].

Sleep is essential for mental and physical well-being, and when it is disrupted, it poses a serious public health risk [2,10]. Sleep disturbances, with the exception of OSA, have received minimal attention in resistant hypertension. All treatment-resistant individuals with hypertension should be evaluated for apnea and, if necessary, OSA therapy (e.g., continuous positive airway pressure (CPAP)) should be started [16,25]. Treating OSA may have a wide range of benefits since it can enhance sleep quality and duration, which can have an impact on other lifestyle goals including food and medical advice. The current study focuses on new findings since that time and concludes with recommendations for future research [8,9,21].

The goal of this study was to summarize the current state of knowledge combined with additional data about the link between OSA and treatment-resistant hypertension. The current study focuses on new evidence that has emerged since that time, and it concludes with recommendations for further research.

Methods

In this cross sectional study we gathered information trying to understand the relationship between obstructive sleep apnea and resistant hypertension among the general population of Calicut

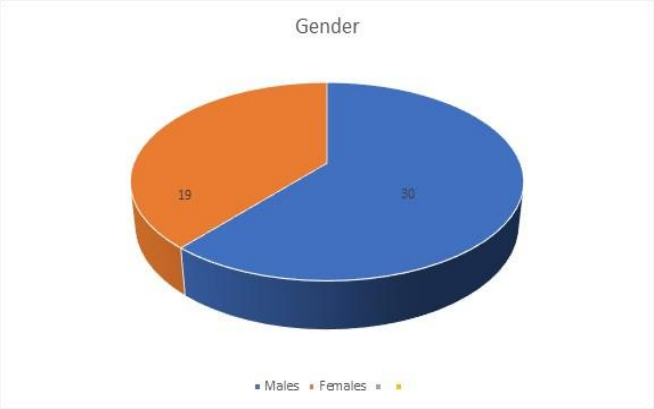
district in Kerala, India. We selected adults between the age of 35-65 to be included in our study. All individuals selected for our study had previously documented resistant hypertension being treated at the Calicut Medical College. Resistant hypertension is defined as a blood pressure that remains above goal despite concurrent use of three antihypertensive agents of different classes taken at maximally tolerated doses, one of which should be a diuretic.

All 49 participants answered self-administered standardized questionnaires about their current lifestyle, and health condition.

The studied screening tool was the STOP-BANG questionnaire which includes four questions related to snoring, tiredness, observed apnea, and high blood pressure. Two or more yes answers to STOP questions indicates high OSA risk. The BANG adds four more questions to the STOP section. The BANG questions assess the OSA risk based on BMI > 35 kg/m2, age > 50 years, (neck circumference > 41 cm for females and neck circumference > 43 for males) and male gender. Three or more yes answers to STOP-BANG questions indicate high OSA risk. In this study, all individuals had one point each for having high blood pressure and the rest of the score was calculated based on individual responses.

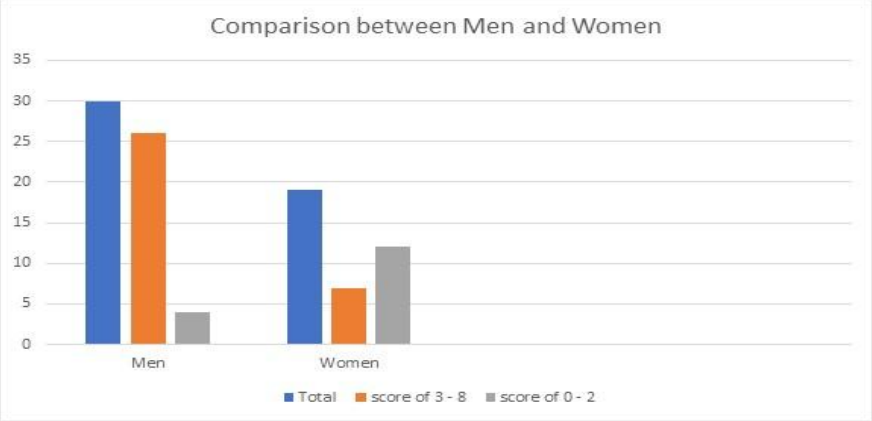
Results

49 participants had come forward and answered the STOP BANG questionnaire which was circulated online. The STOP BANG questionnaire is arranged into scores of 0-2, 3-4 and 5-8. According to the responses acquired from the participants 16 of them were placed in 0-2, 15 were placed in 3-4 and 18 of the participants were placed in the category 5-8. The participants that fall in between the scores of 0-2 were considered to be low risk. Those that fall in between the scores of 3-4 were considered to be in the intermediate zone whereas those that fall in the 5-8 category are said to be a highrisk individual. In our study, those participants that fall in the groups between 3-4, and 5-8 (33 participants) can be considered to have a correlation between resistant hypertension and obstructive sleep apnea.

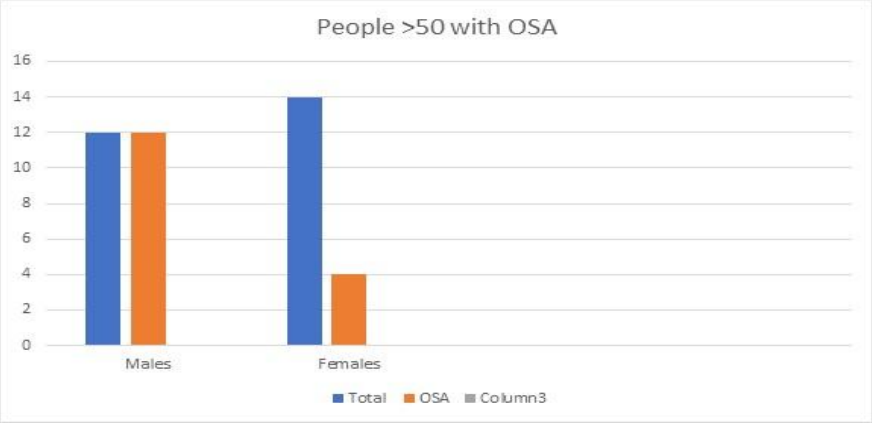


Among 30 males, 26 have a definitive link between resistant hypertension and OSA, and among 19 females 7 of them had a definitive link (based on the scoring criteria).

According to the result, it can be derived that OSA is potentially one of the many causes of resistant hypertension and is more common among men than in women.



According to the data there were 26 participants over the age of 50, in which 16 of them were found to be suffering from Obstructive Sleep Apnea. Of the 16 participants 12 of them were males and 4 were females.



All 16 of the individuals who had a BMI of >35, had a definitive link between resistant hypertension and OSA.

Out of the 33 participants that were linked between resistant hypertension and OSA, 15 individuals (10-males, 5-females) were found to have a large neck circumference, this provides additional support in drawing a link between OSA and resistant hypertension.

As per all of the data that was put together, it can be concluded that patients who suffer from resistant hypertension can have multiple etiologies to their illness. From the feedback obtained through the questionnaire we can draw a relationship between resistant hypertension with other factors such as BMI, male gender and those with a large neck circumference.

Discussion

The data we collected gave rise to several relevant findings. First, there was a significant link between sleep apnea in patients with hypertension, representing nearly all male subjects, and a moderate association in females. Secondly, there was a complete association of all obese patients with OSA. The increased prevalence of OSA among individuals with resistant HTN is likely due to a variety of factors. For starters, both resistant HTN and OSA may have risk factors in common. Obese people constituted nearly one-third of all subjects. Obesity is a well-known risk factor for OSA and is a typical feature of people with resistant hypertension [17]. The research failed to reveal a statistically significant link between neck circumference and resistant hypertension, although a modest association can be made. This can be attributed to the low sample size of 49 subjects.

OSA is known to be linked to high blood pressure [14,22]. The probable processes driving the connections, however, have yet to be fully understood. Several possible explanations may aid in our understanding of the link between OSA and hypertension. OSA generates oxidative stress and intermittent hypoxia, similar to hypoxia/reperfusion damage, resulting in vascular endothelial dysfunction [12]. Meanwhile, blood pressure increase is caused by excessive sympathetic vasoconstrictor output along with decreased nitric oxide bioavailability [18,19]. Furthermore, episodes of OSA increase sympathetic activity, which affects the chemoreflex and can lead to hypertension [6,7]. Individuals with sleep-disordered breathing have higher sympathetic nerve activity, as measured by 24-hour urinary catecholamine excretion, according to clinical observations [26]. Untreated OSA may also diminish pharmaceutical effectiveness due to pharmacokinetic or chronotherapeutic effects, potentially establishing a resistance pathway to antihypertensive medicines [13,23].

This study has certain limitations such as the sample size. We have a relatively small sample size with only 49 patients. The patients were enrolled in the study on the basis of themselves identifying as patients with resistant hypertension however, measuring of blood pressure on different occasions in a clinical setting was not performed. In our study, we found out that 33 individuals had moderate to severe OSA depending on the score from the STOP-BANG questionnaire. However, the gold standard for diagnosis of OSA still remains overnight polysomnography (PSG) owing to the low specificity of sleep questionnaires. The questionnaires also lacked the causative factor for their resistant hypertension, with this additional information, more insight would have been revealed about the several risk factors linked with resistant hypertension.

Overall, our study was able to demonstrate a statistically significant relation between OSA and resistant hypertension along with relevant factors such as gender, age, neck circumference, and obesity. Several of the shortcomings could have been overcome with a larger sample size, specific and precise diagnostic criteria, and a modified comprehensive questionnaire. Further studies must explore the link between the treatment of resistant hypertension and obstructive sleep apnea.

Conclusion

The STOP-BANG questionnaire was sent out to the population of Calicut district in Kerala. The majority of the participants were males (30) and the remaining 19 were females. The strongest correlation between OSA and treatment resistant hypertension was seen in the male population with 26 of the 30 participants and 7 of the 19 female participants. Therefore, in conclusion this study draws a positive relationship between OSA and treatment resistant hypertension among males in the population of Calicut district, Kerala.

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LUKA ABASHISHVILI, ANA GOGOLASHVILI, DIANA KERATISHVILI, MARIAM PESTVENIDZE

INFLUENCE OF GENDER IN DEVELOPING AUTOIMMUNE DISEASES (Review Article)

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Abstract

Autoimmune diseases and their management are one of the most challenging problems of modern society. These types of diseases are far more commonly seen in women than in men. We believe that understanding the reasons for the sex-based differences in autoimmune diseases can play a significant role in managing the patients. This review summarizes the most important contributors to gender-based disparity, such as the X chromosome, sex hormones, micro-RNA, and microbiota.

Introduction

Autoimmune disorders are the result of an exaggerated immune response that is detrimental to multiple organs and tissues. Because of their chronic nature and associated comorbidities, these diseases are a major public health problem. They increase the societal burden in terms of healthcare costs, loss of work productivity, and reduced quality of life; the etiology of autoimmune diseases is still unknown, but based on the available evidence, an interaction between genetic, environmental and lifestyle factors play a key role in disease development.

The prevalence of autoimmune diseases is much higher in females than in males. 80% of autoimmune patients are women [1]. The women were found to have a higher quantity of immunoglobulins and many circulating CD4 T cells that lead to a more effective immune response to infections, allografts, and tumors. The downside of this fact is that females are more likely to mount their immune system against self-antigens [1,2]. For instance, systemic lupus erythematosus (SLE), Sjogren's

syndrome, Grave's disease, and Hashimoto's thyroiditis are seven to ten times more common in women than men; multiple sclerosis (MS), rheumatoid arthritis (RA), and scleroderma are two to three times more common [2]. Moreover, odds of having female sex in patients with SLE was approximately 9 based on data from the Georgia (USA) Lupus registry 2002 [2]. Symptom severity, disease course, response to therapy, and overall survival may also differ between males and females with autoimmune diseases [3]. Understanding the basis of this variance can be vital for the development of future research on this topic.

Several experts speculate that the sex chromosomes, sex hormones, micro-RNA, and sex-specific environmental factors such as dimorphic microbiota are important mechanisms of gender bias in autoimmunity. In this review, we discuss current and foundational studies addressing the possible reasons behind these fundamental differences.

X chromosome and autoimmunity

Female and male karyotype differs from each other. Females have two X chromosomes whereas males have one X and one paternal Y chromosome. The Y chromosome contains approximately 100 genes including the SRY sex-determining gene, whereas the X chromosome has approximately 1,100 genes [4]. To avoid double X chromosome expression, females exhibit phenomena called XCI (X chromosome inactivation), which allows equal gene expression. The role of sex chromosomes in autoimmune diseases has been proposed based on several mechanisms including X chromosome inactivation patterns, fetal microchimerism, and X-chromosome monosomy and duplication [5]. X chromosome inactivation is not complete and about 15% of the genes escape inactivation, leading to over-expression of some X-linked genes in females [5]. A recent study using highly sensitive approaches to measure allele-specific gene expression found that no X-chromosome was 100% inactive in any of the female cells examined. Furthermore, the degree of XCI was heterogeneous between cells [6]. Numerous genes (such as CD40 ligand, chemokine receptor CXCR3, O linked N-acetylglucosamine transferase, Forkhead boxP3 (FOXP3), toll-like receptor (TLR)7, TLR8, IL-2 receptor gamma, tyrosine-protein kinase BTK, and IL-9 receptor) encoded by the X chromosome are shown to influence the immune response in a sex-dependent manner when over-expressed [7]. In this line, a single mutation in the interleukin-1 receptor-associated kinase 1 gene (IRAK1) contributes to an increased risk for lupus [8]. We believe that overexpression of these genes due to incomplete inactivation can be an underlying cause of increased susceptibility to autoimmune diseases.

Fetal microchimerism was first suggested as a possible factor in autoimmunity based on the observation that most autoimmune diseases manifest their peak of incidence following the fertile period. These fetal cells are often hematopoietic and can differentiate into somatic cells in multiple organs, potentially acting as targets for autoimmunity and resembling graft-versus-host disease after stem-cell transplantation. Multiple studies suggest that karyotype abnormalities such as loss of X chromosome or monosomy of it may be an underlying cause of autoimmunity. It is highlighted that absence of a second X chromosome in females (Turner syndrome) is linked with increased susceptibility to autoimmune diseases compared to sex matching individuals in the general population. A Cohort study conducted in Denmark showed that the overall risk of autoimmune disease among women with Turner's syndrome was twice that among Danish women in general [9]. The idea, that those chromosomal abnormalities and especially X chromosome involvement in autoimmunity is further supported by a study result, which revealed that men who have Klinefelter's syndrome (47, XXY genotype) are 14 fold more susceptible to systemic lupus erythematosus, compared to the population with normal karyotype [5].

Thus, the X chromosome, its incomplete inactivation, monosomy, duplication, and fetal microchimerism can be one of the most significant contributors of multiple gene over-expression, which plays an important role in autoimmunity and influences the gender-based differences in the prevalence of autoimmune disorders.

Sex hormones

One of the significant differences between the female and male bodies is the sex hormonal composition. Women and men synthesize the same sex hormones (androgens, estrogens, progesterone) but at different levels, and their effects depend on their concentration levels and the type of target immune cell [10]. These differences may be the one contributing factor to gender dimorphism in immune responses

and the reason why there is a significant difference in the incidence of autoimmune disease development between males and females.

The gender dimorphism in autoimmunity is more evident and apparent after puberty. For example, pre-pubertal onset multiple sclerosis (MS) is rare [11] and gender bias within these cases of MS are absent [12]. After the onset of puberty, however, incidence changes rapidly and pubertal girls are found to be at greater risk of developing MS than pre-pubertal. Moreover, the earlier onset of puberty in girls is also associated with an increased risk of developing MS. Similarly, in SLE, the adult male to female ratio of 1:9 may be as low as 1:2 before puberty [12]. However, many environmental factors also influence the development of autoimmune diseases.

The exact interaction between sex hormones and immune reactivity is incompletely understood, however, many studies investigate the impact of sex hormones on different constituents of our immune system. According to these reports, one of the targets of sex hormones is the autoimmune regulator gene (AIRE gene). Recent studies showed that the androgen/androgen-receptor complex directly binds to the promoter region of the AIRE gene and increases its transcription. This leads to escalated tissue self-antigen expression resulting in a more efficient negative selection of T cells [13]. As a consequence, mice administered dihydrotestosterone were protected from central nervous system autoimmunity. On the other hand, estrogen suppresses AIRE gene expression and gives the opposite result [13]. This different effect of sex steroids on the AIRE gene is a significant mechanism by which sex bias occurs in autoimmunity. In vitro studies claim that sex hormones control the production of a variety of immune cytokines, including interleukin (IL)-1 [14,15], IL-6, IL-2, IL-4, IL-5, interferon-gamma [16], and transforming growth factor-beta [8]. The IFNs are of obvious relevance to this subject because they are well known to be overexpressed in patients with certain autoimmune diseases [1]. The interferon-gamma promoter region has four estrogen response elements and there are odds that higher estrogen levels in females stimulate interferon-gamma production by T-cells, which may increase the susceptibility of developing interferon-gamma mediated autoimmune diseases in females [13].

Estrogens

The effects of estrogens on the immune system are very complex. There are three types of endogenous estrogens: estrogen (E1), estradiol (E2), and estriol (E3, produced only during pregnancy). Each of them has distinct action on intracellular estrogen receptors that are present in all cells of the immune system including T and B lymphocytes, and peripheral NK cells [17]. In particular, estradiol can regulate immune responses acting at multiple levels including cell development, proliferation, cytokine or antibody production, and apoptosis. Estradiol has two main receptors, estrogen receptor α, and estrogen receptor β. As mentioned above, all immune cells express intracellular estrogen receptors, but the proportion of one estrogen receptor subtype to another may be different that may alter the estrogen effect, either by aggravating or alleviating inflammation. Activation of estrogen receptor α results in immune system enhancement while the activation of β receptors has a slightly immunosuppressive effect [18]. The number of intracellular estrogen receptors does not change during the menstrual cycle, with age, or after menopause. Estradiol appears to favor the survival of high-affinity DNA-reactive B cells at both the immature and transitional B cell stages facilitating the maturation of a potentially pathogenic naive auto-reactive B cell [18].

In females, circulating levels of estrogens fluctuate because of the menstrual cycle, pregnancy, and menopause. This is significant because the varying concentration of estrogen may affect immunity differently. For example, a high level of estrogen during pregnancy or the periovulatory phase of the menstrual cycle inhibits pro-inflammatory pathways and stimulates anti-inflammatory ones. Conversely, at low levels (as seen after menopause), estrogen stimulates pro-inflammatory pathways [1].

Estradiol stimulates antibody synthesis from B cells independent of the circulating level. New investigations found out that estradiol promotes the expression of activation-induced deaminase in the B cells that drives antibody diversification and transforms benign antibodies into autoantibodies leading to autoimmunity.

Androgens and Progesterone

In men, androgen levels are higher and the incidence of autoimmune disease is low, which may give us a clue that androgens protect against immunity. Studies showed that orchiectomy of male mice leads to overt autoimmunity, whereas treatment with androgens in ovariectomized female mice reduced mortality [19]. In humans, treatment with testosterone had a positive effect on men with MS (slowed cognitive decline and brain atrophy) [20]. The possible explanation of this effect is that testosterone suppresses the expression of the pro-inflammatory cytokines TNF-α, interleukin (IL)-1β, and IL-6 and promotes the expression of the anti-inflammatory cytokine IL-10 [17,18]. It also down regulates Th1 differentiation by up regulating type 1 protein tyrosine phosphatase (Ptpn1) in both mice and humans, reduces the proliferation and differentiation of lymphocytes, and may suppress immunoglobulin production [17,18]. Overall, these data strongly support an immunosuppressive role for androgens although, since their effects may vary considerably depending on the level of exposure, the exact role of androgens is still unknown [17,18].

The presence of progesterone receptors in immune cells suggests that this hormone has an impact on immune responses. Activation of intracellular progesterone receptors by low physiologic concentrations of progesterone is thought to suppress antibody responses in both sex [17,18]. Understanding and analyzing the impact of sex steroids on immune-mediated diseases could lead to the identification of innovative and readily available therapeutic interventions, such as hormone antagonists or agonists, to manage autoimmune diseases.

Autoimmunity and miRNA

Multiple studies demonstrate the importance of miRNA expression in immune cells. Zhou et al. (2008) observed that dicer-deficient Tregs lost the ability to suppress an immune response [21]. These mice developed an autoimmune disease that closely resembles IPEX syndrome (FOXP3 knockout phenotype). This suggests the importance of miRNA in maintaining a balanced adaptive immune response. Having said that, this article emphasizes the major difference between a female and male expression of miRNA in autoimmune diseases. There are three major contributing factors to sexual dimorphism of miRNA expression: sex chromosome, hormones, and external (environmental) stimuli.

Firstly, according to the miRBase microRNA archive (www.miRBase.org2013), the X chromosome contains approximately 113 miRNA genes, while the Y chromosome only has 2. Skewed XCI may also contribute to the disparity of X-linked genes expressed in females. There has been ongoing research to identify exactly which miRNA is involved in the pathogenesis of autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. Dai and Ahmed (2014) were the ones who tested the splenocytes of female and male NZB/WF1 mice. The NZB/WF1 model closely resembles human lupus. They found a significant increase in expression of the miR-182 cluster, miR-155, miR-31, miR-148a, miR-127, and miR-379 after the onset of lupus in female NZB/WF1 mice [22]. Another example of altered miRNA expression in different diseases is miRNA-223, coded by the X chromosome. There was increased expression of miRNA-223 in rheumatoid arthritis and type 2 diabetes patients, but decreased activity in systemic lupus erythematosus [22].

Secondly, evidence suggests the influence of sex hormones like estrogen and androgens on expressing miRNA. Sex hormones bind to nuclear hormone receptors and alter gene expression [23]. This can be both direct and indirect. The genes influenced by sex hormones may directly contain the miRNA molecule genes or have the promoter regions embodied in them [23]. Moreover, sex hormones alter post-transcriptional modification and expression of miRNA by inducing or inhibiting molecules like export 5, Dosha, or Dicer [22]. Dai et. al (2008) discovered increased miR146a and miR-223 in estrogen-treated splenic lymphocytes which then enhanced the response of the cells to lipopolysaccharide (LPS) [24]. This further supports the hypothesis of different miRNA expressions between genders.

Last but not least, the response to external stimuli differs in females and males. For example, sex-specific miRNAs were down-regulated in female mice but not in male mice after exposure to ionizing radiation [22]. In addition, there has been some difference in the metabolism of different drugs between sex. Females have been shown to have increased activity of Cyp2b9, a subclass of the cytochrome P450 superfamily [22]. Scientists have found a negative correlation between Cyp2b9 and miRNA expression

[22]. This may contribute to decreased expression of these miRNA genes in females compared to males. Thus, environmental influences can also contribute to dimorphic miRNA gene expression between the two groups.

To sum up, there has been an increasing investigation to discover the role of miRNA in causing autoimmunity. There is some evidence showing that miRNA can be a culprit in causing lupus, rheumatoid arthritis, and other autoimmune diseases. All - chromosomal, hormonal, and environmental influences on miRNA are vital. However, we need more investigation to finally understand what is the role of miRNA in causing autoimmunity.

Gut Microbiota

Gut microbiota is the assembly of all the microorganisms, such as bacteria, viruses, fungi, protozoa that live in the digestive tract of humans and other animals. It has long been known about their importance on various levels for the human body [25]: they help us with digestion, ferment dietary components, fight against harmful pathogens, produce vitamins, and immunologically active molecules such as short-chain fatty acids, and many more. However, it is only recently that studies demonstrated that disbalance in the intestinal microbiota is associated with the pathogenesis of some autoimmune diseases. Moreover, the difference in microbiota composition also contributes to sexually dimorphic immunity.

The interaction between gut flora and the immune system involves two main components: molecular mimicry and molecular complementarity. Since the intestinal microbiota significantly contributes to the normal functioning of the human body, it has the utmost significance to protect them from pathogenic bacteria and the host immune system. Through evolutionary processes, these microbes have evolved to look like host antigens, to mimic them. On the other hand, the immune system has developed to make a simplified “body double” [26] of the host and minimal distinctions between “self” and “non-self”. Using molecular mimicry, these bacteria effectively avoid immunological detection and processing. It is well known that complementarity between specific proteins on the cell surface of the microbe and receptors or transporters on the cell surface of host cells is essential for the identification of host cells by microbes. That’s why many commensal microbes produce mimics of receptor ligands, suggesting molecular complementarity.

Based on molecular mimicry and molecular complementarity, many microbial antigens look like host antigens so much that an active immune response to the microbe may cause cross-reaction with the host, causing autoimmune disease. Or in the opposite, if an autoimmune disease is induced against the host, any microbiome components expressing antigens similar to those targeted by the autoimmune disease will also be affected. Anyways, either or both mechanisms could contribute to autoimmune disease development.

Knowing that the microbiome differs in individuals by sex, it is not surprising that the sex bias in autoimmune diseases is also affected by the microbiome and vice versa. Though gender differences in microbiota composition are found both in mice and in human studies, the lack of standardization in human studies may mask the sexual dimorphism in microbiota composition. The reason is simple - many factors such as age, genetic background, BMI, diet, and sex hormones appear to interfere with the sexual dimorphism in microbiota composition. This is the reason why studies have been performed on rodents.

The classic example is considered to be the study performed on NOD [27] (non-obese diabetic) mice. It showed that female NOD mice with normal microbiota were several times more likely to develop type 1 diabetes (T1D) compared to male NOD mice with normal microbiomes. But after comparing female and male germ-free NOD mice, this difference disappeared. It was further confirmed by the microbiota transfer study by Markle et al. according to which, transplantation of microbiota from conventional NOD males to germ-free NOD females resulted in the protection of the female mice against T1D. There can be three models [28] explaining these results: A - suggesting that either through immune or metabolic mechanisms hormones regulate the microbes and that microbes then activate the protective effector mechanisms, B - microbes are regulators of hormonal metabolism and the hormones are the actual effectors, and C - both microbiota and hormones contribute in an additive fashion.

Understanding the contribution of gut microbiota in gender dimorphism of autoimmune diseases, in addition to many other possible causes, can be crucial for the future treatment of these types of

conditions. Developing certain approaches to microbiome manipulations and treatment of microbiome dysbiosis using probiotic replacement therapies can be rather helpful.

Conclusions

In conclusion, this paper has discussed the reasons for the abnormal functioning of the immune system causing autoimmunity and the possible contributors to the sex-based disparity. The evidence suggests that the most important factors, from the huge list of influencers that may underlie this striking gender difference, are the X chromosome, sex hormones, micro-RNA, and microbiota. Analyzing these findings and implementing them in modern therapeutic interventions could become a cornerstone of managing autoimmune diseases.

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OXIDATIVE STRESS AND ANTIOXIDANTS WITH EMPHASIS ON AGEING

(Review Article)

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Abstract

Numerous theories have been postulated to understand the process of aging. One of them links degenerative senescence to reactive oxygen species (ROS), which are natural byproducts of oxygen metabolism. Understanding and supporting this theory may be of great importance due to number of reasons. Firstly, there are well-established risk factors that can increase the production of ROS in our body. Secondly, there are different types of antioxidants that can neutralize toxic effects of ROS. Examples of antioxidants include natural enzymes produced in our body, as well as certain exogenous supplements. Moreover, antioxidant supplementation is already offered by some facilities as a hope to decrease age-related complications. Therefore, it is crucial to understand, how ROS is involved in the process of aging and if there is any evidence how alteration in ROS and antioxidants levels influence the process of ageing.

Introduction

Aging is a natural inevitable process. It starts after a person reaches the maximum level of growth in his or her 20s. After certain age the functioning of the human body declines gradually, involving physical and cognitive aspects of health. Aging is accompanied by many chronic illnesses that are associated with the daily wear and tear, which makes us vulnerable to different cardiovascular, nervous system, reproductive, urinary, metabolic, musculoskeletal and digestive problems, as well as the decrease in cognitive functions. There are many factors, which determine the human body's response to this transitioning process, lifestyle choices and genetics are said to be two of the main contributors.

1. Different theories of aging

Over many years researchers have tried to decode the exact mechanism behind aging and as a result we were presented with seven main theories, which could be discussed as individual reasons or

consequences of aging, or be considered as small components of a very complicated process. The theories mentioned below describe the physiological as well as psychological and social contributors.

The disengagement theory by Elaine Cumming and William Earle Henry [14] gives 9 postulates with the assumptions that disengagement is an inevitable process of cutting the social ties by an older person realizing that he or she does not have much time to live. This is a process, which is caused by losing the previous abilities and skills and can be considered as a defense mechanism for the elderlies to avoid humiliation and damage to reputation. However, at the same time, it can be seen as a balancing necessity as the younger generation is replacing the older one. With the complete disengagement from the society the elderly find a new role in life in order to avoid the crisis of identity and still feel that they are worthy.

The activity theory, by Robert J. Havighurst [17], can be considered an answer to the above-mentioned postulate. It denies the pessimism of the disengagement theory and suggests that there is no withdrawal, as the elderly are feeling happiest when they engage in the social activities, which is reflected in the increased longevity.

While talking about the physiological triggers of aging, the neuroendocrine theory should be discussed. This theory states that with age the functional responsiveness is lost, which is directly translated to the slower current transmission in the neuroendocrine axis. This means slower response of the muscles and delay in obtaining the homeostasis, which is crucial for the person's physical wellbeing [15].

Professor Imre Zs-Nagy from Debrecen University, Hungary described the theory of membrane [57]. According to the Nagy's research the cell membranes solidify more and more and become less lipid with ageing, which subsequently decreases the efficiency of normal function conduction, which further causes the toxic accumulation of lipofuscin, a cellular toxin. According to the theory, as we age, lipofuscin deposits are seen in the brain, heart, liver and skin. There are studies, finding high lipofuscin levels in the patients with myocardial diseases, associated with sudden cardiac death, and its increased levels in the different parts of the brain leading to brain damage [28]. This theory states that this pigment is highly disruptive and has a major effect on the normal organ function [39]. This theory also suggests that as the cell membranes become more solid, the sodium and potassium transport and electrical and heat transfer are impaired, which results in the decreased cell-to-cell communication and is apparently attributed to aging.

The cross-linking theory by Johan Bjorksten [56] is one of the oldest theories of aging and refers to the oxygen-dependent accumulation of the cross-linked glycosylated proteins. With age there is increased possibility for the oxygen to associate with glucose and protein. Thus, glycosylated proteins are damaging to the cells and tissues, which in turn decreases the speed of the physiological bodily processes causing aging.

The mitochondrial theory is a variant of the free radical theory of aging [49]. Its key point is that the accumulation of excessive damage to the mitochondria and the mitochondrial DNA, which happen over time leads to aging. It has been observed that functional deterioration of mitochondria and the mitochondrial DNA mutations, caused by the ROS, which themselves are the result of the age-related respiratory enzyme impairment, are increased in the cells in an age-dependent manner [2,25]. Human mitochondrial DNA, which is not protected by histones and is exposed to high levels of ROS and free radicals in the matrix of mitochondria, is susceptible to oxidative damage and somatic mutations. Multiple different mitochondrial DNA mutations have been found in patients with mitochondrial diseases, and some of them are also observed in aging human cells. The incidence and abundance of these mutant mitochondrial DNAs increase with age, particularly in tissues with great demand for energy. On the contrary, recent studies [31] have demonstrated that the ability of the human cell to deal with oxidative stress is compromised in aging. This theory is supported by the observation that intracellular levels of H2O2 and the oxidative damage to DNA and lipids are significantly increased with age. Moreover, the mitochondrial pool of reduced glutathione declines and the DNA damage is enhanced in aging tissues. Taken together, these observations and our previous findings [8] that mitochondrial DNA mutations and oxidative damage are increased in aging human tissues, suggest that mitochondrial theory of aging is mature.

The last one is the free radical theory of aging, which is the most discussed and well-known out of these seven. It was proposed by Denham Harman and states, that the accumulation of free radicals

produced during aerobic metabolism causes oxidative damage to the cells and subsequent aging [25]. This theory can be linked to the mitochondrial theory, as it states that the ROS primarily damage the mitochondrial DNA and the degree of damage determines the lifespan of an individual.

Majority of the above-mentioned theories support association between ROS and aging. Therefore, further detailed exploration of this topic could bring better understanding of the aging process.

2. What are reactive oxygen species and how they might be involved in ageing process

ROS are natural byproducts of oxygen metabolism. They are mainly produced in mitochondria during oxidative phosphorylation. Normally, most of the oxygen inhaled from air is reduced to produce water, however, 0.1-2% can be incompletely reduced and result in formation of ROS. Examples of ROS include superoxide anions and hydrogen peroxide. In small quantities, they are needed for the maintenance of human physiological processes and are thought to be involved in cellular growth, apoptosis, signaling pathways and generation of the inflammatory response against pathogens [18]. However, in high amounts it can induce damage by oxidizing certain cellular components. This is called oxidative stress.

Researchers have proven that ROS directly damage crucial elements of the cell, specifically lipids, nucleic acids and proteins [2]. Lipid peroxidation leads to the formation of cyclic endoperoxides and unsaturated aldehydes that are toxic for cellular membrane and enzymes. ROS also damages all four bases, resulting in formation of double-strand breaks and cross-links in nucleic acids, they further lead to peptide fragmentation and oxidation of amino acid residues [3].

These findings have served as the foundation for several ageing theories, aiming to prove, that through these processes ROS accelerate degenerative senescence. Free radical theory of aging dates back as 1956 [4] and since then it has been a topic for many researches. However, the exact impact that ROS might have on aging is still controversial. Oxidative stress is known to be a contributing factor for developing certain degenerative and age-related diseases such as Alzheimer's disease, Parkinson's disease, and virtually all cardiovascular diseases [1,4]. However, whether these toxic effects are also the cause for cellular aging is largely debated.

One of the widely accepted theories states that toxic effects on nucleic acids lead to accumulation of somatic DNA mutations that affect cellular longevity [32]. However, presence of DNA repair mechanisms largely questions this theory. Studies have shown that prokaryotic and mammalian cells are capable of successfully repairing mutations induced by oxidative stress [6,8]. But there is no data about whether or not these repair mechanisms decline throughout the years.

In the late 20th century, another theory had been introduced, which linked oxidative stress on mitochondrial DNA to senescence. According to this hypothesis, mtDNA damage can lead to mutations that directly block the replication capacity of the cells [5]. Some research also state that oxidative stress depletes the biochemical pool necessary for cellular division [6].

Experimental studies have also been conducted on birds with different lifespan to identify if there is significant correlation between levels of ROS and life expectancy. One of the studies performed in 2018 demonstrated positive association. According to it, long-lived birds tended to have higher levels of antioxidants [52]. However, another study that linked oxidative status to telomere length, suggested no apparent impact of antioxidants on the senescence of long-lived birds [7,23,41].

Despite the presence of numerous theories, none of them have been sufficient enough to confirm if free radicals produce significant impact on aging. However, they provide a basis for further research and exploration of the topic that might be beneficial for preventing a number of age-related complications. Prevention can be achieved through different types of antioxidants that can neutralize ROS and alleviate its toxic effects or through modifying risk factors that increase oxidative stress.

3. Risk factors that increase ROS

Exogenous factors

There are several exogenous causes that induce oxidative stress, which include environmental pollutants, cigarette smoke, e-cigarettes, ionizing and non-ionizing radiation, drugs, and foods. Chemical substances such as pesticides and heavy metals like mercury, lead, arsenic, cadmium, chromium and organic solvents, are also known contributors of oxidative stress.

3.1 Environmental Pollutants

Environmental pollution significantly affects human health and is among the leading contributors of mortality and morbidity in people [12]. It poses a global threat especially in rapidly developing countries with increasing population and metropolitan development resulting in the decline of air quality, which conversely affects developing countries [12]. Air pollutants contain a mixture of gases, particulate matter, and chemicals whose source and constituents are difficult to determine. Particulate matter with an aerodynamic diameter ≤ of 2.5 μm (PM2.5), originating from combustion processes are considered most harmful and[54], they mostly consist of carbon particles, with other organic molecules like sulfates, nitrates, and polycyclic aromatic hydrocarbons [50]. The physical and chemical properties of the particles i.e., size, structure, composition suggest their significance on health [48].

Experimental and epidemiologic studies have recognized ROS as essential mediators of particle toxicity, with a specific association to respiratory and cardiovascular diseases [1, 19]. Increased mortality from ischemic heart disease, heart failure, and lung diseases, like asthma, chronic obstructive pulmonary disease (COPD) [11, 48].

3.2 Cigarette smoke

Cigarette smoke and smoking is an important risk factor in the generation of ROS. Cigarette smoke is an aerosol that consists of toxic substances, chemicals, and carcinogenic agents [26]. More than 4000 harmful compounds have been identified in cigarette smoke such as carbon material, quinones, organic solutions, heavy metals, polycyclic aromatic hydrocarbons, and N-nitrosamines [44]. There are more than 50 carcinogens in cigarette smoke that have been recognized by the International Agency for Research on Cancer (IARC) with “sufficient evidence for carcinogenicity” [26, 44]. Cigarette smoke can be divided into mainstream smoke (inhaled by the smoker) and side stream smoke (the smoke that goes into the air from a burning cigarette and is the main part of second-hand smoke). Cigarette smoke has two phases: the tar phase and the gas phase. The tar phase contains stable polycyclic aromatic hydrocarbons and nitrosamines, and the gas-phase contains nitric oxide, carbon particles, and peroxyl radicals [51]. In the presence of iron, tar can produce hydroxyl radicals and hydrogen peroxide.

In an experimental study conducted to demonstrate the relationship between cigarette smoking and oxidative stress in patients with coronary artery disease, the results concluded that cigarette smokers have increased oxidative damage and reduced antioxidants than non-smokers and are at an increased risk of developing coronary artery disease [29]/

3.3 E-cigarette

The practice of using e-cigarettes has become widely popular, ever since they have been marketed as a healthier substitute to traditional cigarettes [21, 40]. There are also reports that state that e-cigarettes aid in smoking cessation [24]. As a result, the consumption of e-cigarettes has increased, especially among the youth population. Since 2014, the most commonly used tobacco product in the United States among the youth are e-cigarettes. This raises a concern, as there is a substantial lack of awareness of the detrimental health effects of e-cigarettes in the overall population. There is minimal evidence about the observation that e-cigarettes are safer than tobacco cigarettes and the risks associated with the long-term use of e-cigarettes. Additionally, recent epidemiological studies have shown several adverse health effects associated with inhalation of e-cigarette aerosols regardless of the nicotine levels [9, 38, 42]. E-cigarette aerosols contain ultrafine particles derived from environmental pollution and have shown to cause equal health effects of environmental pollutants by stimulating inflammation, oxidative stress and starting the process of endothelial dysfunction which eventually leads to cardiovascular and lung disease (20, 21, 30, 37].

3.4 Radiation and chemotherapy

Radiation therapy is one of the major treatment modalities for various types of cancers, however it is often presenting with toxic side effects. Types of ionizing radiation include alpha particles, beta particles, gamma rays, and X-rays. These rays can all lead to increased oxidative stress. Alpha particles have less penetrative energy to the outer layer of the skin and is it not a major concern. All the other types of ionizing radiation are indeed penetrative. Gamma rays and X-rays are the most commonly used types of ionizing radiation in a medical setting. Ionizing radiation increases oxidative stress by inducing ROS due to the radiolysis of water molecules.

Chemotherapeutic agents that generate high levels of ROS include anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin), which generate the highest levels of oxidative stress, platinum-containing complexes (e.g., cisplatin, carboplatin), alkylating agents, epipodophyllotoxins (etoposide and teniposide), and the camptothecins (topotecan and irinotecan) [13].

3.5 Drugs

Aspirin, antipyretic agents, antipsychotics, antiretrovirals, and analgesic non-steroidal anti-inflammatory drugs (NSAIDS) generate ROS. The mechanisms of drug-induced oxidative stress differ [16].

3.6 Foods

There are several types of nutritional sources that can lead to an increase in oxidative stress. Evidence suggests that high quantities of macronutrients (carbohydrates, fat and proteins) induce oxidative stress [46]. Dietary carbohydrates are essential to mention as they contain high glycemic load and contribute to the long-term effects of nutritionally mediated inflammation and aid in the development of cardiovascular diseases, diabetes, obesity, and cancer [36,47]. Processed foods such as snack foods and cereals contain trans-fatty acids, which help in the generation of ROS as they have acrylamide which gives rise to oxidative stress [55]. When vegetable or animal lipids are heated in the microwave, free radicals are generated. Meat is considered as part of the normal diet in many developed countries even though meat is high in proteins it can be a source of toxins due to the presence of N-nitroso compounds, heterocyclic amines, and polycyclic aromatic hydrocarbons associated with cooking and grilling the meat in high temperatures [33]. High concentrations of ethanol can be damaging to the body as it generates ROS. Dietary intake of iron and copper can also increase oxidative stress and its buildup in bodily tissues can increase the risk of cancer [22].

Endogenous factors

Dysfunction in the mitochondrial respiratory chain and other enzymes like xanthine oxidase, lipoxygenases, glucose oxidase, myeloperoxidase, cyclooxygenase and nitric oxide synthase are generators of ROS endogenously.

4. Types of antioxidants

Antioxidants are substances that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate. The role of antioxidants, as the definition suggests, is to prevent the damage to cellular components arising as a consequence of chemical reactions involving free radicals. Antioxidants delay or prevent damage mainly by using their free radical scavenging property [34]. There are several groups of antioxidants which include antioxidant enzymes, chain breaking and transition metal binding proteins.

First example of antioxidant enzymes is catalase, which has 2 stage conversion of hydrogen peroxide to water and oxygen. It is mainly located within the cells in peroxisomes and demonstrates the greatest activity in liver and erythrocytes. Another example is superoxide dismutase, which catalyzes the dismutation of superoxide to hydrogen peroxide. Later, hydrogen peroxide is removed by catalase or glutathione peroxidase, which is also another antioxidant enzyme. Glutathione peroxidase catalyzes the oxidation of glutathione using hydroperoxide [53]. Plasma form of this enzyme is mainly synthesized in the kidney and has the highest concentration in the liver. These enzymes are produced in the body, however there are certain exogenous factors that can function as antioxidants. For example, physical exercise leads to increase in antioxidant levels in heart, muscle and liver tissues and reduces free radical production and subsequent damage [53].

Chain breaking antioxidants are small molecules which receive an electron from a radical or donate an electron to radical and form stable byproducts. These are divided into aqueous phase and lipid phase antioxidants. Lipid phase antioxidants react with radicals in lipoprotein particles and in membranes. One of the most notable ones is vitamin E, which has 8 different forms and 2 classes, tocopherols and tocotrienols, both of which have antioxidant function [10, 53]. Another interesting property of Vitamin E is that it helps to structurally stabilize membranes. Vitamin A also exhibits antioxidant property, however it does not show any dependency of oxygen saturation.

Flavonoids are polyphenolic antioxidants found in fruits, vegetables, tea and wine. However, not much is known about their absorption and metabolism. Some studies showed that intake of flavonoids decreases the incidence of coronary heart disease [53].

Aqueous phase chain breaking antioxidants scavenge radicals in aqueous compartment. One of the most important members of this group is Vitamin C. Ascorbate scavenges radicals such as superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid, aqueous peroxyl radicals, and singlet oxygen. Vitamin C undergoes 2 electron reduction to semdehydroascrobyl radical and subsequently to dehydroascorbate, which later on hydrolyses to diketogulonic acid which is broken down to oxalic acid [43].

Another important example is uric acid, which is converted to allantoin. It provides protection against ozone. Albumin bound bilirubin also plays a major role in the protection of a neonate from oxidative damage. Melatonin is unique in the way that it does not undergo redox cycling and cannot be reduced to its former state and it is called a terminal antioxidant [53].

In conclusion, all of these antioxidants can reduce oxidative stress and theoretically can be used as risk reduction agents. It is still debatable and there is even evidence that synthetic antioxidants are dangerous to health. Overall, it is not proven entirely that antioxidants can have an important impact in prevention of free radical damage and more research is required on this matter.

5. Role of antioxidants in preventing age-related complications.

Aging is a very complex topic, thus various studies have arranged for understanding its mechanism and association with oxidative stress. Studies find that the amount of oxidants and antioxidants are balanced in healthy humans. However, there are different genetics, environmental or lifestyle factors that misbalance equilibrium and organisms begin the journey of damage until balance equilibrates. Such factors are cigarette smoking, obesity, air pollutants, ultraviolet B, G6PD deficiency and many more. Studies show that ceasing cigarette smoking, exercising and avoiding obesity are major life extensions.

Increased oxidative stress leads to atherosclerosis, Alzheimer's, dementia, cancer and various diseases that alter quality of life and lifespan. In order to solve or reduce the prevalence of latter disorders, dozens of trials preceded for finding correlation between antioxidant use and decreasing complications of oxidative damage. One of the studies was done from 1993 to 1999 for finding whether long-term supplementation with vitamin E decreases the risk of cancer, cancer death, and major cardiovascular events [27,41]. From the total cancer patients, the control group had 156 (3.3%) deaths compared to placebo group 178 (3.7%). From cardiovascular patients 1022 (21.5%) deaths was in control patients and placebo 985 (20.6%) respectively. As a result, there was no major association between vitamin E and preventing major cancer or cardiovascular events.

In 2003, 980 elderly "free of dementia" subjects showed no association between intake of carotenes, vitamin C, vitamin E antioxidants and decreased rate of Alzheimer [27,35]. Neither higher intake of vitamin C, E and beta carotene showed promising results in 1999 for pulmonary middle aged male patients living in Finland (n = 1248), Italy (n = 1386), and the Netherlands (n = 691) [45]. All three antioxidants had positive results on the pulmonary function before adjustment for energy intake. Cerebral ischemia showed disappointing results for 26593 male smokers, aged 50–69 years taking lycopene, lutein zeaxanthin, vitamin C, flavanols, flavones, vitamin E. However, intake of beta carotene was inversely associated with cerebral ischemic patients [27].

One of the few associations between the antioxidant vitamins and the delayed aging was vitamin C and its inverse relationship with diastolic pressure for >20 years old males and females, and comparison of vitamin A and E levels with higher risk of hypertension for the same cluster of patients.

Solely increased antioxidant intake does not reduce the aging and the complications of oxidative stress. Reducing oxidative stress such as ceasing cigarette smoking and following healthy diet and following lifestyle extends the lifespan and the quality of life in the most effective way.

Conclusion

Numerous studies have found an association between aging and ROS, however, there is some data that contradict this theory. For this reason, it can be concluded that damages caused by ROS can serve as causal factors, but clearly, they do not account for all the consequences that come with aging.

Experiments conducted in order to test a free radical theory of aging gave us an opportunity to expand our understanding of this complex process. It has been proven that there is an increase in oxidative stress with aging which alters cellular longevity and causes cell damage, at the same time antioxidant enzymes have shown some protective effects. However, these processes cannot fully explain the root cause of aging.

Modifying risk factors that can increase oxidative stress is thought to be an important intervention in order to improve the quality of life and increase lifespan to some point. Important risk factors include air pollution, cigarette smoke, processed or reheated food, a diet rich with high quantities of macronutrients (carbohydrates, fat, and proteins), high concentrations of ethanol, certain drugs, chemotherapy, and radiation. These are the risk factors that can be easily modified and if decreased might result in significantly improved health in an aging population.

However, it is still unclear, whether increased antioxidant supplementation can prevent age-related complications. Supplementation of antioxidants such as lycopene, lutein zeaxanthin, vitamin C, flavanols, flavones, and vitamin E have not shown promising results in preventing major cancer, cardiovascular events, or hypertension. All these outlines the fact that aging is a multifactorial process and cannot be reduced to any single cause.

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TAU IMMUNOTHERAPY FOR ALZHEIMER'S (Review Article)

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Abstract

Alzheimer's is a tormenting disease that progressively destroys a person's cognition. Researchers have toiled long and hard to find a cure or slow the course of this disease. Therapies have mainly targeted the two hallmarks of Alzheimer's pathophysiology: amyloid and tau protein. This paper sheds light on the new developments in the field of immunotherapy aimed against tau protein, particularly in comparison to the thus far futile efforts of targeting amyloid. Tau targeting immunotherapy is emerging as a promising therapeutic option.

Introduction

Several advances made by medical science have resulted in the increase of life expectancy. Owing to this, diseases affecting the aging population cropped up. One of the most devastating is Alzheimer's, emerging as the fifth leading cause of death in people over 65 years of age. Without any progress in the

prevention, slowing down or curing of this disease, Alzheimer's will affect more than 13.8 million people by 2050 [1].

A thorough understanding of the pathophysiology of Alzheimer's is required before exploring its therapeutic options. Formation of beta amyloid plaques and neurofibrillary tangles by tau protein are the pathophysiologic hallmarks of this disease [2]. Cleavage of amyloid precursor protein (APP) by an enzyme called "Beta secretase" is responsible for the change in configuration that promotes aggregation [3].

Meanwhile, tau protein naturally functions as a microtubule stabilizer. Upon hyperphosphorylation they are rendered incapable of normal and appropriate function. They aggregate to form oligomers or paired helical filaments, the latter of which is more prominent in Alzheimer's.

Currently there are no effective disease modifying drugs to treat Alzheimer's. Anticholinesterase inhibitors (e.g. donepezil, rivastigmine and galantamine) along with memantine, a glutamate receptor antagonist, are typically used for symptomatic care but do little to prevent the fundamental pathology, namely that amyloid accumulates extracellularly and tau tangles intracellularly. The logical target for initial treatment options is prevention of these processes; amyloid was first targeted [1]. However, these efforts have not been very fruitful. Further research showed that altered Tau function and morphology preceded the clinically evident onset of Alzheimers and that Tau neurofibrillary tangles correlated better with the severity of Alzheimer's [4]. Hence there was a shift in focus to Tau targeting therapies, with an up and coming field being immunotherapy targeting Tau protein. If proven to be successful it can be used as a preventive tool to strike before the detrimental effects of this disease sets in.

Why not amyloid?

The role of β-amyloid in the pathogenesis of Alzheimer's disease has been well known for the past 30 years and, until recently, it has been an attractive therapeutic target [3]. What is well documented is that β-amyloid accumulates through alternate cleaving of amyloid precursor protein (APP), and that increased levels of β-amyloid are associated with the beginning and progression of Alzheimer's. Though It is still unknown whether it triggers cell-surface receptors or binds intracellularly, or whether it triggers nothing at all and simply accumulates. Perhaps even more intriguing is that lowering β-amyloid levels do not necessarily improve cognitive function [5, 6]. This in itself suggests that β-amyloid may not be the end-all be-all of Alzheimer's disease.

There are several prevailing therapeutic approaches currently under study. One employs anti-β-amyloid antibodies, the aim being to bind and remove β-amyloid, thus preventing it from exerting its effects. This strategy looks attractive since it precludes any of its pathologic effects. However, clinical trials have not shown any difference in outcome measures. Bapineuzumab was halted in its Phase III trial because it failed to show any effect, and solanezumab also revealed no effect in two of its Phase III trials [6].A second approach involves blockade of β-site APP cleaving enzyme 1 (BACE1). Blockade of the abnormal cleavage of APP would result in less β-amyloid accumulation. Studies in humans have thus far failed to show any meaningful results, although animal studies are promising.

Other mechanisms of interest are antagonism of receptors that are triggered by β-amyloid and management of risk factors that may accelerate β-amyloid formation.

Until β-amyloid's pathways and mechanisms of disease have been further mapped out, the design and use of therapeutics will likely continue to yield minimal results. Meanwhile, the rise in research surrounding tau protein, another downstream player in Alzheimer's, is perhaps easier and simpler to target. Early results have shown promising safety results, and trials are still ongoing.

Current Tau-Targeting Therapies and Vaccines: Active

Historically, vaccines have saved innumerable lives from infectious diseases. Unconventional vaccines have recently emerged which deals with creating vaccines for non-communicable diseases. Such vaccines work by influencing the immune system to recognize various target proteins and other molecules [7].

Alzheimer's is a disease with a multi-faceted and complex pathology. Due to the failures experienced with therapeutic approaches targeted at amyloid plaques, the focus has shifted to targeting neurofibrillary tangles. Early attempts were made to inhibit kinases or tau aggregation, but these approaches were hampered by the tested drug's toxicity and/or lack of efficacy [8]. Recently, however,

the spotlight has fallen on immunotherapy against Tau protein. The most attractive attribute of vaccines is that it can be used as prevention before full fledged Alzheimer's sets in, thereby averting the whole catastrophe. Since the patient's own immune system is producing the antibodies, the emergence of anti-drug antibodies are avoided as well. Additionally, compared to monoclonal antibodies which required repeated dosing and cost a great deal of funds, active immunization with vaccines will have drastically fewer doses and thus is less costly.

However there are a few pitfalls to using active immunization, the most obvious and fearful threat being developing antibodies against normal functioning host protein leading to autoimmune complications. Nonetheless, a few trials have shown vaccines overcoming these challenges and showing efficacy in preventing tau related pathology [9].

Hitherto, two vaccines in particular have had promising results. The first vaccine called AADvac started trials on rats in the year 2013. Researchers found a certain Tau peptide sequence ^{294}KDNIKHVPGGGS^{305} in the regulatory region which was responsible for the oligomerization of tau. This sequence was found with the help of a monoclonal antibody(mAB) DC8E8. Using enzyme linked immunosorbent assay, the Tau domain that bound to this mAB was found. These domains were also found to be discriminatory between pathologic and physiologic Tau [10]. Following its identification, an immunogenic T lymphocyte activating epitope was needed. This was provided in the form of a carrier protein called keyhole limpet haemocyanin protein [11]. The results of this study were positive. Addressing the previously mentioned aspects in vaccine development, AADvac 1 was able to generate high affinity antibodies specifically against pathologic tau in experimentally immunized animals. Additionally the immune response was found to be predominantly of the Th2 phenotype which is a testament to its safety [12].

The vaccine was then introduced for human trials. Patients with MRI- confirmed Alzheimers with an MMSE score of 15-26 were enrolled for the study. Of the 30 people who received six doses of the vaccine, 29 people developed IgG antibody response. When these IgG antibodies were compared for the reactivity to pathologic and physiologic Tau using ELISA, the discrimination between the two forms seemed to be similar to the parental antibody which was using for the sequencing (DC8E8). This confirms that the AADvac vaccine can induce safe, selective, and specific antibody response to pathologic tau. With just 30 participants receiving the drug for a period of 6 months, the sample size and time period were simply not large enough to show any significant cognitive and functional end point. Additionally, the patients continued to deteriorate at the pace of their disease progression. Injection site reaction was the only significant side effect [13]. In the succeeding year another study with the same patients in the phase 1 trial was done as a follow up. This showed that the IgG response was lasting past 6 months since the last vaccination. The decline in cognition was slowed in proportion with the amount of IgG produced, highlighting the importance of a sound immune system for the success of such a treatment. The next phase of this trial is yet to publish its results [9].

Another vaccine that is worth mentioning is ACI-35. It didn't raise any safety concerns but failed to elicit a robust immune response. Hence it was remade by including another adjuvant to further activate T helper cells. The second version was able to produce a stronger immune response in monkeys and they were specific to phosphorylated Tau. Trials in humans were started in July 2019 and are set to be completed in 2022 (Alzforum.org 2021. ACI-35).

Current Tau-Targeting Therapies and Vaccines: Passive

Given the vital role that tau proteins play in neurons and the neuronal environment, many therapies have been approached to target them. Among the many drugs like small molecule inhibitors, GSK3B inhibitors, microtubule stabilizers, anti-phosphorylation drugs, and tau aggregation inhibitors, immunotherapy has garnered a wide reception owing mainly to its success in animal models due to its varied targetability at early and late-stage diseases and low risk of side effects. The monoclonal antibodies can be made to target various regions and epitopes of the tau protein, including its oligomer or amino acid parts [14]. With the use of active immunization techniques, there has been a concern for immune-mediated long-term side effects due to the body's response [15]. But with passive immunization, it is possible to design monoclonal antibodies against various epitopes of the tau protein and their effects are

transient. Another advantage that has been proposed for the use of passive immunization is the ability to individualize the treatment based on a patient's disease severity and specific tau epitopes that can increase the efficacy of the therapy [16]. Currently, there are more passive immunotherapies in clinical trials than active ones, and also apart from their success in-vitro studies and mouse models in disrupting the pathological tau process, many of the currently ongoing trials also show promise in humans.

In healthy individuals the blood-brain barrier limits entry to circulating antibodies to around only 0.1-0.2%. But the reason behind the effectiveness of the antibodies themselves remains largely speculative and various mechanisms have been put forward. While healthy individuals only have around a hundredth of the immunoglobulin reaching the CNS, patients with Alzheimer's disease have a disrupted blood-brain barrier that changes the efficacy of this therapy [17]. There are currently 8 passive vaccines in clinical trials and below are their details.

BIIB092 (Gosurenamab) is a humanized lgG4 monoclonal anti-tau antibody against the N-terminal of fragmented forms of tau that were initially isolated from familial Alzheimer's Disease patients. This monoclonal antibody has already completed phase I studies on patients with progressive supranuclear palsy. There was a dose-dependent accumulation of the antibody in the serum and CSF. Moreover, it has shown to decrease the levels of CSF free tau effectively [18]. Currently this antibody is undergoing phase II trial in patients with mild AD and a positive amyloid PET scan, during which the patients will receive 3 different doses of infusion or a placebo.

ABBV-8E12 (Tilavonemab) is also a humanized IgG4 monoclonal antibody but it targets certain tau amino acids and also an aggregated extracellular version of tau. This antibody has been shown in animal studies to decrease neurofibrillary tangles, impede seeding of tau and also slow down brain atrophy. The drug has been approved for treatment of PSP and currently has completed phase I trials and is undergoing a multi-center randomized placebocontrolled phase II study.

RO6926496, another humanized monoclonal antibody attacks the phosphorylated portion of tau protein. It has been shown that the phosphorylation of tau at certain sites plays a major role in where the tau protein ends up accumulating and what structure it takes [19]. Animal studies that had targeted this epitope of the tau protein have shown decreased levels of tau and an improvement in cognitive assessments. While this antibody had completed the phase I trial, there has been no phase II trial to date.

RO7105705 (Semorinemab) is an anti-tau antibody that was designed for mainly targeting extracellular tau. The presence of the extracellular tau protein has been implicated at various stages of the disease and also a potential driver for inflammation and atrophy [20]. This antibody has completed phase I studies and is currently undergoing two different phase 2 studies. One of the studies targets patients with mild AD or prodromal AD verified by PET or CSF amyloid. The other focuses on patients with moderate AD and who are positive for the tau ligand GTP1 verified through PET.

JNJ-63733657 is a humanized IgG1 anti-tau antibody that targets residue 217 on the tau that is located in the middle region. This is in stark contrast to other antibodies that usually target N-terminal residues [20]. This antibody was reported to reduce pathological tau seeding. After being deemed safe in two phase I trials, this antibody is currently in phase 2 trial on patients with mild AD and a positive PET scan.

BIIB076 is a recombinant human monoclonal anti-tau antibody. This antibody is known to bind to monomeric and pre-formed tau proteins with a very high affinity. This pan-tau antibody is able to block tau aggregation and tau propagation across neurons [21]. After being assessed as safe from animal studies this drug was tested for safety in a phase I clinical trial in healthy volunteers that ended in March 2020.

UCB0107 (Bepranemab) is another humanized IgG4 monoclonal antibody that targets the central region of tau, specifically amino acids 235-246. Again this approach was shown to reduce tau aggregates. This antibody was evaluated for safety on healthy volunteers and in patients with PSP in two separate phase I studies. The phase 2 trial will be underway in 2021.

LY3303560 (Zagotenemab) is a humanized anti-tau antibody that targets a specific conformational epitope of tau in the N-terminal region. It has been shown that it binds with nanomolar affinity to aggregates over monomers [22]. This drug after animal studies has completed two phase I studies and is currently in phase 2.

Table 1: Summary of passive tau-immunotherapeutic drugs in clinical trials

Drug Name	Mechanism	Trial Identifier	Status
BIIB092 (Gosuranemab)	Humanized IgG4 monoclonal anti-tau antibody against N-terminal fragment	NCT03352557	Phase 2, Active
ABBV-8E12 (Tilavonemab)	Humanized IgG4 monoclonal antibody against extracellular aggregated tau	NCT02880956	Phase 2, Active
RO6926496	Humanized monoclonal antibody targeting the tau phosphoepitope pS422	NCT02281786	Phase 1, Completed
RO7105705 (Semorinemab)	Anti-tau humanized IgG4 antibody against N terminus of extracellular tau. Binds all six isoforms of human tau including oligomeric and monomeric forms	NCT02820896	Phase 2, Active
JNJ-63733657	Humanized IgG1 monoclonal antibody that recognizes the microtubule binding region of tau.	NCT04619420	Phase 2, Recruiting
BIIB076	Human recombinant, monoclonal anti-tau IgG1 antibody that targets the mid-domain of the tau protein. Binds to monomeric and fibrillar forms of tau.	NCT03056729	Phase 1, Completed
UCB0107 (Bepranemab)	Humanized, monoclonal IgG4 antibody that binds the central area of tau, between amino acids 235–246 near tau's microtubule-binding domain.	NCT04867616	Phase 2, Not yet recruiting
LY3303560 (Zagotenemab)	Humanized anti-tau antibody that targets a specific conformational epitope of tau (an early pathological form) in the N-terminal region	NCT03518073	Phase 2

Limitations and the Future

As has been shown, Aβ immunotherapy has been largely ineffective to date. Therefore, Tau immunotherapy may be the most prudent course of action, especially once the symptomatic process is underway. The AN-1792 vaccine seemed to demonstrate some ability to clear Aβ plaque-associated tau lesions by plaque removal in the first successful immunization study, and the anti-Aβ antibody bapineuzumab decreased CSF phospho-tau levels in patients with AD in phase II trials. Unfortunately, these findings did not extend to phase III studies in which bapineuzumab had no effect on tau pathology. These data suggest that any clearance of Aβ during active or passive immunization cannot significantly reduce tau levels to alter the course of the disease, and the next logical step is direct targeting of tau.

Successful tau immunization has shown to reduce tau pathology by targeting single or multiple phospho-epitopes, the amino terminus, full-length, normal and mutant tau. In mice, when given in combination with strong T-helper 1-inducing adjuvants that are not licensed for human use, tau vaccination has been reported to cause toxicity [23]. Due to the occurrence of meningoencephalitis in roughly 6% of the enrolled moderate-to-severe AD patients, the first successful vaccine clinical trial for AD AN-1792 was halted early in 2002 [24]. Although Aβ deposition in the limited number of responders who came to autopsy over the next few years decreased focally in particular brain regions, many were seriously demented at the time of death. Based on in vitro experiments using a monoclonal antibody (DC8E8) which prevents tau oligomerization, the epitope for production of the tau vaccine AADvac1 was selected. Adverse effects were limited to inflammation at the injection site and no deleterious immunological responses were observed during the procedure. To prevent autoimmune-like reactions a large effort was made to test passive immunotherapy using humanized anti-Aβ monoclonal antibodies.

Passive immunization provided a reasonable response to the safety issues arising from active strategies. Patients will not produce their own antibodies and it is possible that the effects of immunization will be transient, thus reducing the possibility of adverse immunological effects. Systemic injection into mice of the Aβ monoclonal antibody specific to the Aβ N-terminus, 3D6 mAb, resulted in the transfer of the antibody to the brain, plaque binding of the antibody, and induction of Fc-receptor-mediated phagocytosis of Aβ deposits [25]. This antibody is the predecessor to Bapineuzumab, a humanized N-terminal-specific mAb, which was later studied in clinical trials in Phase I, II and III. Bapineuzumab has shown no major clinical benefits in 2 large clinical trials in Phase III. Pfeifer et al. has documented increased incidences of cerebral microhemorrhages despite plaque reductions. This has been confirmed by them in other clinical studies in mice. In patients with Alzhiemer's disease those who have one or two Apolipoprotein E ε4 alleles, bapineuzumab treatment has been shown to cause a transient vasogenic edema and microhemorrhage.

In early clinical trials Intravenous immunoglobulin (IVIg) pooled human antibodies showed promise. However, recent studies, including USA's Octapharma Phase II 24-week Octagam 10 percent IVIg study in 58 AD patients showed no major slowing of AD progression [26].

Two new antibodies have been announced for human testing. A phase I clinical trial of JNJ-6373365 passive immunization has been launched by Janssen Pharmaceuticals. The antibody tends to bind to the tau middle region and has been engineered to avoid seeding and spread of tau. UCB0107 is now advancing towards clinical trials. Preclinical research indicated this antibody binds to amino acids 235-246 in the proline rich region of tau and that it was effective in preventing pathological tau spreading. Soon, some other successful tau immunotherapies are expected to enter clinical trials. For example, a large portfolio of anti-tau antibodies is currently being developed by Lundbeck, targeting both total tau and pathological hyperphosphorylated PHF-tau [8].

It is likely that the most powerful antibodies would be those that can attack all pathological tau protein pools, both intracellularly and extracellularly. Finally, it is unclear how closely tau seeding and spread are linked to tau toxicity. An antibody chosen to prevent tau seeding and spread may therefore not inherently block the toxicity of tau.

While the field of AD immunotherapy has developed immensely over the past decade, some problems still remain and will need to be solved in order to see long-term protection and success.

One of the major issues of using active or passive immunization is for the antibodies to cross the blood brain barrier. Usually, only a small amount (roughly 0.1%) of antibodies cross the blood brain barrier, so discovering ways to increase the penetration of antibodies into the brain can be beneficial. The use of chaperone proteins or bi-specific antibodies to transfer therapeutic antibodies to the brain, the temporary opening of the BBB by chemical means, and the direct injection of antibodies into the CNS by means of time-released pumps are some of the possibilities to increase antibody delivery across the BBB.

Second, to prevent clogging of the clearance pathway during long-term treatment, a better understanding of the clearance of Aβ/anti-Aβ immune complexes is required. Active vaccination requires attention to the effects of immunotherapy, including immuno-degeneration in the elderly, the potential for autoimmune effects in self-protein vaccination and the use of very strong, pro-inflammatory adjuvants.

Conclusions

After the failure of amyloid-beta targeted therapy across many different clinical trials, tau immunotherapy has become the focus in the recent treatment of Alzheimers and other taupathies [9]. Tau offers many versatile targeting options that have been implicated as playing key roles in the disease process [27]. As discussed previously there are currently many active and passive immunotherapy vaccines that are in clinical trials or in development. These immunotherapies target intracellular and extracellular tau with varying affinities which could possibly translate into clinical efficacy. In fact, some vaccines that are in clinical trials and some which are also in development have chosen to target aggregated tau proteins in addition to the monomeric form. This represents an important milestone in tau immunotherapy as these newer antibodies have the ability to prevent 'seeding' and 'prionosis', which have been theorized to be the key mechanism behind the progression of the disease [28]. Currently, most of these therapies are in

either phase II or III will complete it within the next two years. Once these promising therapeutics have completed the phase III trial, we can accurately understand the effect these “vaccines'' will have on the clinical syndrome of the disease. And since tau proteins have been identified to be accumulating decades before the symptoms of the disease arise, it enables us to think about screening or even prophylaxis. We might be able to vaccinate the general population against taupathies or even be able to identify early biomarkers that can allow giving this vaccine in the very early stages of the disease. There is also a very significant chance that tau targeting might be an unfruitful avenue to pursue and could yield the same results as amyloid-beta targeting. Given the multifactorial and polygenic nature of tauopathies, we multiple targeting options might have to be combined to possibly halt the progression of the disease [12]. More research into the pathogenesis and pathophysiology of tauopathies can help to refine current targets and to develop novel ones.

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ASSOCIATION BETWEEN CHILDHOOD TRAUMA AND MOOD DISORDERS IN ADULTHOOD

(Review Article)

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Abstract

It is commonly believed that childhood trauma can manifest into mood disorders in adulthood; the aim of our study is to thoroughly review the array of mood disorders that can be caused by childhood trauma and underline the predisposing risk factors and mechanisms of developing negative symptoms. We are trying to investigate the relationship between specific trauma types and mood disorders. For this purpose, we reviewed more than 20 articles on various childhood traumas, mood disorders, their mechanisms, and relationship.

In our research, we specifically focus on the risk of developing mood disorders in the victims of physical abuse, sexual abuse, and neglect, briefly review the statistical data, as well as try to explain the pathophysiologic mechanism by analyzing: genetic factors, neuroplasticity, HPA axis, hippocampal volume and the effect of oxytocin.

Introduction

Many children around the world are exposed to a traumatic event at some point in their lives. These events not only affect children at present time but can also take part in developing mood disorders in adulthood. Nearly 3 in 4 children-or 300 million children- aged 2-4 years regularly suffer physical punishment and/or psychological violence at the hands of parents and caregivers [1]. The numbers are higher for the mood disorders, most common being depression and bipolar disorder which combined affect more than 310 million people worldwide [2]. In 2004, Charles. B Nemeroff conducted the first study

which aimed to investigate neurobiological consequences of childhood trauma. His study showed that repeated early-life stressor leads to alterations in central neurobiological systems, particularly in the corticotropin-releasing factors system, leading to increased responsiveness to stress [3].

After this study, several new ones have been conducted but the information about association of childhood trauma and adulthood mood disorders is still rare. Given the destructive potential of mood disorders, it is important to find how early life stressors affect the psychological condition of the child and if those stressors can really cause adulthood mood disorders.

Types of Childhood Trauma

The World Health Organization (WHO) defines child maltreatment as "all forms of physical and emotional ill-treatment, sexual abuse, neglect, and exploitation that results in actual or potential harm to child's health, development or dignity to children under 18 years of age". There are four main types of abuse: neglect, physical abuse, psychological abuse, and sexual abuse. Abuse is defined as an act of commission and neglect is defined as an act of omission in the care leading to potential or actual harm [2]. In the following paragraphs, different forms of childhood emotional trauma will be discussed.

Sexual Abuse

Child sexual abuse (CSA) is defined by WHO as "the involvement of a child in sexual activity that he or she does not fully comprehend and is unable to give informed consent to, or for which the child is not developmentally prepared, or else that violate the laws of social taboos of society. CSA can include exhibitionism, fondling, oral-genital contact, and rectal or vaginal penetration [4].

The exact measurement of the prevalence of childhood sexual abuse is not easy because the definition of CSA varies among studies. For example, the age used to define childhood might vary from study to study, also the types of acts of sexual abuse might be different (e.g., both contact and non-contact). Survey methods used to evaluate CSA, number, and details of screening questions also influence the resulting prevalence estimates [5]. Even if the surveys are anonymous and the screening questions are as delicate as possible, only an incomplete portion of CSA incidents are reported, even smaller numbers are reported to authorities and the biggest portion of cases remain unreported because of shame, fear, or other factors [6].

The 2006 world report on violence against children provided data, which shows that in 2002 approximately 150 million girls and 73 million boys were subject to contact CSA worldwide [7]. In the US, more than 34,000 adults aged 18 years or older residing in households were face-to-face interviewed in a survey conducted during 2004-2005[8]. Child sexual abuse was defined by the four questions that the Adverse Childhood Events Study used to assess unwanted sexual experience before age 18.: "Before you were 18 years old: 1) How often did an adult or other person touch or fondle you in a sexual way when you didn't want them to or you were too young to know what was happening?; 2) how often did an adult or the other person have you touch their body in a sexual way when you didn't want them to or you were too young to know what was happening?; 3) How often did an adult or other person attempt to have sexual intercourse with you when you didn't want them to or you were too young to know what was happening? And 4) how often did an adult or other person actually have sexual intercourse with you when you didn't want them to or you were too young to know what was happening?" [9] responses to all four questions ranged from 1= "never" to 5= "very often". Individuals who responded never to all these questions were classified as not having a history of CSA. All other individuals were classified as having a history of CSA, resulting in 10.14% (24.8% in men, 75.2% in women) of participants being the victims of CSA [8].

Sexual abuse in childhood often occurs alongside other forms of abuse or neglect and are more common in families with low socioeconomic status. There are several factors that are thought to increase the risk of child sexual abuse and are divided into 4 big categories. These categories include individual, family, environmental and social factors. The psychological health of the child also plays the role in the occurrence of CSA, meaning that children who are impulsive, emotionally needy, and who have learning or physical disabilities and substance use disorder are more prone to be the victim of sexual abuse [10].

Child Neglect

Child neglect includes both isolated incidents, as well as a pattern of failure over time on the part of a parent of other family members to provide for the development and well-being of the child- where the parent is in a position to do so in one or more of the following areas: health, education, emotional development, nutrition, shelter, and safe living conditions. The parents of neglected children are not necessarily poor [11]. There are 7 types of neglect: 1) physical – inadequate food, clothing, shelter, hygiene; 2) medical- failure to provide prescribed medical care or treatment of failure to seek appropriate medical care in a timely manner; 3) dental- failure to provide adequate dental care or treatment;4) supervisional- failure to provide age-appropriate supervision; 5) emotional- failure to provide adequate nurturance of affection, failing to provide necessary psychological support, or allowing children to use drugs and/or alcohol; 6) educational- failure to enroll a child in school or failure to provide adequate homeschooling, failure to comply with recommended special education, allowing chronic truancy; 7) other- includes exposing a child to domestic violence, or engaging or encouraging children to participate in illegal activities such as shoplifting or drug dealing [11].

The main contributor to the neglect is the parental problem, including mental health issues, intellectual deficits, and substance abuse. However, often several factors interact and play a role in the occurrence of neglect- for example, a single parent with substance abuse and mental health problems in a community with low socioeconomic status [12]. Children with complex medical problems and children with developmental disabilities are at the risk of neglect, one of the reasons being the increased requirement of care, financial aid, complicated parent-child relationships, and increased family stress. The community and society in which the child lives is one more contributor to the occurrence of neglect. If the community does not have community centers, mental health support organizations and are impoverished often have a higher prevalence of neglect [13].

The number of research papers about childhood emotional and physical neglect is small. The number of studies reporting about physical neglect is 16, and only 13 for emotional abuse.

The participants for those studies were 59,406 and 59,655 respectively. The results showed the prevalence of physical neglect to be 163/1000 and prevalence of emotional neglect - 184/1000 with no apparent gender differences [14]. These numbers were extremely low in comparison to a meta-analysis published in the same year about child sexual abuse yielding over 200 publications using self-report measures of sexual abuse over 40,000 participants [15].

Physical Abuse

Child physical abuse (CPA) committed by parents or other caregivers is a major public health problem and social welfare problem all around the world (Gilbert et al., 2009; Pinheiro, 2006). To examine the prevalence rates of child abuse committed by parent/caretaker a population-based survey was carried out in 2008 in Sodenmarland Country, Sweden among all pupils in three different grades (n=8494). Children were asked about their exposure to violence. Contact persons in the school as nurses and teachers were responsible for the distribution of the questionnaires. To maintain total confidentiality, the questionnaires were sealed in the envelopes. A total of 15.2% of children reported that they had been hit [16].

Mechanisms

Stress response is created in humans by a complex interplay between several endocrine, autonomic, inflammatory and behavioral components, all stemming from a large neuronal circuit that includes the prefrontal cortex, hippocampus, amygdala, and brainstem regions. Hypothalamus-Pituitary-Adrenal (HPA) axis is a network of central nervous system (CNS) and peripheral nervous system (PNS), combining numerous nuclei, ganglia, and neurotransmitters. Oxytocin is a hormone neuropeptide which assumes an opposite role and can be thought of as a stress-protective. Inflammation, expression of various receptors and growth factors affect CNS neuroplasticity in various ways, often illustrated by hippocampal volume as well as microscopic studies. Finally, genetic and epigenetic factors offer a lot of leads but not too many answers as of yet; only few of them are discussed here. Although each of the above elements is discussed in separate paragraphs, it is of utmost importance to see all these parts as exactly that - “parts” of something whole, even if that “whole” is not perfectly coherent from our current data.

HPA axis

HPA axis is a cornerstone of neuroendocrine stress response in mammals. It was first described in 1956 that plasma cortisol concentrations are elevated in those patients who fit the criteria for a diagnosis of MDD [17]; further, HPA axis is more resistant to negative feedback inhibition (i.e. dexamethasone suppression test) in depressed patients than in non-depressed individuals. Both of the above-described effects – of hypercortisolism and resistance to negative feedback inhibition – are reversible upon resolution of MDD [18]. Hence the association between stress and depression has been defined for the better part of a century. But not everyone under stress develops depression, and pre-existing factor(s) that determine an individual's ability to process the emotional challenge must be at fault. Age at which a person experiences stress seems to be exactly that kind of factor, as demonstrated in a pioneering study in 1994 by Brown and Moran [19]. Several other studies [20,21] in the years that followed further quantified the association between childhood trauma and MDD, including the fact that childhood, but not adolescent, physical or sexual abuse is associated with an increased rate of depression or anxiety symptoms. Experience of childhood trauma sensitizes individuals to stress in such a way that these people are at higher risk for developing depression, especially in response to further stress [22]. In a study conducted in 2008 by Heim et al., subjects subdivided into 4 categories according to presence or absence of childhood stress and current psychiatric disorder were compared by their response to a mild stress stimulus in adulthood. In response to standardized psychosocial protocols (e.g. public speaking, mental arithmetic) that have reliably been shown to induce sympathetic activation in humans [23], the difference was striking – subjects with a history of childhood stress with or without current MDD exhibited higher ACTH, Cortisol, and heart rate than control groups.

The results solidified that childhood stress is the single strongest predictor of ACTH hyperactivity. The number of abuse events, adulthood traumas, and depression were the other, relatively weaker predictors.

Whereas psychosocial stress engages HPA axis starting from the cognitive-emotional processing, pharmacological stress tests (i.e., CRF stimulation) only exerts effect at the level of pituitary. Abused women without depression showed similarly elevated ACTH response to the pharmacological stress test as they did in the psychosocial stress test; depressed women both with or without childhood abuse showed a blunted ACTH response, which is expected in MDD. This result signifies the CRF receptor changes on pituitary corticotropes due to overactivity of the hypothalamic Paraventricular Nucleus (PVN)-median eminence in the HPA axis. As for the glucocorticoid-mediated feedback regulation, a low-dose dexamethasone test in abused women with depression showed increased suppression of cortisol. This finding is believed to be a major mechanism of stress sensitization and is also a prominent finding in PTSD. As an advancement of the simple glucocorticoid suppression test, the combination of dexamethasone/CRF test was performed in study subjects composed of men subdivided into all 4 groups; this combination of negative feedback (by dexamethasone) and escape from suppression (by CRF) is considered to be the most sensitive indicator of HPA axis hyperactivity. Men with a history of childhood abuse responded with higher cortisol levels to the dexamethasone/CRF test than did men without a history of abuse. Similar results had already been previously demonstrated in women with borderline personality disorder [24], signifying that the dexamethasone/CRF test is sensitive in elucidating HPA hypersensitivity regardless of sex.

Apart from assuming the primary role in the HPA axis through the PVN, CRF also acts as a kick-starter for autonomic and behavioral changes of stress. CRF levels in the CSF have been thus found to positively correlate with symptoms of depression [25,26] and levels of escalating perceived stress [27]. Heim et al., 2008, found that CSF CRF concentrations were progressively more elevated in subjects who had undergone childhood abuse, with higher concentrations found in those who had experienced both physical and sexual abuse (rather than sexual alone) and correlated with severity ($r=0.43$, $p=0.004$ for physical; $r=0.33$, $p=0.026$ for sexual) and duration ($r=0.34$, $p=0.023$ for physical; $r=0.29$, $p=0.05$ for sexual) abuse. Elevated CSF CRF levels were also stratified according to the age of the women at the time of abuse; women who had experienced abuse in later childhood had a higher concentration of the neurotransmitter than those abused at or below the age of 6.

Oxytocin

Neurotransmitter Oxytocin has drawn attention in this field of research due to its known role in bonding, trust, social support, and anti-stress properties. During a highly plastic period of the development of the brain, Oxytocin decreases amygdala reactivity [28]. Childhood abuse and disruption of the caretaker-child relationship were shown by Francis et al. in 2000 to affect persistent negative effects in CNS Oxytocin receptor expression in rats. In 2008, Heim et al. provided the first such evidence in humans – markedly decreased CSF Oxytocin concentrations were found in women with a history of childhood abuse. This offers insight into the interplay between stress-inducing (HPA axis) and stress-protective (Oxytocin) in adults, and how disbalance in them may put an adult at particularly high risk when exposed to stress later in life.

Hippocampal volume

As one of the most neuroplastic regions of the brain, the hippocampus also controls aspects of the HPA axis, contributes to memory and specifically contextual memory regarding fear.

Decreased hippocampal volume has been described both in patients with current MDD and in remitted patients [29,30]. Notably, after controlling for childhood abuse, it was revealed that only those subjects who had experienced childhood abuse had left hippocampal volume decreased by 18% from normal, while depressed patients without a history of abuse had a normal volume of hippocampus. Childhood trauma seems to be associated with smaller hippocampal volume, regardless of current or past psychiatric status. CRF and Cortisol hyperactivity acutely in childhood, and/or chronically thereafter (due to HPA axis hyperactivity) is suspected to contribute to this finding. On a molecular level, abnormally high glucocorticoid effects on the hippocampus include a reduction in dendritic branching, loss of dendritic spines, and reduced neurogenesis, particularly in the CA3 region.

Inflammation and neuroplasticity

Described first in 1983 by Kronfol et al. [31], depression has since then many times been demonstrated to have a definitive association with innate immunity inflammation. In 2008, Danese et al. [32] were the first to demonstrate that childhood abuse is also associated with increased inflammation (specifically measured inflammatory marker CRP) – and this association was especially strong in those subjects who proceeded to develop MDD in adult life. Other inflammatory markers, such as IL-6, have also been demonstrated to rise in abused individuals. The inflammatory reaction is most likely attributed to epigenetic changes induced by childhood stress; the most prevalent epigenetic modification is considered to be selective DNA methylation [33]. According to a model proposed by Miller and Chen [34], stress that occurs during early ages permanently influences the inflammatory system. This includes brain inflammatory cells, such as Macrophages, Microglia, and Dendritic cells, which will be over-expressed chronically once the expression of a few key regulator genes have been altered. Some of the better studied of these regulator genes are NF-kB and BDNF, both of which determine levels of pro-inflammatory cytokines, synthesis of neurotrophin (and other neuromodulators), and expression of glucocorticoid receptors. While most of the epigenetic variables concerning early life stress has only been examined in mice and monkey models, human data is starting to catch up; in post-mortem brain studies, early life abuse was associated with increased methylation of the GR exon 1f promoter in the hippocampus, one of the first such findings in humans [35].

Genetic factors

It is also necessary to note that genetic factors predispose each individual to susceptibility to childhood stress-related neuroendocrine changes. For example, the dexamethasone/CRF test is known to vary not only with childhood abuse but also with FKBP5 gene (which codes for

GR-regulating cochaperone hsp-90). Promoter polymorphisms of the Serotonin receptor gene (5HTTLPR) [36], MAO-A gene, Corticotropin-releasing hormone type 1 receptor gene (CRHR1) are only a few examples of the several known genes that moderate the link between childhood stress and dysfunctional emotional regulation.

Psychological supportive data

The above-described combination of endocrine, autonomic, and behavioral components of the stress response encompasses the brainstem, hippocampus, amygdala, and prefrontal cortex. In concert with the physiologic findings already described, psychological research data proves immensely valuable

in illuminating some of the earlier, subtler effects of childhood abuse. Children with a history of abuse have a deeply skewed perception of social and emotional relations; such children tend to interpret ambiguous facial expressions as angry and show increased amygdala activity when reacting to what was perceived as "angry" [37].

EEG findings of abused children further support the evidence of impaired cortical-limbic connectivity [38].

Array of Mood Disorders Caused by Childhood Trauma

Different forms of childhood trauma can have strong associations with a wide variety of mood disorders in adulthood like Major Depressive Disorder, Persistent Depressive Disorder, Bipolar Disorder and even Postpartum Depression. In this review we will try to summarize the different outcomes of childhood trauma based on multiple research articles.

Childhood Trauma and Its Relation to Chronic Depression in Adulthood

All the studies reviewed aimed to examine the prevalence of retrospectively recalled childhood trauma in the group of chronically depressed patients. Reported studies were based on distinct samples and relied on a variety of measures and diagnostic procedures. Therefore, several studies used the Childhood Trauma Questionnaire (CTQ), some used childhood trauma questions and childhood trauma interviews, in some of the articles even criminal court records were used. Some studies relied on diagnostic interviews conducted with DSM-III/IV diagnoses, while others relied on medical diagnoses [39]. Chronicity was operationalized as a function of time: Major depressive episodes were defined to last for at least 12 months already at the beginning of the study, while diagnosis of dysthymia or double depression indicate depressive symptoms for at least 24 months or even longer. BDI-2 and the QIDS-C were used to assess symptom severity. In the end 75.6% of the chronically depressed patients reported being abused at least once (clinically significant abuse) while 37% of the chronically depressed patients experienced multiple childhood traumas. Experiences of multiple traumas also led to much more severe depressive symptoms. Multiplicity turned out to be the only significant predictor for symptom severity for chronic depression. While emotional neglect, psychological abuse, physical abuse, and sexual abuse were significantly associated with the chronicity of depression [40]. Physically abused (OR = 1.54; 95% CI 1.16–2.04), emotionally abused (OR = 3.06; 95% CI 2.43–3.85), and neglected (OR = 2.11; 95% CI 1.61–2.77) people turned out to have a higher risk of developing chronic depression compared to non-abused individuals [41]. The test for heterogeneity showed high significance, with $p<0.01$ for both abuse types and neglect. The OR estimates were slightly higher in males, but it did not show statistical significance, while women were significantly more often exposed to childhood trauma. Even though primary analyses showed a higher risk of drug use associated with physical abuse, emotional abuse or neglect, the significance was only borderline and a dose–response relationship was not consistently seen [42]. Physically abused (OR = 3.00; 95% CI 2.07–4.33), emotionally abused (OR = 3.08; 95% CI 2.42–3.93), and neglected (OR = 1.85; 95% CI 1.25–2.73) individuals had a significantly increased risk for suicidal behavior compared with non-abused individuals, which manifested in both, suicide attempts and ideation [42].

Multiple childhood trauma can be specifically related to chronic depression and we can integrate this trauma aspect for the treatments.

Childhood trauma in Bipolar Disorder

All the studies reviewed here were as well retrospective in nature, studies were conducted using childhood trauma questionnaires in the group of patients diagnosed with bipolar disorder and control groups.

These papers demonstrate strong association between childhood trauma and bipolar disorder. Higher CTQ scores were found in patients, who were diagnosed with either bipolar I or bipolar II disorder compared to controls. The studies included different types of childhood traumas and their outcomes, there turned out to be a strong association between all types of childhood traumas and bipolar disorder, other than sexual abuse [43].

In bipolar patients, who had the symptoms of melancholia, the scores of emotional and physical neglect in childhood were higher. Unlike some older studies, newer studies found that emotional neglect

is the single most important risk factor, instead of physical abuse [44]. However, some of the research done on this topic is small in size, which potentially increases the risk of type II error.

Maternal childhood trauma and Postpartum Depression

Our review is based on the study done on a longitudinal sample of South African women, who were followed throughout the pregnancy and postpartum, it is a prospective study on a woman who experienced childhood trauma based on a childhood trauma questionnaire.

Women with childhood trauma experienced greater depressive symptoms through six months postpartum, which itself predicted negative child outcomes at one year. It even affected maternal-infant bonding and infant growth. This study demonstrated the intergenerational transmission of negative symptoms, it shows that in an environment of high trauma settings perinatal intervention can be of serious importance [45]. This study can have limitations because of the small sample size and potential confounders, but it is one of the rare studies on this topic [46].

Conclusion

We reviewed sociologic, epidemiologic, and neuroendocrine characteristics of childhood trauma and their relation with development of depressive disorders in later life. Although childhood trauma in general is associated with high incidence of depressive symptoms in adulthood, the reviewed literature suggests that there are some tangible differences in incidence when controlling for physical or sexual abuse and child neglect. WHO data makes it clear that a sizable adult population reports a history of abuse, and even larger groups of population are assumed to be experiencing but not reporting abuse; this population has a lifelong high risk for developing depressive symptoms. Some neuroendocrine and inflammatory effects of childhood trauma are very clear and well-defined, but the existing gaps in research make it hard to paint a clear picture. Genetic factors make some children particularly susceptible to developing epigenetic modifications in response to stress; this results in over- and under- expression of receptors, neuromodulators, inflammatory cytokines, and consequently alters the major neuroendocrine axis and neuroplasticity of the CNS. Children with the typical reactive changes are not adequately equipped to healthily cope with stress in adulthood, and are hence susceptible to develop depressive disorders.

Although most of the reviewed literature focuses on MDD, other affective disorders such as Bipolar and Postpartum depression have also been definitively described. Our results demonstrate that the high prevalence of childhood abuse might be strongly linked with the high prevalence of affective disorders (especially MDD); considering the high disease burden of MDD, it is imperative that additional research addresses the complex mechanisms behind the association. Any new advancements in this area stand to shed much light on ways to improve effectiveness of early detection, prevention, and treatment response, of MDD.

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USE OF STEM CELLS IN REGENERATIVE CARDIOVASCULAR MEDICINE

(Review Article)

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Abstract

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide, with its lifetime risk exceeding over 60%. Though various medications and procedures have managed to play a role in reducing mortality, none have shown to be permanent. The idea of stem cells is to generate an original solution that provides normal physiological responses. When applied to cardiology, it holds tremendous promise for rapid myocardial regeneration. The selection of the most appropriate type of cell is essential for its efficient application. If done successfully, it will negate temporary solutions such as a

stent, defibrillators, and medications. This article discusses all the studies that applied stem cells in cardiac pathologies and reveals the benefits as well as outcomes. It helps us understand the limitations one may come across while experimenting in this field and introduces issues that will need further research.

Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, killing 17 million people each year. It is estimated by the World Health Organization (WHO) that this number will reach 24 million by 2020 [1]. CVD contains multifactorial pathologies that are both genetic and environmental. Lifestyle changes, pharmacological or surgical intervention are current strategies against CVD. The effect of drug treatment differs for each individual and surgery is not viable in all patients. New strategies or approaches have to be considered to better understand the pathogenesis of CVD and broaden the diagnostic and therapeutic plan, especially in the case of heart failure (HF).

Stem cells are one of the human body's key cells that can develop into more than 200 cell types. Stem cells are undifferentiated cells that are found in the embryonic, fetal, and adult stages of life and give rise to differentiated cells that create tissue and organ structures. Stem cells construct the foundation for the entire body's tissue and organ system. It mediates various roles in host disease development, growth, and tissue repair processes. There are four types of stem cells, i.e., unipotent, multipotent, pluripotent, and totipotent, depending on the trans-differentiation ability. Self-renewal, clonality, and potency are the key characteristics of stem cells. Between different stem cells, these properties can differ. Blastocyst-derived embryonic stem cells (ESCs) have a greater capacity for self-renewal and potency, whereas adult tissue stem cells have minimal self-renewal because they do not proliferate freely and can only differentiate into tissue-specific cells. Stem cells can be classified as embryonic stem cells (ESCs), tissue-specific progenitor stem cells (TSPSCs), mesenchymal stem cells (MSCs), umbilical cord stem cells (UCSCs), bone marrow stem cells (BMSCs), and iPSCs on the level of regenerative applications. Stem cell transplantation for induction of tissue regeneration of malignant cells may be autologous, allogeneic, and syngeneic. Tissue typing of human leukocyte antigens (HLA) for tissue and organ transplantation as well as the use of immunosuppressants is advised to prevent the effects of host-versus-graft rejection [2,3,4].

In patients with advanced HF after MI, stem cell transplantation has been reported to enhance cardiac function as a new treatment strategy. Stem cell transplantation, as shown in many basic research and clinical trials, can boost tissue perfusion, contribute to angiogenesis, and retain or regenerate myocardial tissue. For myocardial infarction (MI), stem cell-derived sheet engineering provides desirable advantages in comparison to direct stem cell transplantation and scaffold tissue engineering. Induced pluripotent stem cells can form vascularized networks that would allow the manufacturing of thick human cardiac tissue and have proven to be successful in MI therapy compared to other sheets.

History

In the mid-nineteenth century, the evolution of stem cells led to the invention that other cells could be developed by certain cell types. In the following years, it was discovered that the bone marrow contained hematopoietic SC and stromal cells. In the late 1950s, Dr. Thomas conducted the first successful transplant. Identical twins were taken in this case to avoid the concern of graft vs host disease. The first successful nontwin allogeneic transplantation was not conducted until 1968. In 1973, a young boy with a genetic immunodeficiency condition received multiple marrow transplants from a donor recognized as a match in Denmark. That was considered the first successful unrelated donor transplant. In 1979, at the Hutchinson Hospital, the first successful unrelated donor transplant for a patient with leukemia took place [5]. Bone marrow transplantation has since expanded dramatically in the 1990s.

Recent clinical trials have shown that cell sheet technology has enhanced the ejection fraction, restored the dysfunctional cardiac wall, increased vascular genesis, and reduced fibrosis in models of heart disease. Cell sheets would be considered a potential treatment despite issues like lack of nutrition or increased transplant time window.

Methodology

There is a range of stem cells used in cardiovascular therapy the same way, there are multiple pathways to deliver this tissue to the pathological sites.

Some of these cell types include:

A. Human embryonic stem cell-derived cardiomyocytes (hESC-CMs)

Before embryonic stem cells can be differentiated into cardiomyocytes, they need to undergo expansion with the help of fibroblasts.

The expansion phase of undifferentiated hESC is performed on mouse embryonic fibroblasts (MEFs) in a medium consisting of DMEM/F12 supplemented with 20% KnockOut serum replacement (Invitrogen), L-glutamine, non-essential amino acids, beta-mercaptoethanol, and BFGF. Cells are then subjected to collagenase IV and trypsin, as well as the ROCK inhibitor Y-27632 to enhance cell survival. Human ESCs were depleted of MEFs before cryopreservation.

The embryonal carcinoma cells and embryonic stem cells found in mice were dissociated into single drops that then aggregated and formed spheroids with 2 layers (inner ectoderm like layer and outer endodermal layer). These spheroids were then termed embryoid bodies [6].

Cardiac differentiation was induced using an embryoid body for which they were re-suspended into low-attachment plates in StemPro-34 medium (Invitrogen) supplemented with L-glutamine, ascorbic acid, transferrin, and monothioglycerol. For the further direction of cell differentiation toward the cardiovascular lineage, the basal media was supplemented with bone morphogenetic protein 4 (BMP4, R&D) for 24 hours followed by BMP4, 6 ng/ml Activin A (R+D), and BFGF for 3 days. On day 4 of differentiation, the embryoid bodies are to be dissociated into single cells using trypsin and seeded onto Matrigel-coated plates at a density of 105 cells/cm2 in StemPro. The medium needs to be changed every 3–4 days thereafter until day 14 of differentiation [7].

At days 14 of differentiation for hESC-CMs, cultures are heat shocked with a 30-min exposure to 43°C media, followed by a return at 37°C in fresh media supplemented with cyclosporine A. One day later, cultures underwent a 1-hr pretreatment with Y-27632 (Rho-associated kinase inhibitor; 10 μM) and then were harvested with 0.25% trypsin/0.5 mM EDTA (Invitrogen). After being washed with DMEM/F12 supplemented with DNase (Invitrogen, 100 U/ml), 10 × 106 cells were suspended into a syringe that consisted of growth factor-reduced Matrigel in basal StemPro-34 medium (50% vol/vol), supplemented with L-glutamine, ascorbic acid, transferrin, monothioglycerol, cyclosporine A (200 nM, Wako), and Y-27632 (10 μM) [7].

B. Human induced-pluripotent stem cell-derived cardiomyocytes

iPSC colonies can be differentiated into functional cardiomyocytes using a variety of methods, which are very similar to those traditionally employed to produce cardiomyocytes from hESCs as they are both very similar in characteristics and differentiation potential. Currently, the most common method of generating cardiomyocytes from iPSCs is the embryoid body (EB) differentiation system which coaxes the iPSCs to differentiate into the cardiac lineage [8].

C. Adipose tissue-derived mesenchymal stem cell

Human adipose tissue has been developed as a novel source for multipotent stem cells and is considered more suitable in regenerative therapy. Their primary benefit is that they can be harvested quickly and repeatedly using a minimally invasive process. ADSCs can be differentiated from tri-germ lineages into different cell types, comprising, for example, osteocytes, adipocytes, neural cells, endothelial vascular cells, cardiomyocytes, beta-pancreatic cells, and hepatocytes.

Intriguingly, immunosuppressive properties and low immunogenicity characterize ADSCs. Collagenase digestion, accompanied by centrifugal density gradient separation, is the most commonly used technique to separate ADSCs from fat tissue. ADSCs exhibit a spindle-shaped morphology in vitro and lack the intracellular droplets of lipids. Isolated ADSCs are usually expanded with a basal medium containing 10 percent fetal bovine serum.

ADSCs display a stem cell-specific combination of surface markers, such as CD90, CD105, CD73, CD44, and CD166, and lack the expression of hematopoietic markers CD45 and CD344, close to MSCs extracted from the bone marrow [9].

D. Bone marrow-derived mesenchymal stem cells and mononuclear cells

Despite having many sources for mesenchymal cells in the body, the most common mesenchymal cells are derived from bone marrow. They not only show positive outcomes for proliferation but also carry immunosuppressive properties [10].

To isolate MSC from BM, it is fractioned by density and is then isolated in a medium that contains fetal bovine serum. They are given two days to adhere and any cells that are unable to do so are removed. This allows the remaining successful cells to grow for a few weeks. Initially, there will be a heterogeneous adherent cell layer including fibroblast-like and small round-shaped cells, while they appear uniformly spindle-shaped after several passages in culture. Cells that manage to form sheets will undergo a reaction with trypsin to expand further. Later on, a panel of monoclonal antibodies will be used against their expressed antigen to study their phenotype [11].

Unfortunately, research has shown that if these cells are given intravenously, they will be entrapped in other organs like the lungs or spleen. At the same time, if given intracoronary, it will need a long ischemic period to ensure that cells are evenly distributed which has shown to cause myocardial necrosis as a complication [10]. This can contribute as one of the reasons its use is limited.

E. Skeletal myoblasts

Skeletal myoblasts can be found between the basal lamina and sarcolemma. Damage to muscle or any degeneration induced by diseases can act as a trigger for its proliferation. Features like the high proliferative potential observed in vitro under appropriate culture conditions, maintenance of undifferentiated status, and resistance to ischemic stress makes these myoblasts favorable to be used for repair in cardiac insults [12].

A very significant and successful clinical study published in march 2003 where skeletal myoblasts were implanted in humans was done and resulted in areas of myoblast engraftment demonstrating healthy graft morphology even though the cells were located in some cases in a large area containing a mature scar. Furthermore, there was significant angiogenesis in the graft side of a patient. However, it was done in conjunction with coronary revascularization making it difficult to accredit the functional benefits to skeletal myoblast implantation alone, and hence, cell survival becomes difficult to assess.

Skeletal myoblasts are obtained from a biopsy and separated from the connective tissue. It is then digested with enzymes on multiple occasions with trypsin, EDTA, and collagenase at 37 Celsius to release satellite cells. They are then allowed to grow in a serum containing fetal bovine serum, recombinant human epidermal growth factor, and dexamethasone. Cell densities would have to be maintained during the process to avoid any possibility of myotube formation. This results in <75% of the culture surface being occupied by cells [4]. It is then washed and preserved in tuberculin syringes along with cryopreservation. This is done to maintain the integrity of the sample until it reaches the clinic where it is warmed.

Applications of Stem Cells in Cardiovascular Medicine

1. General Applications

a. Paracrine signaling: This function allows stem cells to influence the surrounding cardiac tissue by activating various signaling pathways, without functional cell-cell contact to the host tissue. Transplanted stem cells release biologically active molecules such as VEGF, TGF-B, EGF, that promote processes of regeneration like activating tissue intrinsic progenitor cells, recruitment of cells needed in tissue repair, reducing cardiac myocyte apoptosis and neovascularization [10].

2. Bone Marrow Mononuclear Cells

a. Ischemic Cardiomyopathy

In the setting of ischemic cardiomyopathy stem cells have been studied in patients by employing injections directly into the myocardium. These studies were often non-randomized and their efficacy was denoted in a nonrandomized study of 21 patients whose areas of viable but dysfunctional myocardium were injected with bone marrow-derived stem cells. At the end of 2 months, there was a significant decrease in the region of reversible ischemia (from 15 to 6% of total myocardium) and a 6% increase in ejection fraction in the treated patients but not in controls. During follow up at 4 months, significant mechanical improvement in the injected segments was confirmed with electromechanical mapping.

Similar findings were observed in the IACT study where intracoronary infusions of cells were given. This trial had a sample size of 18 patients with a history of MI and followed a non-blinded observational study method. The controls consisted of a group that did not receive therapy (cellular). Results noted during follow up at 3 months after intracoronary injection of BMMCs were: Reduction in size of infarct by 30%, increased ejection fraction (by 15%), and increased movement velocity of infarct wall by 57%. However, there were no noticeable changes in the control group that did not receive therapy.

This concludes that if stem cell transplantation was applied in ischemic cardiomyopathy, the results were favorable in regards to bone marrow cells [13].

b. Acute Myocardial Infarction

There have been many studies done in the past to check the application of bone marrow infusions in patients with MI. TOPCARE-AMI was a trial conducted by infusing bone marrow-derived mononuclear cells into AMI patients. This research was followed up for 5 years and the end result concluded proving long term safety of intracoronary delivery of BMMNCs, as well as improvement of left ventricular ejection fraction.

Another meta-analysis study of 2626 patients also showed significant results. The infarct size and left ventricular chamber enlargement underwent significant reduction and these findings were consistent in long term follow up. Furthermore, it was deduced that BMMNC therapy reduced the incidence of death, recurrent MI, and stent thrombosis in patients with ischemic heart disease [6].

c. Chronic ischemic cardiomyopathy and heart failure

FOCUS-CCTRN was a phase 2 trial conducted in patients suffering from chronic ischemic cardiomyopathy. This trial studied the 6-month efficacy of trans-endocardial delivery of BMMNCs on myocardial function and perfusion. The results showed significant improvement in stroke volume and LVEF, which correlated with higher bone marrow CD34+ and CD133+ progenitor cell counts. These conclusions were used to derive the notion that certain bone marrow-derived cell populations may provide a greater regenerative benefit and thereby determine clinical efficacy.

Based on this the ACT34-CMI (Adult Autologous CD34+ Stem Cells) investigators conducted a double-blind, randomized, phase II clinical trial to evaluate the safety and efficacy of intra-myocardial injections of autologous CD34+ cells in patients with refractory chronic myocardial ischemia. At 6 months and 12 months, there was good exercise tolerance and reduction in angina episodes compared to the control group. This supported the idea that bone marrow cells played a greater significance in patients with refractory angina [6].

3. Mesenchymal Stem Cells

a. Immunomodulation

MSCs known for their immunomodulatory properties can influence inflammatory processes after AMI and in HF. Studies have shown that MSCs can regress the proliferation and cause apoptosis of T cells. They can stimulate the Treg cell generation and promote a phase that resolves inflammation after MI. This allows for significant wound healing. However, the function of this is highly dependent on pro-inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF-α).

Recently, Luger et al. showed that intravenous application of human BM-derived MSCs reduced the number of NK cells and neutrophils by 25 - 50% in hearts 7 days after MI which was which would avoid adverse remodeling, especially in mice with large infarcts [10].

b. Neovascularization

To replenish the damaged tissue with needed oxygen and nutrients, the process of new blood vessel formation is important. MSCs from different sources are capable of releasing pro-angiogenic factors contributing to the formation of new blood vessels. Additionally, it also showed that the release of cytokines increased capillary density. The notion was heavily backed up by a study by Timmers et al who injected conditioned human MSCs into MI pigs. Post 3 weeks, the animal showed increased capillaries in the area [10].

4. Skeletal Myoblasts

a. Resistance to Ischemia.

- Skeletal myoblasts are resistant to ischemic stress and differentiate into the myogenic lineage. Many clinical trials have been conducted using skeletal myoblasts and the results indicate the reduction of left ventricular modeling and interstitial fibrosis along with improved systolic and diastolic benefits.

b. Use in HF patients.

- The first known use of skeletal myoblasts was in a single patient with severe ischemic HF as reported by Menasche et al. The process involved implanting autologous SMs into post-infarction scar during coronary artery bypass graft (CABG) to remote myocardial areas. On a follow up conducted 5

months later, the Echo and PET scan showed that the contraction and viability of grafter scar were healthy. These benefits were also evident symptomatically in the patient.

- Another Phase I study was conducted recruiting 12 patients and using the trans-epicardial approach to deliver autologous SM. By noticing an increase in LVEF and improved viability in PET, it can be concluded that the use of skeletal myocytes would benefit HF patients via the increase in functional cell mass [12].

5. Human Induced Pluripotent Stem Cells

a. Model a disease

- Human-induced pluripotent stem cells have the ability to model diseases given their ability to differentiate into any cell type within the body. Studies have shown that patients with inherited arrhythmias, such as long QT syndrome can be studied by producing its induced pluripotent cells. These iPSCs can capture the disease phenotype and hence provide a platform for research on the pathology and investigation of different compounds as a means to discover a novel treatment. Modeling the long QT syndrome- An extensive study was done by Moretti et all to show that human-induced pluripotent cells can reiterate the exact phenotype of the disease. They found and targeted an AD inheritance of a 596 G-A missense mutation in the KCNQ1 gene which has been known to be affiliated with QT syndrome. 4 patients, 2 with LSQT1, and 2 as the control group were compared for this study. When the iPSC-CMS cells obtained from these patients were assessed via electrophysiological parameters, a prolonged AP duration and slowed repolarization velocity was observed compared to the control group. These findings are consistent characteristics of long QT syndrome and further prove that iPSC-CMs can model diseases [8]. iPSCs are patient-specific, hence they can bypass the obstacle of tissue rejection often seen in transplant procedures. This gives induced pluripotent stem cells a major advantage over other cell types [8].
- A study was done by Nelson et al where iPSCs were delivered into the myocardium of infarcted hearts in mice. This procedure was followed by ligating the LAD artery. Evidence showed that the graft provided promising results in contractility and wall thickness while also regenerating the surrounding tissues [8].

6. Human Embryonic Stem Cells

a. Gives rise to numerous differentiated cells. hESCs were found to have various advantages over other types: immortality, ability to indefinitely proliferate in culture while maintaining the undifferentiated phenotype, and the capacity to form derivatives of all three germ layers [14].

b. Drug testing

A major part of drug development is testing new products for clinical use which requires affirmation that the product does not have any significant toxicities that can result in cardiac dysfunction or arrhythmias.

Detection of potential cardiac toxicities can help pharmaceuticals save millions of dollars and promote the use of these funds towards beneficial clinical trials. To validate this notion there is a high demand for human model cells, whether healthy or damaged. Here hESCs come into play as recent studies have demonstrated that hESC-CMs allow a great opportunity for electrophysiological drug screening since the cells are tolerant to drugs that are cardiac or not [8].

7. Adipose Tissue Cells

a. When ADSCs were used for studies in animal models they showed improvement in cardiac function and repair. Some of the improvements included successful differentiation into cardiac tissue. There were also paracrine effects such as angiogenesis, recruitment of local cells, reduction in fibrosis, and less induced apoptosis. This helped the infarcted tissues to revascularize and prevent cell death [15].

b. Recent studies have shown that NRGI- Neuregulin-1, an endothelial-derived factor, can synergize with the ADSCs in cardiac repair. These suggest that the ADSC-NRG1 combination could be used for future clinical studies, perhaps with NRG1 expressed from the ADSCs directly, or from co-administered microparticles.

c. A great application of ADSCs was done in the APOLLO trial on patients with ST-elevation. This Phase I clinical trial was based in Spain with a sample size of 9 analyzable patients and was carried

out for 6 months after the infusion of adipocytes cells via the coronary artery. Results showed an increase in EF, better perfusion, and reduction in the infarct by half [15].

Outcome

A major problem seen in stem cell therapy was the common immunologic reaction known to any transplantation. Allogenic stem cells are known for positive outcomes in cardiac function, but the differentiation often results in immunologic rejection [16]. To provide this therapy, research was carried out to study the effects of interleukin 6 on cell differentiation. Interleukin 6 often is secreted as a normal physiological response to transforming growth factor-b. The research observed that the idea of cell differentiation caused a low level of interleukin-6 which increased leukocyte cell mediation damage ($P< 0.01$) [17]. It further proved the idea that the immunologic response may merely be due to cell differentiation rather than the cell phenotype. By defining that interleukin downregulation is one of the factors, a decrease in rejection can be obtained by restoring interleukin.

The second response of immunologic response is due to major histocompatibility classes. Sometimes, the stem cell recipients have performed antibodies against foreign HLA antigens. The use of mismatched allogeneic cells can lead to a reaction and cause graft failure. The mechanism of this reaction can simply be defined as MHC I protein binding to microglobulin beta 2 which is detected by T cells and destroyed [18]. It brought up the idea of whether the absence of B2M would avoid such a reaction. Research conducted showed that when cells were deficient in B2M, there was no mediated reaction by T cells. Moreover, the memory cells which are mediated by MHC II also aided in the rejection of graft in terms of vascularization. This embarked on an idea that if somehow major histocompatibility complexes I and II were knocked out, the cells would not be able to elicit a response. A study created a B2M/CIITA double-knockout mutation and injected the stem cells. After 8-10 days of differentiation, the cell function was confirmed by ELISA. The outcome of the result showed that there was no effect on the differentiation, but the T cell marker was significantly decreased in HLA knockout mutation in comparison to the control group [19]. Both the research brings out a major factor that if certain interleukin factors are restored, and some immune-mediated proteins are knocked out, the immunological rejection can be decreased to a great extent.

Though many factors can alter the results of stem cell transplantation, one of the main modulators is aging. There is evidence that transplant stem cells interact with neighboring cardiac myocytes and play a role in differentiation. Older individuals are often associated with cardiovascular and other diseases that would alter overall body function. Whether it is stressors like reactive oxygen species or a decline in DNA protein turnover, stem cell differentiation is highly compromised by these factors [20]. The ineffective stem cells can risk the stem cells to express senescence-associated factors. Senescence of a cell can be defined as irreversible cell cycle arrest in response to stressors. It leads to molecular and biological changes which overall promotes carcinogenesis. It also leads to the induction of cytokines and growth factors like IL-6, IL-8, and TNF-a [21]. These cytokines are also secreted by atherosclerotic patients at a higher level. The solution is increasing the expression to SIRT3. Sirtuin 3 is a mitochondrial deacetylase that reduces oxidative stress and enhances differentiation [22]. This is usually low in age-related stem cells and increasing the overall expression, promises differentiation.

The incidence of arrhythmia also brings the efficacy of stem cells to great doubt. An experiment done by Dr. Mesache et al. observed the efficacy of skeletal myoblasts in ischemic cardiomyopathy patients. Out of 10 patients, 4 patients experienced episodes of sustained ventricular tachycardia and an internal defibrillator had to be inserted to prevent the risk of arrhythmia [23]. It is said that the lack of gap junction contributes to the onset of arrhythmia. The major adhesion protein known as N-cadherin and gap junction protein called connexin 43 is expressed normally in undifferentiated myoblasts. However, after differentiation, there is evidence that they are down-regulated [24]. Many investigations done such as by Suzuki et. al have managed to increase the overexpression of these proteins and reduce the risk of arrhythmia. However, the effectiveness of skeletal myoblasts as stem cells has been so positive, that risk of arrhythmia incidence outweighs the overall cardiac function [10]. The arrhythmia incidence, although significant, can be managed by medications and a defibrillator.

Conclusions

Progress in the field of regenerative medicine has been remarkable in the past few decades which gives us a promising future in improving the prognosis of cardiovascular insults. Although certain challenges like immunological reactions, expression of senescence factors, and arrhythmia induction persist, newer technologies continue to be developed. Studies managed to show that when different types of stem cells were used to differentiate into cardiac myocytes, it showed favorable results in terms of ejection fraction, wall thickness, contractility. Furthermore, it even introduced the idea of understanding disease by replicating its phenotype and opened doors to drug experimentation on a deeper level.

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PREDICTING MORTALITY OF COVID PATIENTS WITH COMORBIDITIES

(Review Article)

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Abstract

Covid-19, an ongoing pandemic, is an emerging ground with several published studies. In general, Covid-19 infections occurring in pre-existing comorbidities stand with the greatest risk of mortality. Importantly, Age is a significant unmodifiable factor that further worsens outcomes. This paper aims to propose a prediction score for the mortality risk of Covid-19 in certain chronic conditions.

Introduction

In December 2019, in Wuhan, China, Covid-19 infections emerged as unknown pneumonia [97]. The virus's ability to spread through air droplets and a higher infectivity rate soon unfolded it into a threatening pandemic. By February 1st 2021, according to the WHO, the confirmed cases stand at around one hundred million and has claimed more than two million lives [40]. The most common comorbidities that increase the adverse outcomes are hypertension, diabetes and obesity [69]. Despite that, elderly individuals face a high risk for severe Covid-19 infection since Age-related physiological and immunological changes worsen the outcome [60]. Moreover, Age is a crucial undeniable risk factor.

This paper aims to review specific comorbidities and establish a sample scoring system for the mortality rate associated with Age and chronic conditions in Covid-19.

Cardiovascular and hypertension in covid-19 patients

Hypertension and cardiovascular diseases are among the leading comorbidities found in COVID-19 patients. To give an illustration, a nationwide study in Wuhan, China, shows Hypertension and Cardiovascular conditions' prevalence as the first and third common diseases among individuals infected with Covid-19 [31]. Based on few studies, that localized ACE2 receptor [26,75], It is evident that a vast majority of expression appears in type II pneumocytes, cardiac myocytes, GI tract, and blood vessel cells, which potentially explains the predilection of COVID-19 in the lung and cardiovascular system.

Furthermore, coexisting chronic conditions in infections are apparent to persuade worse outcomes. A study conducted in Wuhan [98] depicts the common concurrent comorbidities being hypertension (38.75%) and coronary artery disease (11.3%) in Covid-19. Despite that, the risk of mortality and adverse outcomes in concurrent diseases are still uncertain. Therefore, these vulnerable patient groups need targeted treatment and preventative strategies urgently.

Cardiovascular Disease

Cardiovascular diseases (CVD) are the number one leading cause of death globally. Nevertheless, little is known about the prognosis of CVD patients in Covid 19 infection. The study conducted on the association of CVD and non-CVD patient's prognosis in COVID-19 [55], illustrates that cardiovascular conditions potentially lead to poor outcomes in COVID-19 infection.

Mortality risk in CVD and the course of the disease can be predicted through biomarkers. Affirmatively, a study conducted in Italy [86], with 397 patients, demonstrates the elevation of biomarkers and its associated mortality. Moreover, the study based in China exemplifies that 12% of patients infected with Covid-19 experience myocardial injury [97]. Conclusively, COVID-19 infection causing elevation of cardiac markers such as troponin T and CK MB during cardiac manifestations might be justified.

The cardiac manifestations of COVID-19 can potentially be explained by the below mechanisms:

1. Myocardial damage through activation of inflammatory cytokine release

SARS 2 (COVID-19) is proposed to enter through the ACE2 receptor and causes modification of signalling response. Therefore causes increased release of cytokines and disproportionate inflammatory activation [98,53]

2. Plaque rupture

Viral infections causing inflammation and increased endothelial shear stress destabilizes plaques leading to worse complications such as acute myocardial infarction.

3. Electrolyte imbalance

Electrolyte imbalances are determined to be caused by two main reasons:

- In general, severe inflammatory reactions are self-sufficient to cause electrolyte imbalance.
- Arrhythmias due to hypokalemia through renin-angiotensin-aldosterone system interaction [17].

Notably, any of the above mechanisms can cause acute heart failure while dealing with COVID-19 patients. However, COVID -19 is a new research topic with growing information and new concepts and not definitive findings. We have data from previous viral infections and proven research studies that might relate to our ongoing pandemic.

Based on an analysis [97], 25 patients recovered from the SARS infection (2002 outbreak) after 12 years were considered. The results indicated the association between lipid metabolism disruption and corticosteroid therapy. Hence, cardiovascular patients treated with high doses of methylprednisolone should potentially need consideration for further evaluation in their later life. Importantly, drug usage in COVID-19 infections should be a meticulous process.

Side Effects of trial drugs used in treating COVID-19

Hydroxychloroquine and chloroquine: Widely used antimalarial drugs such as hydroxychloroquine and chloroquine pose a risk of QT prolongation and Torsades de pointes [29,85]. Hydroxychloroquine could increase beta-blocker bioavailability and should be used cautiously.

Antiviral drugs: Lopinavir/ritonavir used as a treatment can potentially cause QT prolongation and potentially cause arrhythmias [14]. Combination with other drugs with similar effects should be avoided.

A combination of drugs such as azithromycin with hydroxychloroquine and or azithromycin with antiviral medication might be detrimental, since azithromycin causes QT prolongation as listed in source – FAERS (FDA Adverse Event Reporting System).

The management plan for pre-existing cardiovascular patients during COVID-19 infection is under evaluation. **Figure 1**. Depicts the proposed treatment algorithm [19].

Biomarkers such as Troponin T, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and brain natriuretic peptide BNP can help diagnose cardiac complications. N-terminal proBNP elevation can help us determine cardiac wall stretching [73]. Elevated biomarkers do not always relate to cardiovascular complications but support the cardiovascular system's involvement and support the clinical diagnosis. **Figure 2**. Biomarkers in COVID-19 [57].

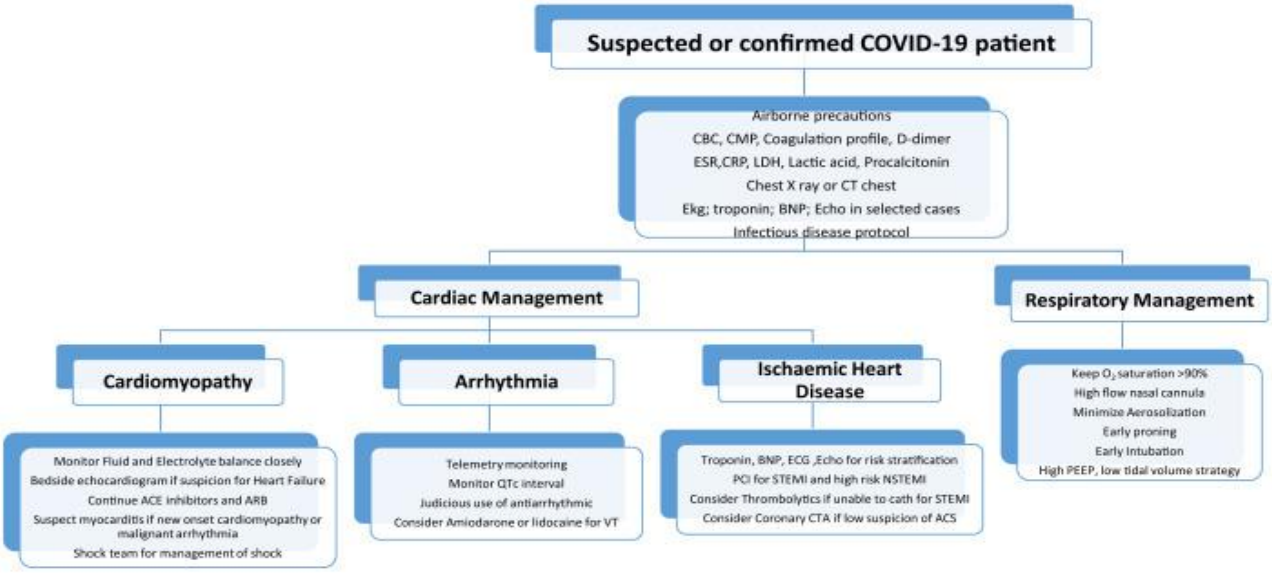
Moreover, Troponin T is mainly being considered as an essential biomarker since it indicates direct myocyte damage.

Troponin T

- Potentially could be used in intensive care units (ICU) to support clinical judgement [36].

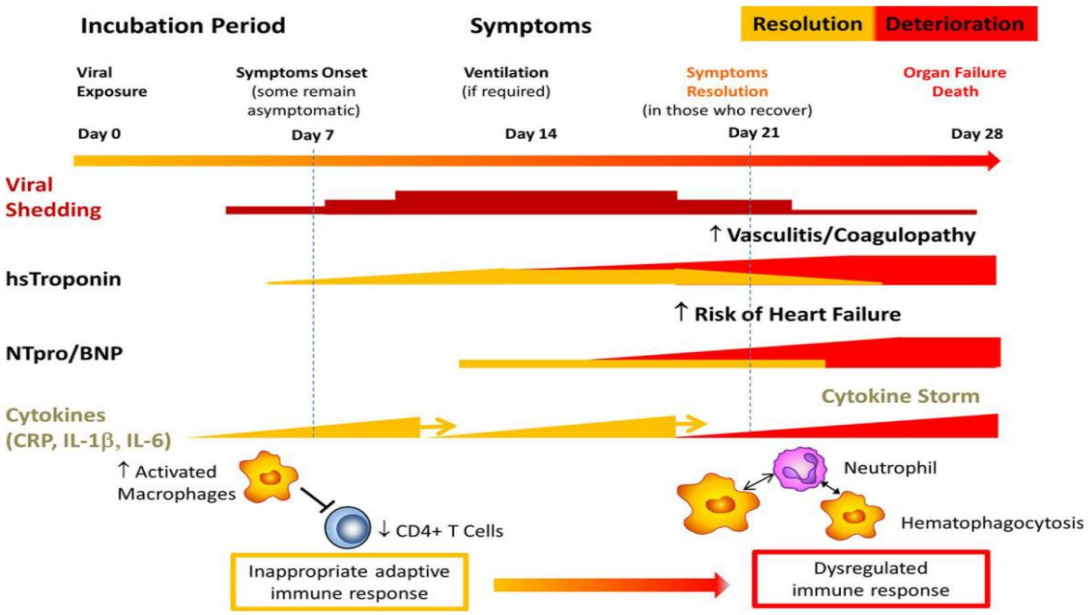
- Elevation in cardiac events. TnT, based on a study [93], signifies the elevation in myocardial injury.
- By contrast, Troponin might be an independent risk factor for mortality, as illustrated in a study, conducted at Mayo clinic [6].

Figure 1. COVID-19 treatment algorithm[19].



Abbreviations: CBC, complete blood count; CMP, complete metabolic profile; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; LDH, lactate dehydrogenase; CT, computed tomography; EKG (also ECG), electrocardiograph; BNP, NTpBNP, N terminal pro brain natriuretic peptide; Echo, echocardiograph; VT, ventricular tachycardia; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; NSTEMI, non ST-elevation myocardial infarction; CTA; computed tomography angiography; ACS, acute coronary syndrome.

Figure 2: Biomarkers in COVID-19[21]



The mortality rate in cardiovascular

With this in mind, a study [33] consisting of 187 individuals had a mortality of 43; among them, patients with comorbid CVD with elevated TnT had the highest mortality rate of 69.4% and elevated TnT but without CVD was 37.5%, indicating cardiac injury being the predominant cause of fatality. Pre-existing cardiovascular conditions intuitively might weaken the body's reserve to fight the occurring disease. The data to predict the mortality rate and analyze the exact mechanism leading to adverse events such as myocardial infarction and myocarditis is far from definitive. Patients with cardiovascular conditions might need aggressive therapy and careful monitoring during the disease.

Hypertension

Hypertension, a medical condition that elevates blood pressure, significantly increases the risks of heart, brain, kidney, and other diseases. Neither uncontrolled blood pressure being a risk factor nor controlled individuals having reduced risk in Covid-19 are unclear. Nevertheless, hypertension is a pivotal chronic condition to research further. Recent studies on Angiotensin-converting enzyme inhibitor (ACE inhibitor) and Angiotensin receptor blocker (ARBs) drug usage in hypertensive patients are arguable.

Renin-angiotensin-aldosterone system and ACE inhibitor/ARB's usage in Hypertension

Studies propose SARS-CoV-2-S(COVID-19) has 80% similarity in spike protein as SARS COV1[38], and speculated to enter through ACE2 receptors [103] during the early phase. The anticipated outcome of severe COVID -19 infections through the usage of antihypertensive drugs are under investigation. ACE inhibitors and ARBs may potentially cause an upregulation of ACE 2 receptors [47]. Chronic usage might increase the viral entry and thus produce a severe infection. That said, these mechanisms are still theoretical from animal studies and not validated in humans. In view of above findings, switching drugs in hypertension may result in effective reduction of viral entry in the near future.

On the other hand, a pathway called ACE-2-Ang-(1–7)-Mas axis counteracts the inflammatory reaction and possibly reduces the detrimental effect [80]. This proposed mechanism is due to the long term administration of the above-said drugs as well. Nonetheless, A study based in Wuhan Central hospital, consisting of 1178 patients, has provided no association between usage of ARB/ACE inhibitors and severity in hypertensive patients [54].

Theoretically, the discontinuation of ACE inhibitor /ARB's drugs might decrease the above said ACE 2 upregulations. But an aftereffect might scale down the preferred ACE-2-Ang-(1–7)-Mas and the resulting anti-inflammatory pathway. Above all, discontinuing life-saving drugs, i.e., ACE inhibitors/ARBs in a vast majority of comorbidities, could be harmful. In summation, whether ARB/ACE inhibitor's usage aids the patient through anti-inflammatory properties through ACE-2-Ang-(1–7)-Mas or promotes upregulation of the ACE 2 receptors causing a severe infection is uncertain.

Moreover, RAAS activation causes vascular complications in many comorbid conditions such as hypertension and diabetes. Given that the most severe forms of COVID-19 infection have resulted in hypertensive patients, long standing RAAS activation potentially links to the pathogenesis of the COVID-19 virus.

Association of severity in Blood group

The relation between ABO blood groups and Hypertension's severity observed in a study [77], signifies that AB blood group (Non-O blood group) vs O group individuals are likely to be associated with:1) Elevated Pro-thrombotic value, 2) Increased rate of death, 3) Increased risk of cardiac injury.

As far as we know, hypertensive patients' deterioration rate is far more significant than other comorbidities, as illustrated in this research which contemplates using the Kaplan-Meier survival curve and insists on a mortality increase in Hypertension [22]. Moreover, several kinds of research indicate the harsh reality about Hypertension causing catastrophic outcomes.

Plasma(ogen)

The plasminogen is theorized one of the common risk factors to increase susceptibility in comorbid individuals [46]. The study illustrates the plasmin role, potentially causing enhanced virulence and infectivity.

Repurposable drugs

The primary objective currently should be to spotlight the management methods followed worldwide and to reduce mortality. Newer drug regimes through analysis are currently ongoing.

Based on previous reports, testing alternative drugs such as carvedilol [82] and verapamil [46] are promising. Carvedilol is illustrated to reduce ACE up-regulation [74], and thus used as a potential drug in Covid-19. Previous studies have suggested that it could potentially reduce inflammation in myocarditis induced by viral infections.

Drugs that are under investigation:

1) Canakinumab [16] - a monoclonal antibody that targets IL-1β explicitly. Thus, it might be beneficial to decrease Th1 mediated cascade.

2) Roflumilast [92] - PPE-4 inhibitor. Proven effective in COPD patients.
3) Drugs targeting Spike protein.

Diabetes and obesity

The prevalence of Diabetes and Obesity in the world make these crucial comorbidities when approaching patient care in the COVID-19 pandemic. Knowing what burden these diseases bring and how they influence a patient's prognosis and overall health can be enlightening. With enough serious research, structuring an integrated, unique prediction medium is not without benefits. Though they are often hand in hand, our review will include the individual factors of Diabetes and Obesity in light of a COVID-19 positive victim.

Diabetes

Some might argue diabetes is a pandemic in and of itself in the world today. The past documentation of diabetic patients proves it has walked the earth with us for some time, though Diabetes Type 2 is more present now than ever. Due to the many stricken by this pathology, numerous studies and treatment regimes have already been implemented with great success. Despite this, there is no cure to this modern ailment. Now enter SARS CoV-2, the new pandemic on block, and we might have to view diabetes in a new light.

COVID-19 goes with comorbidities like winter and the flu. Their combination in exacerbating a patient's condition and causing mortality belies the true meaning of the word synergy. In a study identifying the main proponents of associated comorbidities with COVID-19 cases, diabetes revealed itself to be a front contender, though the authors do admit the lack of concrete data prevents them from figuring out to what extent [88]. Other researchers propose that both were an 'unholy interaction of two pandemics' because of their effect on one another [64]. Diabetes increases the chance of and worsens the prognosis of a SARS CoV-2 infection. Likewise, the reaction to and treatment for coronavirus rapidly deteriorates the glucose control of a diabetic [15].

In order to understand how diabetes affects the prognosis and risk of morality in a COVID-19 patient, the pathophysiology as well as the mechanisms of each must be considered. At the same time, past outbreaks of similar microbes may also be referenced for more calculated conclusions.

In general, SARS CoV-2 enters the cell through surface S-glycoprotein attachment to host cell ACE-2. ACE-2 is expressed in many cells throughout the body, including type 1 and 2 alveolar epithelial cells in the respiratory system [81]. Cells expressing the same entry point include pancreas, intestinal epithelium, rental tubular epithelium, cardiac cells and vascular endothelium.

Once entry has been achieved, inflammatory responses recruit T helper cells and other inflammatory cells. This causes a cytokine storm leading to multi-organ dysfunction and failure. Assuming the patient has no other conditions that can contribute or negate, this is the usual course of the infection.

Now add in diabetes. So far, the research that is out there found that the increased risk and severity of SARS CoV-2 seen in diabetic patients can be attributed to:

1. ***Increased ACE-2 Expression***
   - One study identified increased expression of ACE-2 in renal, liver and pancreas [71].
   - Diabetic patients expressing more ACE-2 might be predisposed to SARS CoV-2.
2. ***Increased Furin***
   - Though ACE-2 is the entry receptor for SARS-2 CoV-2, a pro-protein membrane-bound protease contributes to coronavirus entry into the cell.
   - Diabetics have been found to have more furin [24].
3. ***Impaired immune functionality***
   - Past studies from the MERS-CoV show that diabetic mice experienced a delay in inflammation and immune response.
   - Likewise, decreased amounts of lymphocytes were seen in COVID-19 patients [51].
4. ***Increases in Interleukin-6 (IL-6)***
   - IL-6 is found to be increased in COVID-19 infection as well as in non-COVID-19 patients diagnosed with diabetes.
   - Synergistic effect upon prognosis is a possible method of severity [58].
5. ***SARS infection causing hyperglycemia***

- In a 2010 study, patients with SARS infection were seen to have hyperglycemia with no history of diabetes.
- Further investigation revealed that SARS could bind to pancreatic islet cells, damage them and cause an acute diabetes [101].

6. Anti-diabetic drugs

- Thiazolidinediones (TZD) was seen to increase the risk of pneumonia [29].
- Pioglitazone [102] and liraglutide [71] were found to increase ACE-2 expression.
- DPP4 inhibitors have been associated with increased risk of upper respiratory infection, but more data is needed to correlate that risk with COVID-19 and diabetes outcomes [43].
- Similarly, in cases of diabetic nephropathy or macrovascular disease, ACE/ARBs, statins, calcium channel blockers and aspirin require more evidence before contraindications can be prescribed [81]

Though there is still more to be discovered, these factors influence the outlook of SARS CoV-2 infection in diabetes. This is of course not including the various acute and chronic complications faced in diabetes, each of which could add exponential risk upon the patient depending on how well controlled their diabetes is found to be. Those with progressive, uncontrolled diabetes have higher incidences of HTN, cardiovascular disease and the like. At the same time, having only diabetes with COVID-19 by no means reduces from any potential risk of adverse events. In fact, a study found COVID-19 patients, without any other comorbidity other than diabetes, to be at increased risk of a myriad of critical complications [34] including:

1. Severe pneumonia and ARDS
2. Release of enzymes related to tissue injury
3. Uncontrolled and excessive inflammation responses (due to increases of inflammatory-related biomarkers),
4. Increased hypercoagulable state
5. Dysregulation of glucose metabolism
6. Greater need of tracheal intubation
7. Associated death within 7 days

Yes, these complications look dire and it is exactly why diabetes increases the death rate in COVID-19 patients [1]. Taking it further, what's interesting is these mechanisms and complications are surprisingly similar to the findings of past viral infections. If we compare past data, this allows us to gather further insight into the true risk of diabetes amidst infectious pathologies:

1. Diabetes and associated plasma glucose levels were an independent risk factors in 2002-2003 outbreak of Severe Acute Respiratory Syndrome (SARS-CoV-1) [100].
2. 'Diabetes increased likelihood of medical consultation and risk of death due to influenza' according to researchers studying the Influenza A outbreak of 2009 [3].
3. Patients with underlying diabetes were associated with life-threatening severity and a 35% mortality in the sudden appearance of 2012's Middle East respiratory syndrome coronavirus (MERS-CoV) [7].

As past studies of similar outbreaks (e.g., SARS [100]and H1N1[8]) show, glycemic control could be a predictor of a patient's course and status in regards to COVID-19. This can prove to be arduous as blood glucose readings usually require a bedside manner differentiated from current regulations of lockdowns and social distancing. Those not critically ill may circumvent this obstacle with self-testing and telecommunication with their care provider, but not all have this luxury. The prospect increases if the patient is elderly, lives alone and suffering from physical, mental or emotional afflictions. What's worse, the acute complications of diabetes (namely DKA and HHNS) are both precipitated by stress and improper insulin management. Inadvertent or forgetful management of medication concurrent with an exposure to coronavirus could be the perfect storm that deteriorates a diabetic patient's health before word gets out. All and all, diabetic patients are facing an all too different face of the COVID-19 pandemic. Quantifying the risks and mortality for patients could arm medical providers with a realistic and sure approach to preserve the lives of those in their care.

Obesity

Obesity, or excess of adipose tissue due to imbalance of food intake and energy expenditure, is also a factor that can't be ignored and is very similar to diabetes. What's worse, both are seen in old and younger generations thanks to the rising tide of adolescent obesity [63]. The wide prevalence throughout the world has been partly attributed to the nutrient values of everyday diets, those high in saturated fats, sugars and refined carbohydrates [11]. These more available, yet unhealthy foods leave many at risk of the current COVID-19 pandemic situation. In fact, obesity was found in a New York study to be one of the leading clinical features amongst 4,103 patients being treated for COVI-19 [68]. Furthermore, a separate study conducted with 1,317 diabetic participants hospitalized for COIVD-19 in France associated the link of obesity with severe outcomes. Patients who required mechanical ventilation via tracheal intubation and/or experienced death within 7 days of admission were more positively associated with increased BMI rather than glucose control [15]. This leads us to believe that obesity, often analogous to diabetes, is in a class all its own as seen in the high risk of mortality is associated with COVID-19 infection in patients with obesity [42].

Charting out the mechanisms of increased risk in obesity and COVID includes:

1. *Increase risk of other risk factors*
   - Increased fat mass was causally related to hypertension, diabetes mellitus, coronary artery disease, stroke, atrial fibrillation, renal disease, and heart failure [78].
2. *Impaired immune response*
   - Similar to diabetes, obese individuals have an altered immune function perhaps due to the metabolic and nutrient from insulin resistance and reduced beta-cell function [61].
   - SARS CoV-19 causes a 'cytokine storm', further weakening the immune system [50].
   - Fat depots have also been linked to disruption of immune cells, preventing interactions, survival and proliferation [84].
3. *Increase thrombotic complications and endothelium imbalance*
   - Amongst COVID-19 infections, risk of disseminated intravascular coagulation and venous thrombosis have been associated [91].
4. *Decreased respiratory performance*
   - Decreased lungs volumes (forced expiratory, forced vital capacity, etc) hamper the recovery period of obese individuals [78]
   - Furthermore, obesity decreases lung compliance and hinders normal diaphragm movement [20].
5. *Increased inflammatory status*
   - Like diabetes, interleukin-6 and C-reactive protein levels are increased.
   - This could be on account of adipose tissue being highly inflammatory, with increased expression of cytokines (eg, TNF-alpha, IL-1, adipokin) [87].
   - Increased oxidative stress is also a contributing factor [12].
6. *Difficulty in receiving healthcare*
   - Medical providers often aren't able to properly perform examinations and treatments for obese patients, such as performing imaging and overall handling [24].
7. *ACE-2 expression in adipose tissue*
   - Adipose tissue is generally found to have more ACE-2; its accentuated presence in obesity allows further ease of of SARS CoV-2 infection [76].

Smoking and Respiratory Comorbidities:

As COVID-19 is a respiratory disease by itself, Smoking and COPD play a role in the prognosis of affected patients. Smoking cannot be called comorbidity but is the primary cause of an array of respiratory conditions, including COPD. So we have to look into smoking as a separate condition rather than a cause of several respiratory problems. Respiratory Failure is the primary cause of death in patients affected with SARS-CoV-2, patients with preexisting respiratory problems, such as COPD, have a higher risk of mortality. Our review will attempt to explain the effect of these conditions in a patient affected with COVID- 19.

Smoking

There are more than 1.2 billion smokers worldwide [95]. Though smoking is not a comorbidity, it is the primary cause of a variety of lung problems. So therein lies the novelty to explore this topic further. ACE 2 is the receptor used by SARS-CoV-2 to enter the host cell [38]. A study conducted by Leung et al. [52] revealed that there is a higher expression of protein ACE 2 in the small airway epithelia of smokers than in nonsmokers. They concluded that this might predispose smokers to an increased risk of COVID-19 infection. But a review study conducted by Marco et al. [72] says that current smoking is not a risk factor for neither acquisition nor severity of COVID-19. Two other studies [32,56] support the conclusion of the study by Marco et al. [72]. Thus, it is safe to assume current smoking may not be a problem, but that does not mean smokers are not at risk.

Since the primary cause of death in COVID-19 is respiratory failure, smoking is the most common cause of lung damage. An array of lung problems have their effect on the prognosis of COVID-19 infection. Since the damage accumulates over a long period, it is almost certain that smokers who have smoked for a long time have an increased risk of progression if they get infected by SARS-CoV-2. A meta-analysis conducted by Patanavich R and Glantz A [66] supports this theory by concluding that "smoking is a risk factor for the progression of COVID-19, with smokers having higher odds of COVID progression than never smokers". To conclude, we can say that current smoking does not increase the rate of mortality, but the damage of lungs and increased ACE 2 expression due to smoking puts patients at a higher risk of progression of COVID- 19 infection. Thus it becomes a high risk when the patient has a history of smoking.

Respiratory Comorbidities

COVID-19 is a respiratory infection, which causes a great deal of damage to the lungs. If a patient already has damaged lungs, it makes the prognosis of COVID-19 even worse. There are a lot of conditions that can damage the lungs. Two of them are COPD, and Asthma. In this review, we will look into these specific conditions as comorbidities.

COPD

SARS-CoV-2 is transmitted mainly by respiratory droplets. The virus uses ACE 2 protein in the respiratory epithelium as the entry point. A study [52] shows there is more ACE 2 gene expression in patients with COPD than patients without COPD. Cai et al. [13] found out that there is increased ACE 2 gene expression in COPD patients to smokers. Another study by Smith et al. [83] concluded that ACE 2 expression was higher in the whole lung tissue of COPD patients. From these studies, we can infer that their COPD patients have increased ACE 2 expression, which is a receptor used by SARS-CoV-2 to enter the host cell.

All these studies focus on the relationship between SARS-CoV-2 and ACE 2 in COPD patients, but we should also consider that COPD patients are already predisposed to respiratory infections. This could be because of decreased type 1 interferon production [39] or immunosenescence because of increased exhausted T cells and reduced memory T cells [27,48]. When we put all this together, we can say the prognosis of COPD patients is worse than a previously healthy patient.

There is a report of endothelial cell dysfunction in COPD patients, which results in increased apoptotic endothelial cells [49]. Increased permeability of airway microvasculature is also related to airflow limitations in COPD patients [62]. These show that COPD patients are susceptible to vascular injury. There is an increased amount of circulating coagulation factors in COPD patients [41]. This amount further increases during exacerbations [90]. COVID-19 infection will be the cause of exacerbation here. These studies show COPD patients are prone to coagulopathies. It also explains why there is an increased occurrence of Pulmonary embolism in COPD patients [2]. Considering all these studies, we can say that COPD patients are at higher risk of mortality.

According to a few studies done in China and America [4,32,59], the prevalence of COVID-19 in COPD patients is around 2-3%. A meta-analysis of 11 case series [5, 32, 59, 67, 69,18, 89] by Andrew et al. [38] showed an 88% increase in the risk of ICU admission or death in COVID-19 patients with COPD. Moreover, it also showed a 45% higher chance of developing severe complications. We cannot take this data at face value because most patients in these studies with COPD were also of age greater than 60. Age is one of the non-modifiable risk factors and comorbidity for the prediction of the outcome in COVID-19

infected patients. An Italian study [9] reported that patients with COPD were at a higher risk of severe respiratory failure. A Spanish study [89] observed a 70% increase in the risk of death among COVID-19 infected patients with COPD. A cohort study by ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) [21] with a sample of over 20000 showed chronic pulmonary diseases are associated with increased risk of death.

Thus with compiled information, we can say that COPD increases the risk of mortality in patients with COVID-19. It is also crucial to note that many of these comorbidities can appear together in a single patient. Though COPD may not increase the rate of mortality as much as hypertension or obesity, it plays a role significant enough to notice.

Asthma

It is safe to say that COVID-19, unlike many other respiratory infections, did not affect patients with asthma to the same extent. A study by José et al. [44] with a sample size of over 70000 concluded that significantly fewer patients with asthma are affected by COVID-19 than other comorbidities. It also concluded that the use of Inhaled Corticosteroids (ICS) showed a safer profile, implying the protective effect of ICS against SARS-CoV-2 infection. ICS may prevent SARS-CoV-2 entry because it can downregulate ACE 2 [25,67]. In these studies, it is found that ACE 2 gene expression is lower in the sputum of COPD and Asthma patients using ICS.

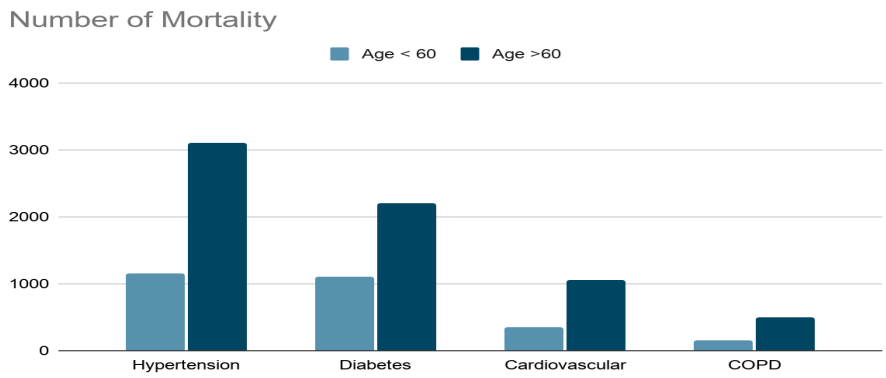
But a literature review by David et al. [35] concluded that there is no evidence of benefits or harms of ICS in COVID-19. An observational study from London [79] found increased mortality in COPD and Asthma patients using ICS. But a sensitivity analysis showed that the cause of mortality was simply due to the severity of the disease itself rather than ICS use. So the conclusion by David et al. [35] and José et al. [45] still stands correct. With this information, we can conclude that asthma will not play a significant role as comorbidity when predicting the mortality of COVID-19 infected patients.

Prediction of mortality

By understanding the interactions of the major comorbidities with COVID-19, we can appreciate how a predictive mortality score method would be beneficial to healthcare providers. Each patient presents a unique challenge and therefore requires a personalized approach. This, however, is difficult in a pandemic situation where clinicals and wards can be easily overwhelmed. By calculating the risk of mortality for patients with SARS-Covid infection, both triage and focused care can be acceptably achieved in any part of the world.

Generating a predictive mortality method still calls for applicable and accurate data to the geographical locations of patients. What comorbidity afflicting one nation will not always align with another nation's. A pertinent example can be seen in approaches by 2 different studies, one based in Wuhan, China[2] and another in Mexico [10]. Each had different statistics in terms of mortality/recovery rate, coexisting disorders and other differential data. But they were able to formulate predictive information to assist future, similar cases. We believe what they achieved was not only innovative and admirable, but also reproducible for all areas of the world.

As an example, we formed a similar approach using figures from West Bengal who diligently assemble and update their demographic data daily [95]. Due to lack of info we were unable to include Obesity and Smoking as fatal cofactors, though this doesn't eliminate their significance to a patient's prognosis. Below we surmised this data into a bar graph about mortality based on age, which statistically is already known to be the greatest modifier, and comorbidities.



Then we created a table for a predictive score report based on age range and applicable chronic disorders. A higher value proportionally infers that this patient is at a higher risk compared to others whose score is less.

Mortality Prediction Table (based on West Bengal data)	
Comorbidities	**Predictive Score**
Age >60	+2
Age <60	-2
HTN	4
Diabetes	3
Cardiac Disease	2
COPD	1
Mortality Predictive Score	
Low risk	0-3
Medium Risk	4-6
High Risk	7-9
Very High Risk	≥10

Conclusion

COVID-19 is a pandemic we can't ignore, especially when afflicting patients with compromising chronic illnesses. Cardiac Disease, HTN, Diabetes, Obesity, Respiratory Disease and Smoking are amongst the considerable coexisting conditions that threaten the prognosis of victims of SARS-CoV-2. We have included the pathways of how they interact with the virus and a solution of structuring a method of predicting mortality risk to give doctors a practical tool. This method was simulated from cited analyses conducted in Wuhan and Mexico respectively.

Unlike the many studies we cited in our investigation, our goal was to create a unique, world-wide predictive score report for determining mortality and adverse events in COVID-19 patients with comorbidities. Our research draws upon their contributions, of which we give them full credit and accolades. This study was compiled in an attempt to present a simple and universal tool for medical providers to apply in their own idiosyncratic situation. We understand that our own predictions are indeed fallible, due to inability to collect on-the-ground statistics as well as observing this data through the lens of ignorance. Despite that, we hope this score sheet allows healthcare experts with the means to make verdicts regarding the health of those in their care.

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GUT-MICROBIOME-BRAIN AXIS AND ITS INFLUENCE ON PARKINSON'S DISEASE

(Review Article)

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Abstract

The relationship between the nervous system and microbiota opened up research opportunities that can significantly change the management of several neurological disorders. The discovery of the microbiota-gut-brain axis helped us understand how the information is relayed between the brain and the enteric nervous system. This connection demonstrated how, in Parkinson's disease, the alpha- synuclein accumulates in different organs, resulting in a wide array of symptoms. Moreover, pathways such as the hypothalamic-pituitary axis, the neuroinflammatory pathway, and neuroactive molecules are associated with the pathophysiology of PD. The manifestation of gastrointestinal symptoms and altered gut microbiota have been noted in patients several years before other significant clinical symptoms appeared. The role of alpha-synuclein accumulation and environmental factors that disrupt the natural flora of the gut in the overall progression of PD has been observed through well documented clinical studies on mice. With the current knowledge that has been established, the alteration of gut microbiota using recently explored treatment options such as probiotics, fecal microbiota transplantation, dietary changes, and certain antibiotics can prevent the progressive symptoms of Parkinson's disease.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that is a consequence of the loss of dopaminergic neurons in substantia nigra pars compacta. More than 10 million people suffer from PD worldwide, and about 4 percent of them are diagnosed before the age of 50. Men are1.5 times more likely to develop the condition, with the likelihood increasing with age [18].

The genetic predilection plays an important role in the development of PD in conjunction with environmental factors. Mutations in LRRK2 Gene can be found in as much as 2% of the PD population in certain ethnic groups such as North African Arab Berber, Ashkenazi Jews, and Basque groups with a prevalence that is far higher than the general population (Common Genetic Mutations, 2021). There are more than 20 different mutations that can occur within this gene, the most common one being G2019S. Mutations in the GBA gene account for about 5-10%, with more than 380 different mutations already discovered. The specific GBA1 mutation has been linked to a large accumulation of alpha-synuclein clumps. Mutations in the SNCA gene can lead to increased production of alpha-synuclein which can be toxic and accumulate in the brain [17].

Several environmental factors have been linked to the development of PD, including head trauma, exposure to metals, industrial solvents such as Trichloroethylene (TCE), and Polychlorinated Biphenyls (PCBs). The herbicide Paraquat has been largely linked to PD and banned in many countries [19]. Moreover, the area of livelihood and occupation has factors that link to PD. Several vascular events have also been linked to PD.

People living with PD experience a variety of symptoms, including movement disorders such as bradykinesia, tremor, rigidity, dyskinesia, sialorrhea (drooling), hypophonia (soft speech), and micrographia (small handwriting). They can also develop neuropsychiatric nonmovement disorders such as decreased attention span, difficulty with planning, language, and memory which can progress to dementia. They can also suffer from psychiatric disorders such as depression, anxiety, apathy, sleep disorders, hallucinations, and delusions. Gastrointestinal symptoms include constipation and early satiety. Additional symptoms include lightheadedness, sexual dysfunction, loss of taste and smell, vision problems, urinary incontinence, and weight loss. These manifestations happen due to the loss of neurotransmitter dopamine which is largely responsible for motor movement, pleasure, and emotional response.

Histopathologically, PD is characterized by the deposition of Lewy bodies, an intracellular eosinophilic structure, composed of a misfolded protein called α-synuclein which accumulates in the basal ganglia, particularly in the caudate nucleus and the putamen. Lewy bodies become cytotoxic and damage

brain cells, especially substantia nigra pars compacta in the midbrain which results in a defect in the thalamic relay to the cerebral cortex. This loss of dopaminergic neurons leads to the symptoms of PD.

Normal Gut Microbiome

Microbiota refers to the entire population of organisms that colonizes a particular location. The gut microbiome is a collective population of bacteria, viruses, protozoa, fungi, other microorganisms, and their genetic material present in the gastrointestinal tract. The relationship between gut flora and the human body is a mutualistic one. The main four phyla in the gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. The composition of these species varies across the digestive tract, playing different roles, including nutrient and mineral absorption, production of enzymes, vitamins, and amino acids, and synthesis of short- chain fatty acids. The normal gut microbiome has a significant impact on the metabolism, immune function, neuroendocrine responses, and intestinal-barrier function [9]. The overall effectiveness of the microbiome has a significant role in the gut-brain axis, hence, playing a crucial role in the pathophysiology of several neurodegenerative disorders [5].

The Gut-Brain axis

The discovery of gastrointestinal diseases associated with different mental health conditions has led to the acknowledgment that there is not just an information pathway from the brain to the gut but also vice versa. This resulted in the establishment of this bidirectional pathway called the gut-brain axis [3].

The communication between the gut and the brain involves several systems including the central nervous system (CNS), enteric nervous system (ENS), autonomic nervous system with both parasympathetic (PNS) and sympathetic (SNS) branches, the endocrine and the inflammatory system [18]. It also includes neurotransmitters and neuroregulators by the bacteria and barriers like the blood-brain barrier and the mucosal barrier in the intestine [11]. The brain interacts with the gut through CNS to regulate its motility and its secretory and sensory functions whereas the gut interacts with the brain through the neuroimmune and neuroendocrine pathways, activating the vagus nerve which then will send signals to the brain [14].

Enteric Nervous System

The gastrointestinal tract, from the esophagus to the anus is innervated by the ENS. Any change in the population of the gut microbiome causes the nerves to change the neuronal physiology and neuronal gene expression to adapt to it. This shows that ENS is plastic, which means that it can sense and react to the changes in the microbiome which makes it an integral part of this axis [14].

HPA Axis

The HPA (Hypothalamic-Pituitary-Adrenal) axis is another form of communication in the gut-brain axis. Its main function is to respond to stress but it also controls digestion, the immune system, mental health, sexuality, and energy transfers [14]. When there is stress the paraventricular nucleus (PVN) of the hypothalamus releases corticotropin-releasing factor (CRF) which acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH) which then acts on the zona fasciculata of the adrenal glands to release cortisol, this redirects the blood to major organs like the brain, heart and also the major limbs through the SNS which means that the GI tract receives less blood for its functions [11]. In germ-free mice, the HPA axis is seen to be hyperactive along with alterations in the hippocampal NMDA and 5-HT1A receptor mRNA expression which are known to regulate the CRF release. Furthermore, vagal stimulation studies done on rodents showed an increase in CRF mRNA expression and an elevation in ACTH and corticosterone levels in the plasma which shows that there is an interaction between the HPA axis and the vagus nerve [4].

Neuroinflammatory pathway

The gut microbiome also interacts with the brain through the neuroinflammatory pathway but this interaction is species-specific [14]. When there is any kind of stressor or any pathologic state, the gut permeability increases either through leak and pore pathway in the tight junctions or through unrestricted pathways through apoptotic leaks which results in the bacteria or its toxins entering the mucosa and the bloodstream, this is known as leaky gut [14]. This activates the pro-inflammatory factors like cytokines

(IL-1, IL-6, TNF-α) to activate the HPA axis through receptors [3, 4] Therefore the neuroinflammatory and the HPA axis goes hand in hand to crosstalk with the brain. The immune system's hyperresponsiveness can also lead to the inflammation of the CNS and can increase the permeability of the BBB through LPS [15,16,24].

Neuroactive molecules

The enteric bacteria produce many neuroactive molecules like serotonin, catecholamines, glutamate, γ-aminobutyric acid (GABA), and short-chain fatty acids (SCFAs). Some reports show that these bacteria can modulate the level of neurotransmitters through TLRs and heat shock proteins through these neuroactive molecules. However, it is still undetermined if these molecules have any other effects on the host or if they can be delivered to CNS via systemic circulation [24].

SCFAs play an important role in the maturation and adequate functioning of the microglia which includes myelination and neurogenesis [14,16]. The bacteria that produce them include Faecalibacteriumprausnitzii, Clostridium leptum, Eubacterium rectale, and Roseburiaspp [15]. The germ-free mice studies show a decrease in the number and its maturity whereas antibiotic- treated mice show a decrease in its maturity alone. This shows that there is more information yet to be determined on how the SCFAs affect the microglia. Since it affects the maturity of the microglia, preclinical evidence shows that it is a signaling metabolite required for the microbiome-related development and maintenance of BBB via epigenetic modification [16]. Other functions of SCFAs also includes the alteration of chemotaxis, phagocytosis and gut integrity, induction of reactive oxygen species (ROS), they also have anti-inflammatory, antitumorigenic, and antimicrobial effects [23].

Enterochromaffin cells

The communication between ECCs and the CNS is a true example of the bidirectional interaction of the gut-brain axis. The main function of ECCs is the production of 5-HT and its storage. 5-HT is produced from dietary tryptophan and it is responsible for gut motility and secretion. ECCs 5- HT production is additionally regulated by SCFAs and 2 BA (bile metabolites).

Thus, the presence of all these pathways shows a clear indication of the bidirectional gut microbiome brain axis.

The relation between alpha-synuclein and PD

The major neuropathological indication of PD 1) the accumulation of intraneuronal cytoplasmic inclusions called Lewy bodies which contain aggregates called alpha-synuclein and other proteins like E3-ubiquitin ligase parkin and 2) the reduced levels of dopamine in the striatum due to degeneration of the dopamine neurons in the substantia nigra pars compacta [8].

Synuclein is a relatively small protein (14kda) that is predominantly expressed in the presynaptic terminals and is mostly found in the cerebral cortex, hippocampus, substantia nigra, striatum, and olfactory bulb, and also outside the CNS in biological fluids like the CSF, blood, and plasma. It plays an important role in maintaining the stability of the neuronal membrane, synaptic signaling, and dopaminergic transmission which is dysregulated in PD.

Modification in the α-syn structure occurs secondary to oxidative stress, proteolysis, or fatty acid concentration and phospholipids, which results in misfolded proteins that oligomerize and form fibrils (which develop into inclusion bodies) and may sometimes have prion-like properties. A study conducted where intracerebral injections of sarkosyl-insoluble α-synuclein from brains of patients with dementia with Lewy bodies showed to induce hyperphosphorylated α-synuclein pathology in wild-type mice can help provide a novel model for the pathology progression [13].

A “dual-hit hypothesis” postulated by Braak and colleagues states that the initial alpha- synuclein aggregates occur outside the basal ganglia in the olfactory pathways, namely the gigantocellular reticular nucleus, caudal raphe nuclei, coeruleus-subcoeruleus complex, glosso- pharyngeal vagal complex, and the Enteric Nervous System (ENS) secondary to insult from toxins or microorganisms. The vagus nerve provides a medium for the ascending transportation of the alpha-synuclein from the ENS to the brainstem up to the cortical area.

The most common GI manifestation in 80% of PD patients is constipation which may be explained by the neurodegeneration of the alpha-synuclein aggregates in the ENS occurring likely due to increased

intestinal permeability or inflammation. Additionally, it is frequently observed that these changes manifest before the appearance of motor symptoms strengthening the hypothesis that PD has a primary connection to the gut [8,13].

Patients with PD have shown higher levels of intestinal alpha-synuclein when compared to healthy controls and this finding is of significance as it shows that overexpression may lead to increased alpha-synuclein aggregation in the intestines and brains of mice and humans. Several studies conducted have shown the presence of phosphorylated α-synuclein in 61.6% of PD samples and Lewy bodies/Lewy neurites in 72.4–100.0% of PD samples when compared to the presence of just 0.0–33.0% of α-synuclein in the healthy population.

Recent research has shown that, when biopsies of intestines were conducted on healthy individuals who later would develop PD, increased synuclein immunoreactivity was observed which further establishes the presence of abnormal alpha-synuclein accumulation before motor symptoms due to CNS degeneration occurs [2].

Microbiome alterations in PD patients

Studies conducted have demonstrated increased intestinal permeability (leaky gut) in patients with PD and it speculated that this is sufficient to expose the enteric neurons to the pro- inflammatory bacterial products like Lipopolysaccharide (LPS) which in turn can induce an inflammatory response and oxidative stress thereby resulting in the accumulation of pathological alpha-synuclein in the ENS [22].

Fecal samples of PD patients analyzed showed gait and postural instability which was associated with increased levels of Enterobacteriaceae [2]. Prevotellaceae are organisms responsible for mucin production in the mucosal layer of the gut, production of SCFA's, and release of thiamine and folate. PD patients also revealed decreased levels of Prevotellaceae which is linked to reduced mucin synthesis, and production of alpha synucleinopathies via disruption SCFA modulated clearance mechanisms. Increased levels of Lactobacillaceae is shown to reduce the hormone ghrelin which is involved in physiological nigrostriatal dopamine activity [1].

Additional studies showed an increase in the pro-inflammatory Proteobacteria of genus Ralstoniain the mucosal and fecal composition and reduced anti-inflammatory properties due to an SCFA butyrate because of reduced levels of bacteria such as Blautia, Coprococcus, and Roseburia [10].

Finally, a study conducted in rotenone (pesticide) induced mouse-model of PD showed alteration in the cecal mucosa and luminal microbiota with a decrease in beneficial bacterial genus Bifidobacterium [20].

With the given findings and studies done to establish a correlation between alteration in gut microbiome and development of PD symptoms, it can be advocated that the enteric nervous system plays an important role in neurodegenerative diseases.

Upcoming Treatment Options for PD

The current mainstay treatment of PD is L-Dopa/Carbidopa (DOPA decarboxylase inhibitors) combination. Other drugs like Bromocriptine (Dopamine agonists), Selegiline (MAO-B inhibitor), and Entacapone (COMT inhibitor) all work primarily on increasing dopamine levels in the brain to diminish motor symptoms. To date, there are no disease-modifying therapies available. Accumulating evidence has established that alterations in the Enteric Nervous System (ENS) affect the progression of PD. Consequently, investigations on medication that alter the gut microbiome and their effects on the progression of PD are ongoing. The influence of Probiotics, dietary changes, and Fecal Microbiota Transplant are a few of the popular topics that researchers venture into [6,7,12,21,25,26]

Conclusion

Parkinson's disease is a neurodegenerative disease caused by the accumulation of inclusions called Lewy bodies in the brain. It is characterized by bradykinesia, tremor, rigidity, etc., and is a disease with a grim outcome. In the past few years, there has been a growing field of research trying to establish the connection between the Enteric Nervous System and the development of neurological diseases. This paper tries to bring more clarity and provide in-depth information about the relationship between our gut and PD, by focusing on how alteration in the gut microbiome is a root cause of the problem.

Recent studies, several of which are included in this review, have established that the gut and brain are interconnected to each other by more than one pathway. The most common hypothesis is related to the modification of the gut microbiome. This entails Prevotellaceae, Bifidobacterium, and Enterobacteriaceae causing oxidative stress which leads to the disruption of SCFAs, which results in the formation of alpha-synuclein aggregates. These aggregates then travel up to the cortical areas of the brain in an ascending fashion using the vagus nerve and result in the dysfunction seen in PD. Further Studies will better elucidate the role of gut microbiome in the development and progression of PD.

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DIETARY IMPACT ON THE GUT MICROBIOME AND ITS EFFECTS ON CLOSTRIDIUM DIFFICILE, INFLAMMATORY BOWEL DISEASES, AND METABOLIC SYNDROMES

(Review Article)

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Abstract

Gut microbiomes have long been known to have diverse effects on normal human health. Medical researchers and microbiologists have studied how it is maintained, about its composition, and how it can be altered. Different types of diets have varied implications on the gut microbiota and its homeostasis. This review paper discusses the different studies conducted on the matters of how diets influence the gut microbiome and also its effect on certain medical conditions, Clostridium difficile infection, Inflammatory bowel disease, and metabolic syndrome.

Diet and its effects on the gut microbiome and how it impacts human health

The diet is the source of the microbiome's fuel. But the diet also seeds the microbiota. The fascinating aspect of the role of diet in the microbiome is that some research has shown that it is almost 5 times more important than the genetics of the host [50]. So, what we eat is 5 times more important in terms of microbiome and health effects than the genes we were given at birth.

Usually, short term diet modifications are temporary [50]. Long-term diet changes can dramatically alter the composition of the microbiota [39,50]. A microbiota that is more complex benefits from a more diverse diet. The only widely recognized marker of a balanced microbiome is the more complex microbiome [48].

With the human host, the microbiome has a symbiotic relationship [9]. Around 15% of the carbohydrates we consume go to the microbiota of the intestine. And that's mostly what we'd only consider fiber-a microbiome power [5]. The gut also contains a very small amount of fat and 5-34% protein. There is a reduction in diversity if we increase our caloric intake while retaining the carbohydrates, protein, and fat proportions [11,50]. In general, indicators of detrimental effects on the microbiome are known to be a subsequent rise in firmicutes and a decrease in bacteroidetes [11]. There would be an increase in diversity if there were a reduction in calorie consumption while keeping the same diet [11].

Fiber and Short Chain Fatty Acids

In the upper part of the digestive tract, macro and micronutrient absorption take place. The lower part is where the microbiome is in the digestive tract. They harvest energy from foods we wouldn't be

able to consume otherwise [45]. And that raises our energy needs by up to 10 percent and that is mainly the digestion of fiber. They contain short-chain-fatty-acids such as butyrate, acetate, and propionate when they digest fiber [27]. Fiber is a dietary bulk-forming factor and is essential for regularity. And that's controlled by intestinal transit time. We decrease the intestinal transit period when we take a high fiber diet. This contributes to an increase in short-chain-fatty-acid production [27]. Interestingly, increased short-chain-fatty-acid development improves motility, which then decreases the time of intestinal transit. So, to keep the system normal, we now have a constructive feedback loop. Another side effect is that the pH is also decreased, which may help to absorb some nutrients, such as iron.

The positive feedback loop contributes to a rise in the prevalence of butyrate-producing bacteria, which is one of the short-chain-fatty acids that are safe. It also contributes to a reduction in the incidence of pathogenic bacteria and the commensals are spared. So, above all, healthy bacteria can flourish.

Fats and Proteins

In a high fiber diet, what we see is just the reverse: a decline in diversity and a reduction in a healthy microbiome. It is possible to see an improvement in the microbiome that thrives off fat and protein, rather than fiber. This suggests that short-chain-fatty-acids are decreasing. The healthy fat in a high-fat diet can be productive [44]. The diversity of the microbiome is not lost if the diet is high in healthy fats.

Micronutrients

Nutrients are formed in the microbiome. There are also B vitamins that come from our microbiome. Since a large proportion comes from the gut microbiota, the stocks of these vitamins in the body are greater than we can expect from consuming food [51]. Interestingly enough, one of the major risk factors is SIBO- Small Intestinal Bowel Overgrowth linked to the microbiome [26]. Thiamine is synthesized and made in higher stores by the microbiome and contributes to the nutritional status. If the good microbiome is eliminated from the body, adequate thiamine will not be produced by the body [18]. For the microbiome, vitamin B6 is important because they need it for their enzymatic activities and so our diet depends on their vitamin B6 diet. Research has shown the possible relationship between how virulent and how basic H. Pylori and its capacity to make B6 are going around [11]. It becomes more virulent and more modal if it can supply its B6, which is potentially harmful to a patient.

The amount of folate that the microbiome produces is considerable. In the production of folate, resistant starch is indeed essential. If the body does not have enough resistant starch in the diet, a significant amount of folate is possibly not developed by the intestinal microbiome [18].

There is also vitamin B12 provided by the gut microbiome. But it's still important for the microbiome's metabolism. They depend on a Vitamin B12 diet. It has everything to do with our epigenetics and maybe the relationship between the host and the microbiome in an epigenomic fashion. In one study, vitamin B12 was shown to require 83 percent of the gut microbiota. So, if the diet does not supply the gut microbiota with adequate vitamin B12, they are likely to die [18,51].

As an electron carrier, the microbiota uses vitamin K, and thus supplying vitamin K via diet is essential for microbiota sustenance [12]. It is proven that beta carotene can be produced by the microbiome. But the human body requires retinol, the activated form. And we haven't shown that retinol activates bacterial beta carotene yet. Besides, no evidence exists as to whether they contribute to vitamin A status. Between the microbiota, in modern Western babies, B. infantis are found to be largely absent. Supplementing B. Infantis cures all conditions pretty much because it is strongly anti-inflammatory and removes autoimmune disorders, asthma, atopic dermatitis. But the impact of B. Infantis depends on providing enough vitamin A. Supplementing B. Infantis, thus, does little for a child with vitamin A deficiency [20].

As they don't have a vitamin D receptor, vitamin D is not important to the microbiome. But for the immune system, vitamin D is essential and the way our immune system responds to commensal bacteria depends on vitamin D [10].

Iron is not well explored, in large part. What is known from the research is that there appears to be a shortage of Lactobacilli in iron-deficient patients [51]. The directionality of the Iron-Lactobacilli relationship is not well known. We recognize, however, that Lactobacilli requires iron. An iron-deficient

patient wouldn't provide the gut microbiome with enough iron. There were no supplementation trials, however, showing that supplementing with iron would boost this.

Polyphenols

A lot of health benefits have been related to polyphenols. The body mainly does not absorb polyphenols. 90 percent of them make it to the large intestine, where the gut microbiome is likely to bioactivate them [51]. So, many of the polyphenols' effects may be attributable to their effects on the microbiome and through it. To find out the role of polyphenols in red wine and the effect of alcohol, a scientific study was carried out. It was carried out by the distribution of de-alkalized wine and pure alcohol. The result showed an improvement in microbiota and the wine as a whole meal showed a substantial increase. Synergistic, it was. The effect of the wine and the effect of the alcohol added together did not, however, match the effect of the wine as a whole. The effect of bringing them together was far greater [51].

Clostridium difficile infection

As shown above, diet influences the human gut through different means, which then also affects human health conditions [19]. Clostridium difficile, an anaerobic bacteria through the exotoxins,

TcdA and TcdB produced by them are accountable for the c.difficileinfections (CDI) worldwide. In murine model studies conducted for CDI, associations between antibiotic consumption and intestinal microbiome are noted along with non-antibiotic disruption of the gut microbiome [41]. The human gut microbiome resists the invasion of pathogenic bacterias through colonization resistance mechanisms of competing with harmful invasive species [46]. The bacteriocin produced by the species B.thuringiensis has shown efficacy in inhibiting the growth of, particularly spore-forming Clostridium difficile [34].

There have been indications that multiple unexplored environmental factors, like nutrients, and host factors probably impact CDI through various mechanisms. Because diets containing high amounts of carbohydrates are used for the survival of different bacterial species, we can hypothesize the role diets can play a vital role in the gut microbiome regulation and hence influences CDI. Several studies have demonstrated that diet can alter and manipulate the gut's microbiome make-up and its functions [54].

Most of the research on CDI is done on murine models so as to understand the mechanisms of nutrient component breakdowns, and detect bacterial involvement, and manipulate different gut colonization. Studies on mice have shown traditional diets to be fermentable food, mainly wheat and other starches which are mainly composed of fructooligosaccharides (FOS). Although not digested by mammals, anaerobic bacterial species like clostridium and lactobacillus can ferment them by cleaving their bonds. This is suggestive of inhabitant gut microbes to thrive and promotes resistance to the pathogenic invasion of c.difficile [32]. Contrary to traditional diets, defined diets consist of mainly “non-fermentable” fibers, and carbohydrates that are easily metabolized. The contents of these defined diets are absorbed relatively easily by the GI tracts of mammals [32]. In another study directed at the more common higher fat-protein diet, and carbohydrate richer diet, the results showed that the higher carbohydrate diet was readily metabolized to monosaccharides and although they encourage the colonization of c.difficile they also mitigate the overgrowth. Additionally, results revealed that despite the type of carbohydrate, a low protein-fat and a relatively higher carbohydrate diet was preventative to a certain extent [30]. The same study also showed mice undertaking antibiotic treatment for CDI, low protein diets showed resident gut bacterias competing with invasive pathogenic clostridium species for amino acids thereby helping combat CDI in the presence of antibiotic use [30].

Zinc also has a significant role in the modulation of the gut microbiota. Higher levels of zinc is seen to aggravate the severity of CDI and worsen conditions. Adversely, lesser quantities of zinc in the diets of the patients with CDI or those at risk of developing CDI have displayed protective effects. Calprotectin, a protein complex that participates in the sequestering of zinc, has antimicrobial properties against Clostridium difficile making it a significant element of the innate immune response to CDI. By restricting the availability of Zinc, calprotectin helps in confronting c.difficile [52,53].

Symptomatic relief for CDI patients can also be achieved by adding probiotics to their diet. Imbalances in the gut microbiota caused by antibiotic treatment frequently tend to exacerbate diarrhea and vomiting, symptoms mediated by toxins produced by c.difficile. The use of probiotics in CDI patients

remarkably decreased the relapse of the infection and also proved to decrease the number of diarrheal episodes post-antibiotic treatment [13].

Inflammatory bowel diseases

As we have seen in the case of Clostridium difficile infections, diet also tends to play an immense role in inflammatory bowel disease (IBD). IBD is a general term for Crohn'sdisease (CD) and ulcerative colitis (UC), which are chronic inflammatory disorders of the gastrointestinal (GI) tract. Over the years, there has seen an increase in incidence all over the world and can be said as a global disease even [3]. Like several other illnesses, IBD is also multifactorial. The ultimate cause of the conditions is still unclear, but several studies conducted thus far have suggested both immune dysregulation and dysbiosis as the probable factors for the inflammations. There is limited evidence on IBD onset and genetic influences on how they correlate, considering all the developments in genetics over the years [21]. The Crohn's and Colitis Foundation is currently undertaking numerous projects to concentrate on preclinical IBD mechanisms, including genetic and microbiome-based mechanisms. Among the priorities to be assessed, environmental stimuli, primarily the diet are included.

Influence of diet on the Etiopathogenesis of Inflammatory bowel disease

In recent reviews of various literature on IBD, the most ubiquitous environmental factor found in IBD was diet. In all regional settings, diet is either a protective or a risk factor in both, UC and CD. It must certainly be the most ubiquitous environmental factor in IBD patients, taking into account the fact that all patients with IBDs are exposed to food and diet also affects the gut microbiota, which is significant in all IBD patients [8]. It is well known that gut microbiota presence is imperative for gut inflammation and additionally it is also known that diet plays a crucial role in its formation. Over the years several different types of approaches were made to understand the part diet plays in IBD, reviewing these data, we can observe that there were noticeable risk factors based on individual diets.

High consumption of plant-based fibers showed a low risk of CD, whereas a deficiency in zinc in CD patients was noted in a cohort study of IBD patients [1]. Dietary fibers tend to be fermentable and make them easier to be metabolized by the gut microbiome into short-chain fatty acids which can inhibit NFκβ and the transcription of pro-inflammatory markers [1]. Zinc however plays role in intestinal epithelial cell integrity mainly through the delocalization of E-cadherin and β–catenin in intestinal epithelial cells and also in an inhibitory effect on NFκβ which is associated with inflammation and also reduction of myeloperoxidase activity, the latter mechanism was noted in animal models of IBDs [1]. High potassium and high sodium diets have also been established as having anti-inflammatory through their effects on inducing Foxp3 and on TH17 cells, which also on cohort data analysis showed inverse effects on IBD [23]. The dietary modification that causes a disparity in the fatty acid levels and also improves the permeability also tends to sulfur production which can manipulate the gut microbiota to grow more sulfate-reducing bacterias like firmicutes [22].

Curative effect diet may have on inflammatory bowel disease

The objectives for the treatment of IBD are to promote and sustain remission, decrease the need for long-term use of anti-inflammatory medications, enhance the quality of life and improve the prognosis. Anti-inflammatory agents are the cornerstones of the current treatment. In approximately 30 percent of patients who undergo surgical interventions, the procedures may result in a pouch or end ileostomy, although they are curative for UC [36]. None of these surgical choices are without complications and pouchitis will occur in up to 70 percent of patients with a pouch [14]. With more than 80% of patients needing surgical resection, an even higher number of CD patients require surgery, which is not even completely effective [36]. During the course of further understanding IBD and finding different approaches to manage and treat IBD, diets have become studied and researched to a greater extent. Various components of our diet play vital roles in bringing a balance to the gut microbiome, which is most important for IBD.

Diet as a therapy for IBD must be taken into consideration with multiple studies showcasing the differences associated with increased and decreased risk of IBD. Studies have shown that animal fat and protein-rich diets compared to plant-based fiber-rich diets have a higher risk of IBD. As mentioned above the plant-based diet has protective factors that are helpful for the gut microbiome to induce anti-

inflammatory functions of cells by metabolizing the nutrients into short-chain fatty acids [28]. A plant-based diet also blocks bacterial translocation across the Peyer's patches, which prevents invasion of certain bacterias which contributes to some of the pathogenesis of IBD [35].

Exclusive enteral nutrition is used as a supplemental treatment for IBD patients. Phytonutrients and phytochemicals are sometimes added to some of the formulas because they have shown positive results in controlling inflammation. Xanthohumol and curcumin have both demonstrated inhibition of COX, LOX, suppress TNFα, and downregulate NF-κB activation which is beneficial for IBD patients [37]. An anti-inflammatory diet (IBD-AID) for IBD has shown positive results [33]. The IBD-AID emphasizes on modification of certain carbohydrates because they provide a substrate for bacterias to proliferate, consumptions of pre and probiotic foods to rejuvenate the lost gut microbiota, and differentiating between fats which are beneficial for treating IBD [33]. Probiotic consumption can induce remission according to studies conducted on bacterial secretion butyric acid, which showed significant effects on cellular proliferation and apoptosis. It also has anti-inflammatory properties and helps in the strengthening of mucosal barriers, which are both supportive in IBD treatment [47]. Butyrate-producing bacteria are found to be promising therapeutic probiotic bacterial strains [47]. Lastly, certain probiotics raise the levels of vitamin D, which influences the gut by maintaining gut homeostasis, improving epithelial cell's integrity, immune responses, and also the composition of the gut microbiome [6]. Vitamin D is beneficial in treatment especially because multiple immune cell types express Vitamin D receptors (VDR) and it has been evidenced through murine model studies that VDR deficiency increases the risk for developing colitis [17].

Metabolic syndrome

As mentioned in the sections above, we know that diet influences the physiology and metabolism of the host by interacting with the gut microbiota [43]. Current evidence has shown that gut microbiota is altered in people with obesity, type 2 diabetes, and stroke. This indicates that the gut microbiota could be a significant environmental factor leading to the progression of metabolic diseases. Dietary interventions are also a possible method for modulating intestinal microbiota and further impacting the health of the host.

A decrease in consumption of indigestible carbohydrates may result in the loss of certain bacteria residing in the human gut, which depends on these sources and thereby decreases the production of short-chain fatty acids [40]. The connection between dietary fiber and the incidence of type 2 diabetes, and dietary fiber and whole grains has shown the increased diversity of the microbiota of the human gut. High fiber intake is associated with the increased levels of the bacterial genus Prevotella.

A study performed by Kovatcheva-Datchary et al observed that changes in postprandial glucose and insulin response following a 3-day barley kernel bread intervention are based on the enrichment of P. copri in the participant's microbiota. This was functionally related to improved efficiency in digesting complex polysaccharides in the barley kernel bread. In those with no improvement in glucose metabolism after the intervention, it was observed that their gut microbiota was neither enriched with P. copri nor their ability to ferment complex polysaccharides improved after the trial. These results suggest that gut microbiota analysis could be used to understand how each individual responds to the dietary intervention [24].

Bile acid is an endocrine molecule, which in addition to the absorption of fat-soluble nutrients, also regulates numerous metabolic processes, including glucose, lipid, and energy balance [31]. Through the direct or indirect activation of a nuclear receptor, bile acids play a role in the metabolism of glucose and lipids: Farnesol X (FXR) and a membrane receptor: G protein-coupled membrane receptor 5. (TGR5). When natural bile acids are supplied as FXR activators, the metabolism of gut microorganisms can produce TGR5 ligands. The gut microbiota induces the inflammation of adipocytes by increasing the expression of genes involved in the absorption of lipids in the liver. The microbial community interacts dynamically with bile acids. This interaction may have positive or negative effects on host metabolism through dietary changes [38,42]. Increasingly, the gastrointestinal tract's metabolic ability and its microbiota are regarded as promising goals for developing glycaemic regulation and managing type 2 diabetes. More specifically,

recent data have shown that the glucose-lowering effects of metformin are influenced by improvements in gut microbiota composition and function [16].

Several gut-targeting treatment strategies to reduce blood sugar are emerging and promising initially, but there is a need to better understand the mechanisms that underlie these effects in humans.

Dyslipidemia and atherosclerotic plaque remain a significant risk factor for cardiovascular disease and is often closely linked to impaired glucose metabolism and obesity [29]. In reducing the effects of long-standing dyslipidemia short-chain fatty acids (SCFAs) have a potentially promising therapeutic role [4]. The human gut lacks the enzymatic ability to break down certain foods, including complex carbohydrates as dietary fiber. But certain anaerobic bacteria present in the large intestine ferment these fibers into many by-products like SCFAs. By activating the free fatty acid receptor (FFAR) 2 coupled with G proteins, SCFAs induce the activation of the gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). Among SCFAs, propionate shows the greatest affinity for FFAR 2.

A randomized, cross-over study was conducted to determine the effects of propionate on appetite regulation. Propionate has been shown to stimulate the gut release of the PYY and GLP-1, leading to a reduction in energy consumption in humans. A decrease in weight gain, intra-abdominal adipose tissue, and hepatic fat distribution, and prevented the decline in insulin sensitivity was seen in the subject group after 24 weeks of propionate supplementation [7]. Optimizing the development of colonic propionate by choosing propionate rich dietary fibers may be a new way of preventing lifelong weight gain and improving health status.

To evaluate the preventive effects of different types of fiber like lupin kernel, legume, and citrus on cardiovascular disease, a randomized crossover study was performed [15]. Subjects on high-fiber diets (i.e., lupin or citrus fiber) underwent a reduction in C-reactive protein, systolic blood pressure, and blood lipid circulation. The authors proposed that the hypolipidemic effects of a fiber-rich diet were due to the production of SCFA. Although SCFAs can have significant tropic effects on the body and health while LPS and peptidoglycan, the components of the bacterial cell wall can contribute to cardiovascular disease risk [2]. For example, LPS-injected mice showed decreased HDL cholesterol in plasma and elevated plasma triglycerides [49]. A retrospective human study of 587 people in the Finnish Diabetic Neuropathy cohort showed that those with the highest serum LPS levels also had substantially higher serum triglyceride and blood pressure levels [25]. Microbial components can also present a risk for metabolic syndrome.

Conclusion and future perspective

In conclusion, we recognize that the abundance of researches and literature on how diet impacts the human gut microbiome has opened up new possibilities to approach not only in treating Clostridium difficile, Inflammatory bowel disease, and metabolic syndrome but also in understanding how it is associated with the conditions itself. Significant associations of the gutmicrobiome with host metabolism, inflammatory, endocrine, and immune homeostasis, and suspected gut microbiome upregulation or alteration have shown to be enormous. In effect, the disturbance of such a fragile equilibrium would contribute to the disease's manifestation. However, owing to a lack of direct evidence and mechanistic information, the causal or association relationship remains debatable. Our knowledge of the gut microbiome is still at a very preliminary level, to better explain the relation, with many weaknesses and research holes worth further exploration. In addition, the commensal microbial composition will impact the host factors' growth, maturation, and normal functioning in turn. A better understanding of the human microbiota and how commensal microbes communicate with the host is certainly relevant in order to elucidate the pathophysiology and metabolic aspects of many human diseases and to provide a more efficient therapeutic framework to tackle the limitations of existing therapies. In short, there is still a relatively new but rapidly growing area of research in the Human Microbiome, showing many preliminary but encouraging studies on the modulatory role of the human microbiome in human well-being and disease. Future applications for the detection of microbiome-based diseases, monitoring of prognosis, prophylaxis, and therapies that have great potential to revolutionize existing disease prevention and treatment measures are certainly worth anticipating. More research is warranted on how diet can be used as an adjunct therapy for these conditions and given the multiple effects diet has on not only the gut

microbiome but also on human immune responses studies can also be made on unearthing prevalence of various conditions due to dietary modifications.

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SELECTED NEUROLOGIC AND PSYCHIATRIC OUTCOMES IN PATIENTS TREATED WITH CLOZAPIN

(Review Article)

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Abstract

Clozapine is a second-generation antipsychotic mostly used for treatment-resistant schizophrenia and acute suicidality. Although clozapine's efficacy is well-documented, it has various side effects which need to be promptly addressed. In this article, we focus on neurological and psychiatric side effects. Among these side effects, seizure is a well-known complication of clozapine therapy. The articles that we reviewed concerning seizures and EEG changes in association with clozapine plasma levels and doses, found significant relations in some but not all cases. In addition to seizures, extrapyramidal side effects are common adverse effects of clozapine which can be an acute or chronic condition. Clozapine is also associated with ocular disturbances such as retinal pigmentation. It has also been shown to cause cataplexy. Moreover, clozapine can cause significant sedation. Effects of clozapine on sleep is still a controversial subject. The studies demonstrate inconsistent results. Cholinergic rebound syndrome is a rare complication of longterm clozapine use, which itself can be the inducer of insomnia. Apart from this, hypersalivation is one of the most frequent side effects with a paradoxical pathophysiology. Finally, clozapine is associated with severe psychiatric side effects, some of which need prompt intervention.

EEG Changes and Seizures

Introduction

A seizure is an important and well-known side effect of clozapine treatment. Patients on this drug can develop seizures at all phases of the course: at low doses during titration and at higher doses during maintenance [1]. We reviewed the articles that assessed plasma levels of clozapine, doses of clozapine, or both and their relation to seizures and electroencephalogram changes.

EEG abnormalities

EEG changes can be epileptiform (focal, generalized spikes, or sharp waves) or nonepileptiform (focal or generalized slowing). One of the articles that we reviewed analyzed 12 different papers that provided data about EEG changes in 565 patients on clozapine treatment [1] Another source that we utilized was a study on 26 patients from Japan [2]. In both papers, the authors described the EEG abnormalities. Varma et al. found that although a variety of changes were noted, the most common abnormality was nonspecific generalized slowing waves, while Kikuchi et al. found spike and wave complexes to be more common.

Clozapine dose and EEG relationship

Using the regression model Varma et al. found a significant relationship between the mean dose of clozapine and the percentage of patients with EEG changes. According to the paper, every increase in mean clozapine dose by 100mg resulted in 8% increase in patients with abnormal EEG findings (0.08, 95% confidence interval [CI] 0.01-0.15, p=0.022) [1]. On the other hand, Kikuchi et al. describe EEG changes in 10 out of 26 patients studied (38.5%) with a daily dose of clozapine varying from 125mg to 600mg with a mean of 305mg daily. In this case, a significant relationship was not seen between EEG changes and mean dose of clozapine but found that mean age and mean illness duration in the abnormal EEG group were significantly lower ($p<0.01$) [2].

Clozapine plasma levels and EEG relationship

According to Varma et al., a significant relationship was found between the two with regression analysis [1]. A 12% increase was noted in the percentage of patients with abnormal EEG with each 100μg/l increase in clozapine plasma level (0.12, 95% CI 0.03-0.21, p=0.023). Kikuchi et al. study did not investigate plasma level relation to EEG changes [1].

EEG changes and seizure relationship

Some of the studies that Varma et al. used theorized that seizures are not necessarily predicted by EEG changes (exemplifying a case report about a patient with myoclonic seizure with a normal EEG before the event). On the other hand, they conversed in a study that found EEG changes as a sensitive indicator to seizures [1].

Seizures

Varma et al. used 10 different studies where 113 patients out of 6344 seizures. According to Kikuchi et al., 6 out of 26 had seizures. Varma et al. states that in all studies that they reviewed clozapine-induced seizure risk was higher compared to 1% risk associated with conventional antipsychotics [1]. In one of the papers, premarketing studies were analyzed that reported seizures at a crude rate of 3.5% over the course of a year. Other studies found that seizure risk was increasing cumulatively and reached 10% in 3.8 years of the drug therapy. Post marketing study in the US reported seizures in 1.3% of patients in the first 6 months of drug release. In terms of seizure types, the majority of them were generalized tonic-clonic type, while 4 out of 6 patients had myoclonic seizures as stated by Kikuchi et al [2].

Clozapine dose and seizure relationship

Varma et al. carried out a regression analysis and did not find a significant relationship between clozapine dose and seizures ($p=0.353$), although, in general, higher clozapine dose is correlated with increased risk of seizures [1]. Most case reports described seizures in patients taking more than 600mg of clozapine daily. However, a post marketing study failed to find dose-related risk for seizures. Kikuchi et al. found that clozapine dose varied from 300mg to 600mg with a mean of 383.3mg/day at the time of a first seizure experienced by the patients [2].

Clozapine plasma levels and seizure relationship

Varma et al. found a statistically significant relationship between plasma levels of clozapine and seizures. They based this on 3 case reports about 4 patients. According to the study, clozapine plasma levels of more than 1300μg/l were associated with increased risk of seizures [1]. It is worth mentioning that patients with preexisting seizure disorder are at increased risk of seizures with lower concentrations of clozapine in plasma.

Movement Disorders

Antipsychotic medications are commonly associated with movement disorders while taking the drug, nevertheless studies exist that suggest that some antipsychotics in this scenario Clozapine can be beneficial in treating selected movement disorders. Extrapyramidal side effects are set in two different groups: acute which includes: Parkinsonism, acute akathisia, acute dystonia and chronic: Tardive dystonia, chronic akathisia, Tardive dyskinesia. Tardive dyskinesia has been listed as the most common movement side effect of Clozapine. Tardive dyskinesia (TD) is a disorder that results in involuntary, repetitive body movements, which may include grimacing, sticking out the tongue, or smacking the lips. Additionally, there may be rapid jerking movements or slow writhing movements. If we take a closer look, Clozapine is associated with lower rates of TD compared to other antipsychotics such as Haloperidol. One study done in 1993 by J M Kane, M G Woerner, S Pollack, A Z Safferman, J A Lieberman which was done to detect any causative relationship between Clozapine and TD shown that two out of 28 schizophrenic patients who has never had any history of TD were rated to have mild dyskinesia by Simpson dyskinesia scale, although this study was not able to conclude whether clozapine was primary associative factor in development of TD in these patients it has shown that patients treated with Clozapine have lower rates of developing TD compared to other typical neuroleptics [3]. Moreover, another study done by C A Tamminga, G K Thaker, M Moran, T Kakigi, X M Gao comparing Haloperidol and Clozapine has shown that dyskinesia rebound which occurred equally in both study groups was subsided in Clozapine group but sustained in Haloperidol group [4]. Another interesting topic is switching to Clozapine from other antipsychotic medication which decreases TD, for that we take a closer look to studies showing that Offending antipsychotics administered at the time of TMS onset were second-generation antipsychotics in 88.6% of patients. Tardive movement syndrome symptoms were remitted in 65.7% of patients after switching to clozapine this raises the point that Clozapine seems to be an excellent treatment option for

TMS in the era of second-generation antipsychotics, especially for younger patients with mild tardive dyskinesia. Clinical trials comparing the effect of switching antipsychotics to clozapine with add-on therapy of new drugs targeting TMS are difficult to design in ordinary clinical settings. Therefore, more naturalistic observational studies are warranted to identify predictors of TMS response to clozapine. Akathisia consists of motor restlessness accompanied by subjective feelings of inner tension and discomfort, mainly in the limbs another study which was done to detect the prevalence of akathisia and in patients receiving stable doses of clozapine has shown that 6.8% of study population has shown signs of akathisia. Multiple studies have been done to find out solution of akathisia caused by Clozapine [5-7], of which one interesting study is a case report of a patient who had Clozapine-induced akathisia that was treated by Gabapentin [6]. 39 y.o female who has been diagnosed with paranoid schizophrenia for 4 years, at first 3 years she has been treated with other antipsychotic medications than Clozapine, but drug was introduced at the age of 38. The treatment at the beginning included lower dosage of the drug but got increased to 425 mg/day in the end which caused increased akathisia, which was not that severe before, that is why 600 mg/day Gabapentin enacarbil was introduced to his medication list that subsided akathisia caused by higher doses of Clozapine. Study has not concluded the mechanism by which Gabapentin decreased akathisia but an opinion was made that it might manage dopamine dysregulation by increasing GABA activity in the brain, however, future research should evaluate and verify this mechanism.

Controversial theory was made by study which has shown possible effectiveness of Clozapine treating resting tremor in Parkinson's disease, bringing a table from this study shows significant decrease in resting tremor, but as it was suggested it was a theory that needs further studies are needed to understand its mechanism and effectiveness (**Figure 1**).

Pre- and post-treatment total tremor score in clozapine-treated PD patients with tremor as an indication

No.	Age	Medication Before Clozapine and Changes After the Addition of Clozapine	Total (Rest and Action) Tremor Score (UPDRS) Preclozapine Treatment[a]	Total tremor Score (UPDRS) Postclozapine Treatment at Last Assessment[a]
1	52	Total L-dopa dose: 2,500 mg/day, sertraline 100 mg/day; no change in medication	8	0
2	62	Total L-dopa dose: 875 mg/day, quetiapine 75 mg/day (on stable dose with no effect on tremor), stopped just before initiation of clozapine. Rivastigmine 3 mg/day was added and L-dopa was decreased to a total L-dopa dose of 625 mg/day between assessments of tremor score	15	0
3	75	L-dopa/benserazide and LD/CD: total L-dopa 1,750 mg/day in 11 doses amantadine 200 mg/day; no change in medication	8	8
4	56	Total L-dopa dose: 1250 mg/day, total pramipexole dose 1.25 mg/day, lorazepam 1 mg at 3 AM; no change in medication	6	4
5	74	Total l-dopa dose: 1,600 mg/day, total pramipexole dose: 3 mg/day; no change in medication	4	0
6[b]	77	Total L-dopa dose: 800 mg/day, trihexyphenidyl 1.5 mg/day, pramipexole 2 mg/day, entacapone 800 mg/day,clonazepam 0.5 mg/day, and quetiapine 25 mg/day; no change in medication	6	5
7	70	Total L-dopa dose: 300 mg, pramipexole 1.5 mg/day, and clonazepam 1 mg/day; no change in medication	24	9
8 (case report)	75	Total L-dopa dose: 1,050 mg/day, pramipexole 0.75 mg/day, and levodopa reduced by 450 mg/day	6	1

[a]Items 20 and 21 of UPDRS; maximum possible score = 28.
[b]Clozapine was stopped 3 months after initiation because of seizures.

CR, controlled release.

Figure 1: Pre- and Post-treatment total tremor score in clozapine-treated PDF patients with tremor as an indication

Ocular disturbances, cataplexy, and sedation

Clozapine and ocular side effects

Clozapine is rarely associated with ocular side effects. Even though it is a second generation antipsychotic, it has been found to be associated with the ocular disturbances classically seen with both high and low potency first generation antipsychotics. Clozapine-related ocular side effects can be divided into structural and functional which will be discussed in the following paragraphs [8-12].

Case report from Borovik, Bosch, et al. describes a 55 years old female patient with 16 year history of taking clozapine (800mg/day) for treatment resistant schizophrenia who developed decreased visual

acuity in both eyes (OS 6/60 and OD 6/9). Bilateral deposits in corneal epithelium and stellate cataract were discovered on ocular exam. Fundoscopic exam revealed macular atrophy and retinal pigmentation. Patient's dose was reduced from 800mg/day to 600mg/day but her ocular defects did not improve. This case report lets us draw two important conclusions about clozapine-related structural ocular abnormalities: 1. Corneal deposits and cataract are classically associated with a low-potency first generation antipsychotic, namely chlorpromazine while retinal pigmentation and macular atrophy are associated with another low-potency first generation antipsychotic, thioridazine. Hence, clozapine shares several side effects of typical antipsychotics. 2. Clozapine-related structural eye defects are largely irreversible (**Figure 2**).

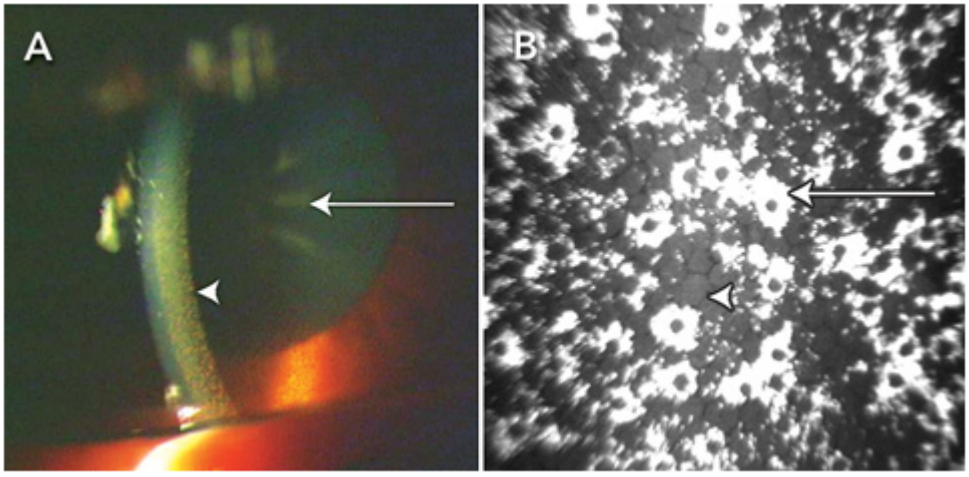

Figure 2: Clozapine-induced ocular defects. Right: corneal band deposit. Left: electron microscopy of retina affected by clozapine.

Case report from Nebhinani, Avasthi et. Al describes a 25 years old male patient with 7 years history of treatment resistant schizophrenia who started taking clozapine (300mg/day) after failing therapy with risperidone (6-12 mg/day), chlorpromazine (800mg/day) and quetiapine (800 mg/day). After a year he started experiencing an oculogyric crisis (OGC), i.e. reduced mobility and painful fixation of eyeballs in upward vertical position. Patient used to develop OGC episodes 4-5 times/week regardless of situation, time, diet, stress. After thorough discussion of risks and benefits of reducing the dosage, patient decided to reduce the clozapine dosage from 300mg/day to 150mg/day. After dose adjustment, the frequency of OGC declined from 4-5/week to 4-5/month but at the same time, the patient started experiencing symptoms of his underlying schizophrenia, namely mood swings, suicidal ideas and irritable mood. This case report lets us draw two important conclusions about clozapine-related functional ocular abnormalities: 1. OGC is dose-dependent side effect of clozapine. 2. Reduction in dosage of clozapine causes significant improvement in frequency of OGC episodes but it causes reemergence of schizophrenic symptoms (**Figure 3**).

Author	Demographics	Diagnosis	Presentation	Treatment and outcome
OGC on clozapine therapy				
Uzun and Doruk, 2007	38 years female 19 years female 45 years female	Schizophrenia	Experienced multiple episodes of OGC on clozapine (dose unknown). Onset 6 months to 2 years after starting clozapine	Follow-up details were not mentioned
Chakraborty and Chatterjee, 2007	37 years male	Schizophrenia	Experienced episode of OGC on clozapine (150 mg/day) at 9th day of treatment	Treated successfully with stat IM promethazine (50 mg). Recurrence on discontinuing trihexyphenidyl (4 mg/day)
Hoseini Sheikh Moonesi, 2007	27 years female	Schizophrenia	Experienced multiple episodes of OGC on clozapine (dose unknown)	Treated successfully with anticholinergic medication (Artane)
Salehifar and Hosseini, 2007	42 years female	Schizophrenia	Experienced two episodes of OGC on clozapine (150 mg/day)	Treated successfully with biperiden
Dave, 1994	Male	Schizophrenia	Experienced multiple episodes of OGC on clozapine (dose unknown), and earlier also experienced with perphenazine	Treated successfully with anticholinergic agents
OGC on clozapine discontinuation				
Mendhekar and Duggal, 2006	18 years female	Mental retardation	OGC after 2 days of clozapine discontinuation (given 300 mg for 6 weeks)	Treated successfully with reinstatement of clozapine

OGC – Oculogyric crisis

Figure 3: Summary of case reports about clozapine-treated schizophrenia.

Clozapine-induced cataplexy

Cataplexy is a neurologic phenomenon characterized by sudden loss of generalized or local muscle tone in the body frequently resulting in a fall. Even though Cataplexy is classically associated with a neuropsychiatric disease called narcolepsy, it was also discovered to be a rare side effect of clozapine. As it is described in the next two case reports, clozapine-induced cataplexy involves mostly hands and knees.

One of the earliest sources of case reports about clozapine-induced cataplexy is the one from Chiles, Cohn et.al, who described 4 patients under their direct therapeutic supervision who developed clozapine induced cataplexy. One of their case reports is about 45 years old female diagnosed with chronic, undifferentiated schizophrenia who started taking clozapine after failing nine different antipsychotic medications. Due to the severe nature of her condition, she was directly started on 550mg/day of clozapine. Her symptoms seemed to be controlled but after 1 year she reported reemergence of auditory hallucinations which prompted physicians to increase her dose from 550mg/day to 600 mg/day. 6 months after the last dose adjustment, patients reported sudden episodes of involuntarily dropping the objects and knee buckling. Dose of clozapine was reduced back to 550 mg/day which caused cessation of these cataplectic episodes.

Another Case report from Desarkar, Goyal et.al describes a 29 years old female with treatment-resistant schizoaffective disorder, manic type who started taking clozapine after 4 years of failing multiple antipsychotics and mood stabilizers. Her dose of clozapine was gradually increased to 150mg/day and although this dose adjustment controlled her psychiatric symptoms, she started experiencing sudden onset knee weakness and dropping of the objects. These episodes were neither triggered by emotional stress (as in narcolepsy) nor followed by loss of consciousness. These episodes of cataplexy used to happen 2-3 times per day. Once the clozapine dose was reduced from 150mg/day to 125 mg/day, the frequency of cataplectic attacks was reduced to 1-2 episodes on alternate days. Despite this improvement, the patient was not able to tolerate cataplexy attacks and she discontinued clozapine which resulted in complete cessation of cataplexy. Even though the exact pathophysiologic mechanism of clozapine induced cataplexy is unknown, Desarkar, Goyal et.al conclude that this rare side effect is supposedly related to clozapine's ability of blocking alpha 1 and 2 receptors which in turn affects presynaptic D2 and D3 receptors.

In light of the above case reports, we can conclude that although it is a rare side effect, clozapine induced cataplexy significantly interferes with patients' activities of daily living and lowers their quality of life.

Clozapine-induced sedation

Clozapine is well known for its sedating nature. According to multiple different studies, 10% to 58% of patients were found to feel sedated, drowsy, and fatigued shortly after starting clozapine therapy (Fitzsimons et.al, 2005; Joseph & Lieberman, 2004). Although sedation can potentially occur at any time along the course of treatment, it is most common at the beginning of therapy and it gradually decreases in the first 4-6 weeks.

There is a clear, well-established neurochemical explanation for clozapine-induced sedation. In addition to its effects on dopaminergic and serotonergic pathways in the brain, clozapine has a significant antagonistic effect on Histamine H1 receptor (Kane, Honigfeld, Singer & Melitzer, 1988) and muscarinic M1 receptor. The blockade of these two receptors mediates sedation and drowsiness.

There are several practical ways of minimizing clozapine-induced drowsiness without using other medications: 1. Physicians can titrate the dose slowly to allow the patient enough time for adaptation 2. Physicians can encourage the patient to take clozapine at night (especially if the patient suffers from insomnia) to benefit from its sedating properties. 3. Physicians might decrease the maintenance dose if the patient's condition is considered to be well-controllable after receiving a larger dose.

While we can manipulate the dose and the timing of clozapine to make sedation more tolerable for patients, there are several drugs that can be used in combination with clozapine to counteract its drowsiness. Stimulants such as dextroamphetamine, modafinil, modafinil or antidepressants with stimulant effects such as bupropion decrease sedation and increase wakefulness. However, in light of their side effects, these medications are used only for patients with refractory or severe clozapine-induced sedation.

From the information above, we can conclude that sedation and drowsiness are very prevalent side effects of clozapine and we can minimize them by slowly titrating the dose or using the drugs with stimulant effects to counteract sedative properties.

Clozapine and sleep

Effects of clozapine on sleep have been a subject of discussion for quite a long time. There are mixed and inconsistent ideas about the effects being positive or negative. Psychiatric disorders, especially schizophrenia and schizoaffective disorder, are strongly associated with impaired sleep continuity. Clozapine, and generally the second generation antipsychotics have been reported to improve sleeping patterns, but some results have been questionable.

Fifteen people (11 women, 4 men), all of them with DSM-IV criteria diagnosed bipolar or schizoaffective disorder participated in the study. Their sleep was monitored at baseline and late after 6 months of clozapine therapy. According to the results of the study, clozapine elongated sleep latency and increased the amount of awakenings, while total sleep period (TSP) and time in bed (TIB) were significantly increased (range: $F=6.2\text{-}17.9$; df=l,12; $p<0.05$). As for individual sleeping stages, stage 2 and slow-wave sleep were both increased, which demonstrates that clozapine is more of a NREM sleep enhancer. Sleeping pattern was clearly better after the drug administration with small but significant improvement, in how refreshed and rested participants felt after awakening ($t=-2.1$; df=26; $p<0.05$) [13]. Primarily the results were positive, but the increased number of awakenings is still a matter of debate.

According to the second study, restless leg syndrome (RLS) during sleeping has been reported as one of the effects of clozapine. Other than anti-serotonergic and dopaminergic activity it has significant antihistamine properties, which is one of the contributing factors of inducing restless leg syndrome. Reports suggest that this is a dose dependent phenomenon. The patient bore 100mg/d clozapine for a long time without ever inducing RLS, but after the increase in a dosage she developed symptoms [14]. Studies stated variable dosages ranging between 50–325 mg/d, proving that the threshold of RLS induction is distinct in different individuals. RLS is a significantly underdiagnosed condition in primary care settings. Therefore, primary care physicians should be warned about this side effect of clozapine.

Sleeping disturbances have been reported as a symptom of clozapine withdrawal. Rebound cholinergic syndrome is usually very rare, but there have been cases of patients developing it after abrupt discontinuation of clozapine. The study describes a case of a 66 years old Spanish male, who was treated with clozapine for bipolar disorder type I. Three days after clozapine termination he developed insomnia, mutism, dysphagia and trismus. He was catatonic, hypertensive and tachycardic [14]. Some of the other symptoms of cholinergic rebound syndrome are agitation, anxiety, sialorrhea, confusion, psychosis, diarrhea, nausea (with or without vomiting) and sweating. Multiple cases of the syndrome are described in the literature, with the patients on high dose treatment for schizophrenia. This case was quite unusual because the patient was taking 50mg/d of clozapine which is considered as a low dose. Another study describes a case of sleep polygraphically documented rebound insomnia after long-term use of clozapine in a 30 years old schizophrenic male [15]. The patient was taking high doses of clozapine for a long period of time and developed withdrawal symptoms one day after the discontinuation, which could be stopped by clozapine administration. In this case withdrawal symptoms were not explained by cholinergic rebound or dopaminergic sensitivity, but indicated participation of GABAergic and antiglutamatergic activities of clozapine.

Clozapine and hypersalivation

Hypersalivation is a well-known side effect of clozapine affecting 30-80% patients taking this medication. One study reported an even higher rate of 91% [16]. It has a huge toll on the person affected, because of how stigmatizing it can be. It is associated with notable social embarrassment, humiliation, in severe cases aspiration pneumonia and the most importantly higher chance of a patient's poor compliance with the medication. Extreme drooling has also been noted to induce insomnia, because of the uncomfortable state patients are in.

Since clozapine has anticholinergic activity that should reduce secretions, this specific side effect is quite paradoxical. Clozapine-induced hypersalivation (CIH) can be explained by understanding its pathophysiology on a molecular level. Muscarinic receptors both M3 and M4 are expressed in salivary

glands and have opposing actions on one another. When M3 is blocked and M4 activated, they both induce salivation. With all the other previously mentioned properties, clozapine has anticholinergic activity affecting muscarinic receptors (M1, M2, M3, M5) but it stimulates M4 receptors. The combined cholinergic activity of clozapine can be the explanation of CIH paradox.

Clozapine and Its Psychiatric adverse effects

There is a significant association of clozapine with obsessive-compulsive symptoms (OCS) [17-22]. Antipsychotic can lead to de novo or aggravation of OCS in patients who receive clozapine for long term but these symptoms may be managed with reduction in dose or by addition of SSRIs. So far there is no exact theory explaining this fact however long-term case observation and its hypothesis assumes that OCS may be the side effect of second-generation antipsychotics and mostly clozapine. The case where a 35 years old man with treatment-resistant schizophrenia was prescribed 200 mg/d clozapine is the best example of everything mentioned above. Three weeks later, his psychosis improved substantially, but obsessions and cleaning compulsions developed. Patient's family history was positive for OCD. After investigation clozapine was thought to be the cause of his symptoms, so the dose was reduced to 300 mg/d over a period of 1 week. Although the OCS decreased, his psychosis worsened. After that the conclusion was made that Clozapine was re-increased back to 400 mg/d however Aripiprazole (15 mg/d) was added, and the OCS gradually diminished, with complete resolution after 5 weeks. (Alasdair et.al.)

Overall there is about 20-30% risk of primary OCS in patients with schizophrenia. This is considerably the high incidence and it deserves explanation, but until now pathological connection of these comorbidities is not clearly understood. However, everyday there are new case reports, case series and systematic evaluations mention the new de-novo obsessive compulsive symptoms or aggravation of primary symptoms after receiving clozapine.

Some patients with pre-existing OCS/OCD had worsening symptoms with clozapine while others remained at the same level or even improved moreover the number of de-novo OCS in patients within 12 months are highly noticed. But as I mentioned already symptoms are reversible with reduction in dose of medication or by addition of SSRIs.

Clozapine remains the drug of choice in patients with resistant schizophrenia however it is also well known for its side effects. One of the life-threatening psychiatric side effects of the drug is delirium. Several studies were done to show the reality of delirium complication of clozapine, in one of them 139 patients were rolled in from which 72 were women and 67 men. Their ages were 40 +/- 12 years, with clozapine given gradually increased dose to an average daily dose of 282 +/- 203 mg for approximately 20 days. Out of 139 patients delirium was diagnosed in 14 (10.1% incidence). A sum up report has shown that in 10 % of clozapine-treated inpatients delirium occurred.

Delirium as complication has higher rate of occurrence in patients who mostly are older, patients who mainly are exposed to other central anticholinergics, or have other medical co-morbidity though delirium was also clinically recognized in milder cases with clozapine alone and was associated with increased length-of-stay and higher costs, and lower clinical outcome. There are also cases of delirium with clozapine withdrawal, even low dose of clozapine as well as an increasing dosage of clozapine has been noted to precipitate delirium, and what is more, few case studies report the complication after restarting clozapine. Delirium despite being a fatal complication is an under-recognized potential adverse effect of clozapine therapy treatment and has been poorly studied.

With all this data we might conclude that mostly clozapine-induced encephalopathy risk escalates with rapid dosage increases, high dose therapies, and when the patient takes many medications in combination with clozapine however these don't exclude clozapine-induced delirium in milder cases.

Neuroleptic malignant syndrome (NMS) is a life-threatening complication of antipsychotic drugs and is characterized by high fever, altered mental status, peripheral muscle rigidity and autonomic dysfunction, irregular pulse, tachypnea, tachycardia. Clozapine was initially thought not to cause NMS. But over time some cases report NMS with the association of clozapine as a single agent that developed NMS. Case report of a 28-year-old male with nine-year history of paranoid schizophrenia and no prior history of NMS was receiving only aripiprazole for seven months; however because of resistance, treatment with clozapine was started. On day 8 the patient developed confusion, high fever, tachypnea,

yet no general muscle stiffness was noted on the exam. Creatinine levels increased one day later. Patients oral medications were discontinued except as needed-lorazepam, the patient received intravenous hydration and supportive measures. He recovered within ten days (Andrew Farah, Department of Psychiatry, UNC). The features of clozapine-induced NMS may be somehow different, with fewer muscle rigidity and a lower rise in creatine kinase levels. Waiting for muscle rigidity or fever may delay or even prevent the diagnosis and treatment of NMS.

NMS usually occurs within two weeks after starting clozapine however may occur at any time. Significant risk factors of developing this complication increases in patients using other mood stabilizer along with clozapine (The combination of clozapine and aripiprazole has induced NMS in five case reports, including the one above) and in patients who had a previous history of NMS (found to reoccur in 50% of those patients) however it may occur de-novo without any previous history or aggravating factors.

Conclusion

Varma et al. found a relationship between mean clozapine levels and EEG changes but Kikuchi et al. failed to find a statistical significance that we believe is due to their small number of cases studied. Clozapine plasma levels and EEG changes were found to be in relation. Although higher clozapine dose is correlated with higher risk of seizures, Varma et al. did not find significance. As for clozapine plasma levels and seizure occurrence, significant relationship was found [1]. Other common acute adverse effects include: Parkinsonism, acute akathisia, acute dystonia while chronic ones are: tardive dystonia, chronic akathisia, tardive dyskinesia. We have listed some common extrapyramidal adverse effects of Clozapine and also brought some interesting studies which show comparison to other antipsychotics, association with movement disorders and controversial theories about using the drug. Clozapine's ocular side effects are similar to those observed with chlorpromazine use. As for the cataplexy, it is still controversial whether it is a true cataplexy or negative myoclonus. Sedation significantly decreases the patient's quality of life. Speaking of sleep disturbances, clozapine increases the number of awakenings, but at the same time the total sleep period is significantly prolonged. Patients report being more refreshed and relaxed upon waking up. The strong association between clozapine and hypersalivation is reported by a great deal of studies. Finally, as other neuroleptics, clozapine is prone to elicit psychiatric side effects such as emotional indifference, depression, restlessness however among all these are several severe complications that should be emergently recognizes and treated, those are clozapine induced obsessive-compulsive symptoms, delirium and the neuroleptic malignant syndrome.

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THE EFFICACY OF CGRP ANTAGONISTS IN THE TREATMENT OF MIGRAINE

(Review Article)

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Abstract

Migraine is one of the most common and disabling diseases in the world. The usage of Calcitonin Gene-Related Peptide (CGRP) antagonist drugs has been on the horizon and looks to be a promising treatment option. Growing evidence suggests that CGRP plays a key role in the development of peripheral sensitization and associated enhanced pain. CGRP is involved in the development of neurogenic inflammation and is upregulated in inflammatory and neuropathic pain conditions. Drugs like Erenumab, Rimegepant, Olcegepant and others of the same category have shown significantly fewer side effects along with high therapeutic effects, which makes this group of drugs a useful addition to the existing therapeutic options.

Introduction

With a prevalence of 11.6% worldwide, 13.8% in females, 6.9% in males, the World Health Organization is listing migraine as the fifth most disabling disease in the world. Migraine is one of the top

10 causes of disability that affects work productivity and social functioning, regardless of ethnicity, geography and socioeconomic status [2].

According to the International Headache Society, a migraine is a headache that lasts for 4 to 72 hours and has at least two of the following characteristics: unilateral localization, pulsating quality of pain, moderate to severe intensity, and aggravation by motion. In addition, at least one of the following two symptoms has to accompany the headache: nausea and/or vomiting, or photophobia and/or phonophobia. Typically, migraine is episodic, but some patients experience chronic migraine headaches occurring at least 15 days a month. The migraine headache is almost always preceded by the premonitory phase that can last for hours. Tiredness, gastrointestinal problems, and mood changes are the most commonly reported symptoms, and these can persist for the entire migraine attack. A recovery or postdrome phase, which is characterized by fatigue and continued sensory disturbances, often follows the headache [10].

There have been well-established studies showing acute and prophylactic treatment, and even Botulinum toxin to be effective for migraine, however, because of their considerable side effects (e.g., selective vasoconstriction in case of triptans), these drugs have somewhat restricted use. Hence the need for newer better alternatives [10].

A CGRP receptor is a new therapeutic target for migraine treatment. In the underlying pathophysiology of migraine, the release of the CGRP from trigeminal nerves is now thought to play a central role. Recent studies have shown that the CGRP levels are elevated during a migraine episode and that an infusion of CGRP can in fact trigger a migraine attack. Furthermore, it is seen that the serum level of CGRP in the external jugular vein is elevated in patients with all forms of vascular headaches, including migraine and cluster headaches. In addition, the absence of vasoconstrictor activity may prove to be a major benefit of the use of CGRP receptor antagonists in migraine treatment [4,9,10].

In this article, we have reviewed selected studies that show the efficacy of CGRP antagonists for the prophylaxis and treatment of migraine.

Etiology

The pathophysiology of migraine is associated with the trigeminal innervation of pain-producing intracranial structures. The ophthalmic division of the trigeminal ganglion gives rise to a plexus of largely unmyelinated fibres which surround the large cerebral vessels, venous sinuses and dura mater [5]. The trigeminal branches that innervate cerebral vessels arise from neurons, which are located in the trigeminal ganglion. These cells contain vasoactive substances, namely substance P and calcitonin gene-related peptide (CGRP). A study conducted in 1988 concluded that these vasoactive substances are released in the extracerebral circulation during the activation of the trigeminal nerve ganglion. Migraine is thought to be caused by the irritation of the trigeminal nerve followed by the increased release of CGRP which has vasodilatory effects on cerebral vessels [6]. Nerves that innervate the cranial vessels consist of myelinated and unmyelinated fibres, which are responsible for the severe pain experienced during a migraine attack. The activation of trigeminovascular systems increases the blood level of CGRP by 85% [11].

The pathophysiological basis of CGRP paves way for the treatment of migraine. Modulation of the release of CGRP from trigeminal ganglions can aid in the treatment of severe migraine.

Andreou et al. report that glutamate plays a role in the pathophysiology of CGRP release. According to this study, receptors with the GluR5 subunit (iGluR5 glutamate receptor) are present on the trigeminal ganglion and could potentially be involved in mediating pain in migraine attacks. Activation of the iGluR5 kainate receptor with its agonist called iodowillardiine causes inhibition of the vasodilation effect that is mediated with CGRP. Therefore, it can be concluded that activation of the glutamate receptors can have a therapeutic effect in migraine attacks [1]. Another interesting way to treat migraine attacks is by directly inhibiting the CGRP receptors via CGRP receptor antagonists. For example, Olcegepant is a highly potent and a very specific antagonist of CGRP receptors [8].

Existing data

Olsen et al. showed that 2.5 mg of Olcegepant had a 66% success rate [9].

Another trial by Goadsby et al. showed a significant reduction of the migraine attack frequency using a monthly dose of 70 or 140 mg of Erenumab Migraine attacks [7].

The knowledge about CGRP receptors in the CNS is limited but their functions on the enteric and peripheral nervous system has been well established. The side effects that have been detected so far have been mild, including paresthesia, nausea, headache, dry mouth and unspecific visual disturbances. However, further observations are needed to evaluate the long-term side effects of these novel medications. [3].

Conclusions

The CGRP plays a major role in the pathogenesis of migraine headaches. CGRP antagonists are effective anti-migraine drugs showing efficacy for both migraine treatment and prophylaxis.

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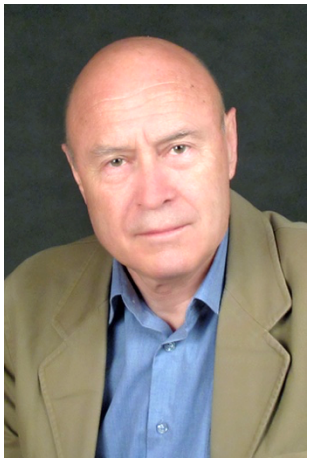

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