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ABSTRACTS

MAKA GONGADZE, NUNU GOGIA, TSITSINO ATAMASHVILI, MAKA MACHAVARIANI, MAIA ENUKIDZE, MANANA IOBADZE

IL10$^{+/+}$ AND IL10$^{-/-}$ TROPHOBLAST CELLS PROLIFERATION, MIGRATION AND INVASION DURING HYPERGLYCEMIA AND EXPRESSION OF CENTRAL MOLECULES

TSMU Institute of Medical Biotechnology, Tbilisi, Georgia

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მაკა გონგაძე, ნუნუ გოგია, ციცინო ათამაშვილი, მაკა მაჭავარიანი, მაია ენუქიძე, მანანა იობაძე

IL10$^{+/+}$ და IL10$^{-/-}$ ტროფობლასტის უჯრედების პროლიფერაცია, მიგრაცია და ინვაზია ჰიპერგლიკემიის დროს და ცენტრალური მოლეკულების ექსპრესია

თსსუ სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი, საქართველო

რეზიუმე

ემბრიონის წარმატებული იმპლანტაციისთვის აუცილებელია ფუნქციური კომუნიკაცია ბლასტოცისტსა და მიმღებლიან ენდომეტრიუმს შორის, ხანმოკლე პერიოდში, რომელიც ცნობილია როგორც იმპლანტაციის ფანჯარა. დიაბეტის დროს იმპლანტაციის ფანჯარა ირღვევა, ხელშემშლელი პირობებია ნორმალურ იმპლანტაციისთვის, რასაც მივყავართ სპონტანურ აბორტებამდე. წინამდებარე კვლევის მიზანი იყო იმპლანტაციის პროცესში IL-10, TNF-α, LIF, MMP-9 და NO-ს როლის შეფასება და მათი ზეგავლენის დადგენა ტროფობლასტური უჯრედების ფუნქციურ აქტივობაზე ჰიპერგლიკემიის დროს. ორსული IL-10$^{+/+}$ (C57BL/6J) და IL-10$^{-/-}$ (B6.129P2-Il10tm1Cgn/J) თაგვების პლაცენტებიდან გამოყოფილ ტროფობლასტების უჯრედებზე ჩატარებულ იქნა ექსპერიმენტები. შესწავლილ იქნა გლუკოზის გავლენა მათ ფუნქციურ აქტივობაზე და ზემოაღნიშნული მარკერების ექსპრესიაზე, უჯრედების IL-10, TNF-α, LIF-ით სტიმულაციისას. ჩვენი კვლევის შედეგებიდან ჩანს, რომ ჰიპერგლიკემიის დროს, ანტიანთებითი ციტოკინი IL-10 მოქმედებს როგორც TNF-α-ს ინჰიბიტორი და ამცირებს ოქსიდაციურ სტრესს. ის არეგულირებს LIF და MMP-9-ის ექსპრესიას და გავლენას ახდენს ტროფობლასტური უჯრედების ინვაზიისა და პროლიფერაციის უნარზე. მიღებული შედეგებიდან შეიძლება დავასკვნათ, რომ ჰიპერგლიკემიის დროს IL-10 და TNF-α მნიშვნელოვან როლს თამაშობენ იმპლანტაციის პროცესებში. გარდა ამისა, IL-10 ამცირებს ჰიპერგლიკემიით გამოწვეულ ტროფობლასტური უჯრედების დისფუნქციას და შესაძლოა ამ პროცესში გადამწყვეტ როლს LIF, MMP-9 და NO ასრულებენ.

Introduction

Successful embryo implantation requires a functional communication between a blastocyst and a receptive endometrium during a brief period known as the window of implantation. During the window of implantation, the blastocyst can attach to the endometrial epithelial cells and invade the endometrial stroma and vasculature. This process can only occur when the endometrium is receptive [10]. The process of implantation in humans involves a coordinated sequence of events that are critical for the establishment of pregnancy.

On the one hand, the success of embryo implantation depends on achieving the orchestration of trophoblast proliferation, migration, and invasion into the endometrium to establish not only the anchoring to the uterine wall but also a blood supply for the conceptus [3,9]. This angiogenic process depends on spiral arteries invasion, occlusion, and endothelial remodeling by a highly invasive and migratory sub-population known as extravillous trophoblast (EVT). They invade the uterus and remodel its vasculature to establish an adequate exchange of key molecules between maternal and fetal circulation.

On the other hand, the feto-maternal interface is constituted by a complex net of cytokines, which regulates immunomodulation as well as the vascularization process [1]. They play an important role in the adhesion of the blastocyst to the luminal epithelium, facilitating the physical contact between embryo and uterus and promoting placental development [8]. Implantation can be characterized as an inflammatory response and cytokines are responsible for this response. It seems to be crucial to identify which molecules are implicated in the transition from an inflammatory process before implantation to an anti-inflammatory response necessary for placental vascularization.

Leukemia inhibitory factor (LIF) has been demonstrated to be an important factor in relation to endometrial receptivity [14]. Women with recurrent implantation failure have shown a decrease in LIF production [7]. LIF regulates trophoblast cell adhesion and might be important for embryo invasion and placental development [19]. The invasion of trophoblast cells is regulated by many factors. Matrix metalloproteinase family (MMP), mainly MMP- 9 is closely related to the invasion of trophoblast cells [16]. There are several pro- and anti-inflammatory cytokines which are required for proper implantation, but we are more interested in the cytokines, which play a role in diabetes.

Diabetes is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia), causing many complications. Among such complications, diabetes in a pregnant mother can cause reproductive abnormalities, abortion, congenital anomalies, alterations of fetal growth, and neonatal morbidity and mortality [5,12]. However, there is little information about the influences of diabetes on reproductive performance, placental morphology, and immune responses at the feto-maternal interface.

At present, most researchers accept that a hyperglycemia-induced increase in the production of reactive oxygen species (ROS) is an initial key event in the pathogenesis of diabetes-induced abnormalities. [11,15]. However, as ROS are capable of regulating numerous intracellular signal transduction pathways [2], subsequent pathological events seem to be far from completely understood. Key molecules, like interleukin-10 and tumor necrosis factor-alpha (TNFα), are involved in these processes. IL-10 inhibits TNFα production [13] and its expression is regulated by ROS and it regulates ROS production in turn [18]. The aim of our current study is to evaluate the role of IL-10, TNFα, LIF, MMP-9, and NO in implantation and to establish their possible impact on the functional ability of trophoblast cells under hyperglycemic conditions.

Materials and Methods

Experimental animals: IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$(B6.129P2-*Il10*^{tm1Cgn}/J) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed under specific pathogen-free (SPF) conditions in an animal facility with fresh food and water ad libitum, temperature (22.0°C ± 1°C), humidity (40%-60%), and light (12-hour/12-hour light/dark). 8–14 weeks old female mice were caged with males for 3 hr., from 7 to 10 am (dark period) and the presence of a vaginal plug (11 am) was determined as the first day of pregnancy. On day 11 placentas were removed and prepared for trophoblast cells purification.

Purification of trophoblast cells: Villous cytotrophoblasts were isolated by a procedure established previously [4] and used in our laboratory with slight modifications. Briefly, term placentas were first subjected to limited digestion with 0.125% trypsin (Life Technologies, Taastrup, Denmark) and 0.01% DNase I (Roche Molecular Biochemicals, Hvidovre, Denmark). The trophoblast cells were then enriched by centrifugation on a discontinuous gradient of Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) consisting of 70 and 25% concentrations, and finally purified by negative immunoselection to remove contaminating cells. This procedure involved a mouse monoclonal antibody (MAb) against the monomorphic determinant of MHC class I antigen (clone W6/32; Dako, Glostrup, Denmark) and paramagnetic beads (Dynabeads M-450 goat anti-mouse IgG; Dynal, Oslo, Norway).

Trophoblast cell culture: Isolated trophoblasts were cultured in Dulbecco's Modified Eagle's Media (DMEM) (Cell Biology media facility, Yale University) supplemented with 10% fetal calf serum and 25 mmol/L Hepes, 4 mmol/L glutamine, and 50 mg/ml penicillin, streptomycin, neomycin (Gibco BRL, Grand Island, NY). The cells were plated at a density of 1x104cells/cm2 and cultured for 18h under a humidified 5% CO2/95% air atmosphere at 37^{0}C in the respective medium supplemented with either 5,5 mmol/l (normoglycemic control) or 25mmol/l D-glucose (hyperglycaemic group). The influence of IL-10, TNFα, or LIF on cells cytokines expression ability and cells functionality were studied and the media was supplemented with each cytokine in doses of 100ng/ml, 100ng/ml, or 10ng/ml respectively. These cells and their supernatants were examined in further experiments.

Cell proliferation assay: The isolated trophoblast cells were plated onto 96-well chambers in a volume of 100µl of the medium (5X104 cells/ml). Thereafter, the cell viability was analyzed by Cell Titer 96 AQueous One Solution cell proliferation assay kit (Promega KK, Tokyo, Japan) according to the manufacturer's instructions. Three independent assays were performed from at least triplicate samples.

Invasion and migration assay: The trophoblast cells were used for the invasion or migration assays. These procedures were performed as previously reported [22]. Briefly, 24-well Matrigel invasion kits (BD Biosciences) or 24-well Transwell cell culture chambers (Costar, Ettlingen, Germany) were used for the invasion or migration assays, respectively. The incubation period of the migration assay was 20 h, whereas that of the invasion assay was 24 h. The number of migrated or invaded cells was counted at X100 magnification. Four individual experiments were performed in triplicate.

IL-10, TNF-α, LIF, and MMP-9 measurement: All these markers concentrations were quantified in cells supernatants using specific Quantikine® enzyme-linked immunosorbent sandwich assays (ELISA) (R & D Systems, Minneapolis, USA) according to manufacturer's instructions. The TNF-α assay was linear from 15 to 960 pg/mL and sensitivity was 5.0 pg/mL; and for the IL-10 assay, a standard curve was developed from 1.25 to 2000 pg/mL, with a sensitivity of 1.0 pg/mL. LIF and MMP-9 expressions were analyzed and absorbance was read at 450 nm with wavelength correction at 590 nm.

Measurement of Nitric Oxide production: NO production was analyzed by measuring nitrite/nitrate in the lysates by Griess reaction-based colorimetric assay (R & D Systems, Minneapolis, USA) according to the manufacturer's instructions. The absorbance was read at 540 nm with wavelength correction at 690 nm.

Statistical analysis: The data were expressed as the mean ± SD and the statistical analyses were performed with Student's test. The differences were considered statistically significant when P was < 0.05.

Results

Cell Proliferation

To investigate the effect of hyperglycemia on trophoblast cells proliferation activity and the influence of IL-10, TNF-α and LIF stimulation on this process, a series of experiments were conducted. For this purpose, Villous cytotrophoblasts were isolated from placentas of IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$ (B6.129P2-*Il10*^{tm1Cgn}/J) mice on day 11 of pregnancy, and cell viability was analyzed by the cell proliferation assay kit. As shown in Figure 1, in a normal environment proliferation of trophoblasts cells from wild-type and knockout mice placentas are similar.

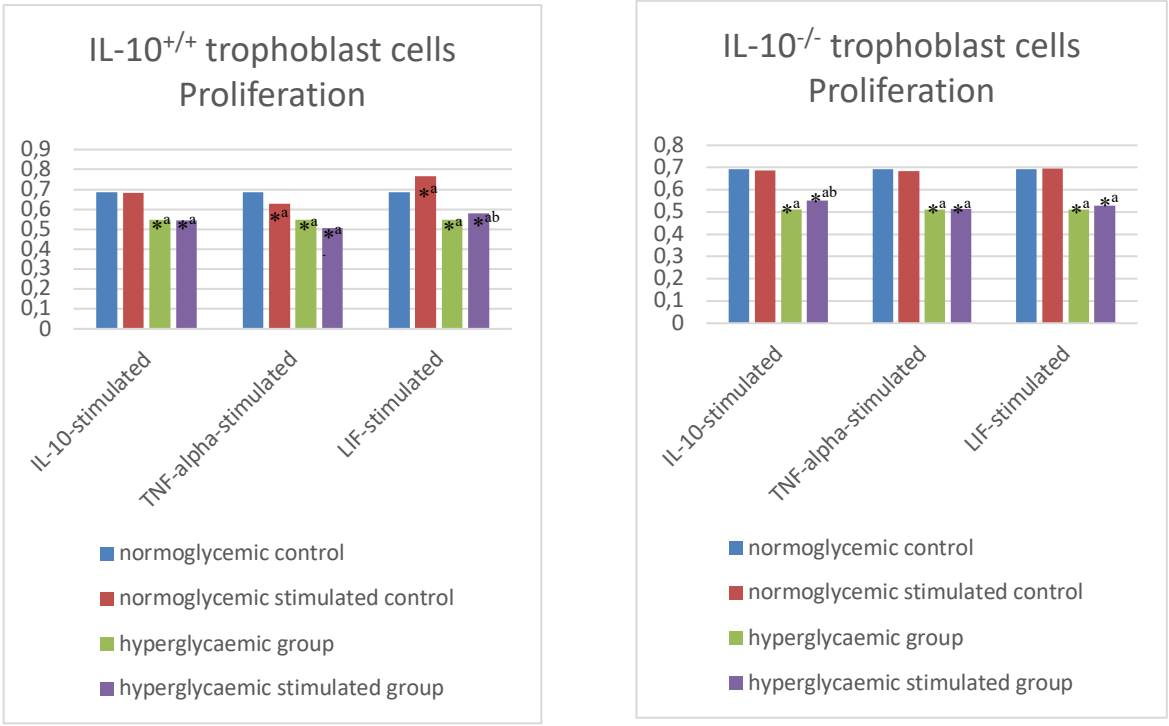

Figure 1. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on proliferation of trophoblast cells was measured by AQueous One Solution cell proliferation assay kit. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^{a}$ $p < 0.05$ vs. normoglycemic control group; $*^{b}$ $p < 0.05$ vs. hyperglycemic group.

Hyperglycemia induces retardation of trophoblast cells growth and reduces proliferation rate in both wild-type and knockout cells approximately by 20% and 26%, respectively. Wild-type cells stimulation with IL-10 didn't have any effect on proliferation capacity, whereas in a hyperglycemic environment proliferation rate of knockout cells was increased by 10%. The opposite effect was observed with TNF-α stimulation, it decreases wild cells proliferation activity in both, normoglycemic and hyperglycemic media, while doesn't affect knockout cells growth. Also, as shown in figure 1, LIF stimulation increases cells proliferation activity; there are significant differences between the growth of wild-type cells in normal media, as well as in media with a high content of D-glucose, with or without the addition of LIF. However, there is not enough significance in knockout cells.

Cell Invasion Capacity

For the same reason, as described above, the experiments were conducted and the trophoblast cells invasion ability was investigated. In these experiments, the incubation period was 24 h. and a 24-well Matrigel invasion kit was used. The number of invaded cells was counted and presented in Figure 2. As shown, D-glucose at doses of 25 mmol/l significantly reduces the invasion capacity of trophoblasts cells from the placenta of wild-type and knockout mice, up to 87% and 80% respectively. The stimulation of wild-type cells with IL-10 affects the cells' invasion capacity in normoglycemic media and decreases it by 11%, while under conditions of hyperglycemia does not affect the ability to invade these cells, as well as the ability of knockout cells under both normoglycemic and hyperglycemic states. A strong suppressive effect was observed with TNF-α stimulation; it reduces both types of cells invasion capacity in the normoglycemic and hyperglycemic environment. In addition, as shown in figure 2, LIF stimulation increases the invasion ability of wild-type cells in normal media, while it does not affect knockout cells.

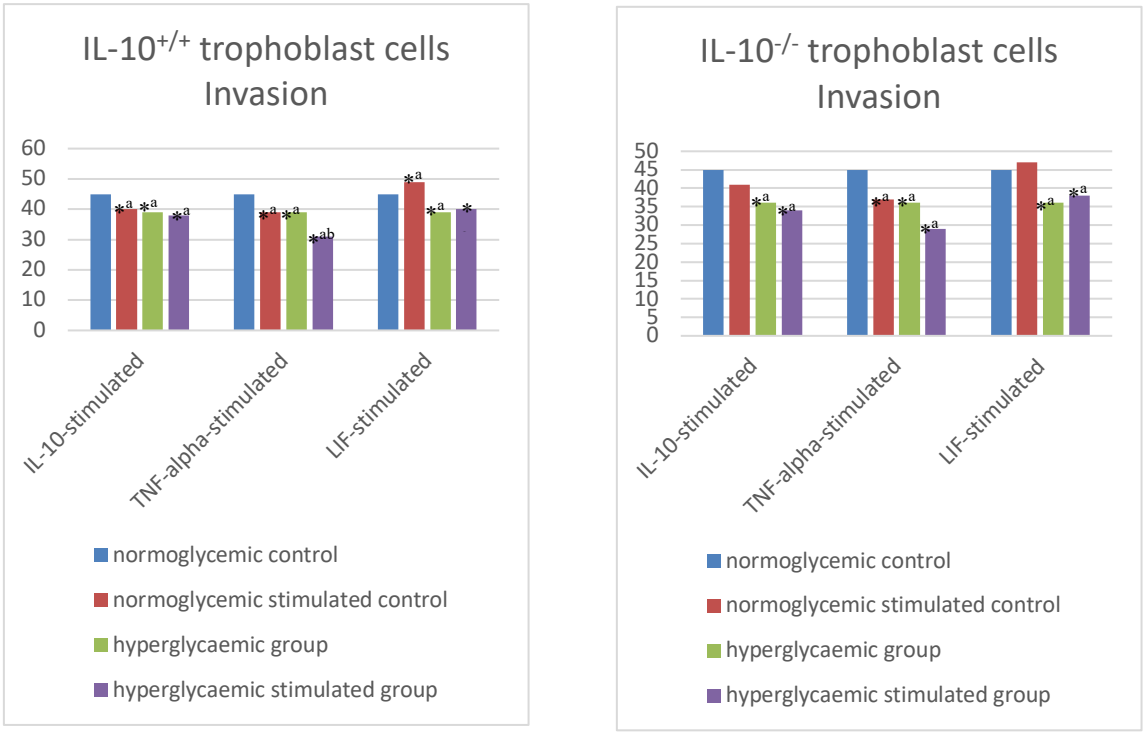

Figure 2. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on invasion capacity of trophoblast cells was performed by 24-well Matrigel invasion kit. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^{a}$ $p < 0.05$ vs. normoglycemic control group; $*^{b}$ $p < 0.05$ vs. hyperglycemic group.

Cell Migration Capacity

The effect of hyperglycemia on trophoblast cells migration ability and the influence of IL-10, TNF-α and LIF stimulation on this process were investigated. For this reason, 24-well Transwell cell culture chambers were used and migrated cells were counted after 20 h of incubation. The data show that the

concentration of D-glucose in the medium influences cells migration capacity (Figure 3.) The ability of wild-type and knockout trophoblast cells to migrate is significantly reduced by glucose (approximately 85% and 92% respectively). Stimulation of these cells by IL-10 and LIF does not affect cells migration capacity. However, TNF-alpha has a decreasing effect on migration ability and these indices of wild-type and knockout mice trophoblast cells in normal media are approximately 86% and 87% of their control levels. The cells migration in media supplemented with TNF-α and high glucose concentration are decreased up to 77% and 85% compared with hyperglycemic group functionality, without the addition of TNF-α.

IL-10, TNF-α, LIF, MMP-9 and Nitric Oxide expression

The effect of hyperglycemia and/or IL-10, TNF-α, and LIF stimulation on the expression of several markers, mainly IL-10, TNF-α, LIF, MMP-9, and Nitric Oxide by the trophoblast cells of the placentas of wild-type and knockout mice were investigated. For this reason, the trophoblasts cells from wild-type and knockout mice were cultured for 18 h in normoglycemic (control) and hyperglycemic media and supernatants were analyzed using ELISA or Griess reaction-based colorimetric assay. As shown in Figure 4 and Figure 5, the results are presented in a fold of control. Wild-type trophoblast cells in normal media produce IL-10, TNF-α, LIF, MMP-9, and Nitric Oxide and their expression levels are changed by hyperglycemic medium; It is high glucose level reduces the expression of IL-10 and LIF up to approximately 60% and 80%, and increases the production of TNF-α, MMP-9 and NO by 2.6, 2.8 and 3.6 times, respectively (Figure 4). Whenever the medium was supplemented with cytokines (namely IL-10, TNF-alpha, or LIF), this picture was modified completely.

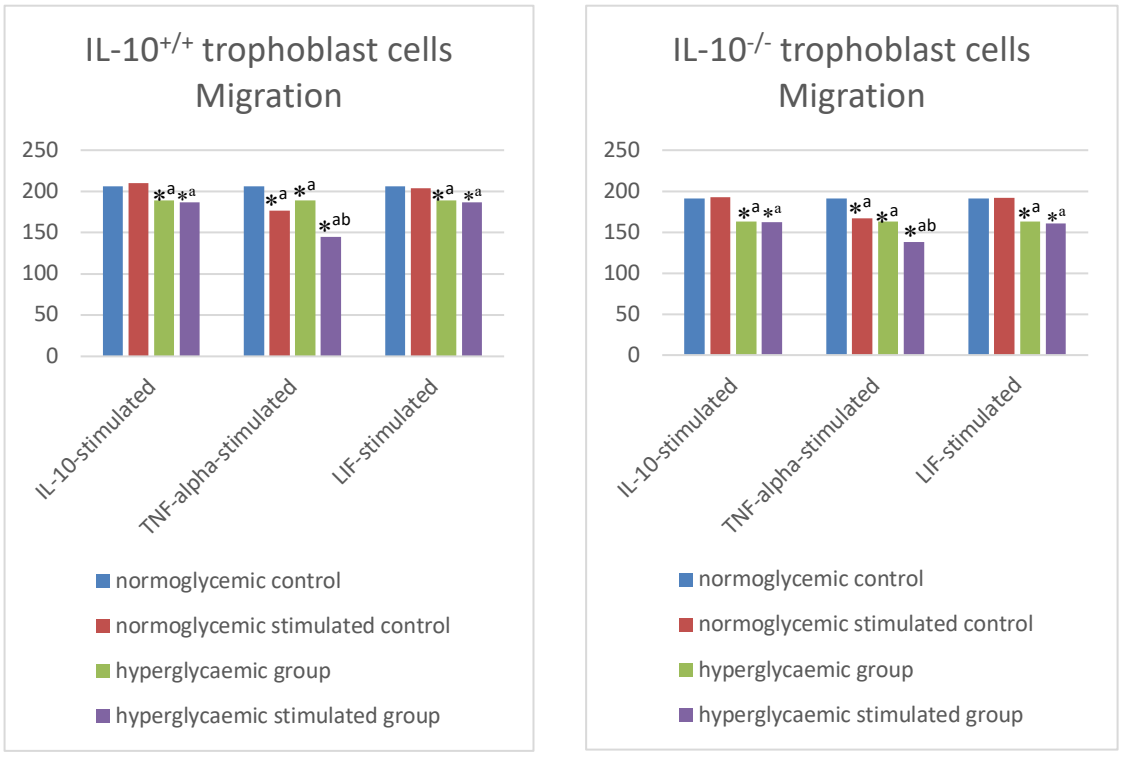

Figure 3. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on migration capacity of trophoblast cells was performed by using 24-well Transwell cell culture chambers. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

Wild-type cells stimulation with IL-10 and LIF under normal conditions induces the release of higher levels of IL-10 and LIF, while the opposite effect was observed with stimulation by TNF-α. It is also important to mention that stimulation with TNF-α increases NO and MMP-9 production in the normoglycemic state. The stimulation with IL-10 in a hyperglycemic environment significantly reduces the differences between the expression levels of IL-10, TNF-α, LIF, MMP-9 and Nitric Oxide in control

and glycemic groups; trophoblast cells produce approximately 1.6, 1.6 and 1.7-times more TNF-α, MMP-9 and NO respectively, also LIF level grows up to checkpoint and IL-10 to 80% (Figure 4). Stimulation of wild-type cells with LIF doesn't affect the production of MMP-9 and NO productivity in a hyperglycemic environment, but it downregulates glucose-induced contrast for IL-10, TNF-α and LIF. The opposite effect was observed with TNF-α stimulation, it increases the contrast between control and hyperglycemic groups.

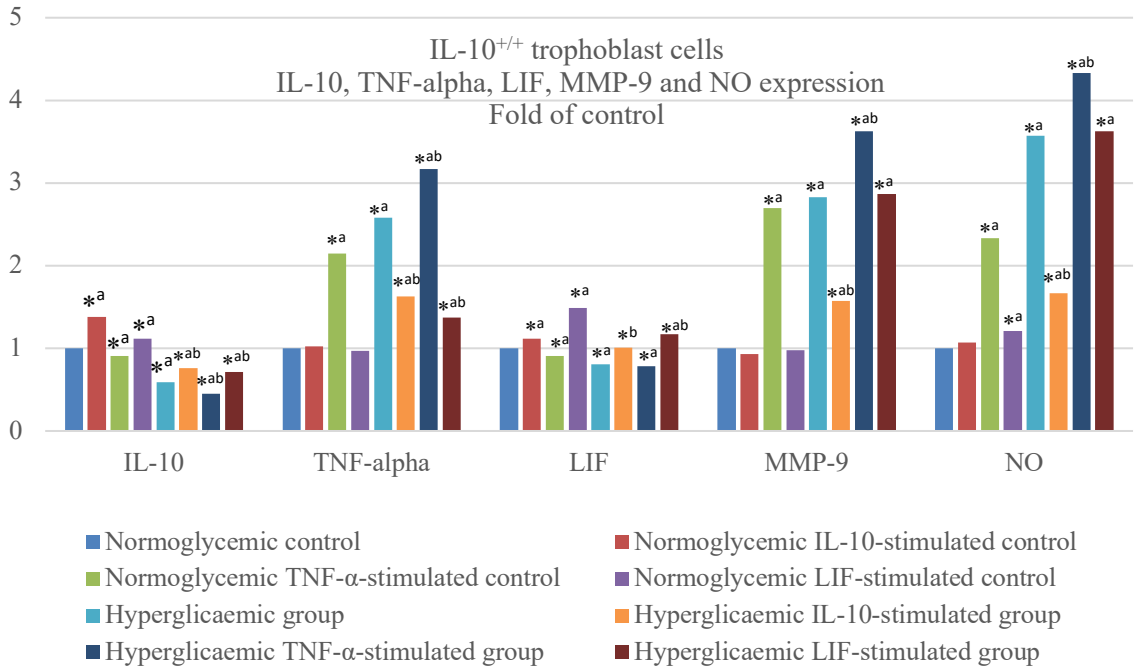

Figure 4. The effect of hyperglycemia and/or IL-10, TNF-α or LIF stimulation on expression of several markers, such as IL-10, TNF-α, LIF, MMP-9 and NO, in wild type trophoblast cells was analyzed by using "R&D systems" assay kits. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed in fold of control. $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

As shown in Figure 5, Our results verify that IL-10 null mutant mice used in experiments were authentic knockout; their trophoblast cells did not produce IL-10, and only after IL-10 stimulation was this cytokine detected. High glucose concentration alters the expression ability of IL-10-/- trophoblast cells to produce TNF-α, LIF, MMP-9 and Nitric Oxide and it seems, that knockout cells are more sensitive to hyperglycemic insult. There is a decrease in LIF expression to about 70% and an increase in the production of TNF-α, MMP-9 and NO by 3.25, 3.43 and 4.94 times, respectively. Stimulation of these cells by cytokines, such as IL-10, TNF-alpha or LIF induces this pattern modification. knockout cells production rates of investigated markers weren't changed under normal conditions after IL-10 or LIF stimulation, except for their levels. The opposite effect was observed with TNF-α stimulation under normoglycemic conditions, it increases the production of NO, MMP-9 and TNF-α approximately by 2.8, 2,7, and 2,2 respectively, and reduces LIF expression by 10%. The stimulation with IL-10 in a hyperglycemic environment significantly regulates the production of these markers, upregulates LIF expression to the checkpoint, and downregulates the expression levels of TNF-α, MMP-9 and Nitric Oxide up to approximately 2.6, 2.7 and 3.3 fold of control. As shown in Figure 5, there are no significant differences between control and hyperglycemic groups after LIF stimulation of these cells in a hyperglycemic state, other than intrinsic concentration. A strong effect was observed when TNF-α and 25mmol/l D-glucose were supplemented in the medium, knockout trophoblast cells released higher levels of TNF-α, MMP-9 and NO; these measures divided by the control level are 3.7, 3.9 and 5.7 respectively.

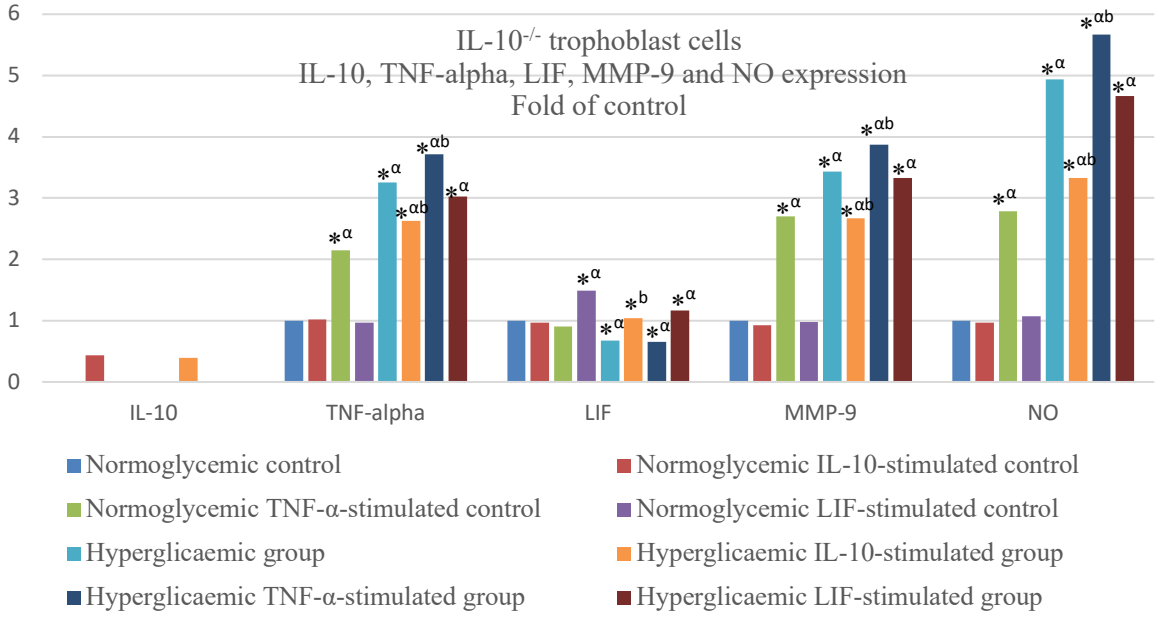

Figure 5. The effect of hyperglycemia and/or stimulation of any cytokines IL-10, TNF-α or LIF on expression of several markers, such as IL-10, TNF-α, LIF, MMP-9 and NO, by knockout (IL-$10^{-/-}$) trophoblast cells was investigated. The production of these markers was analyzed by using "R&D systems" assay kits. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean. $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

Discussion

Successful implantation requires a receptive endometrium, a functional embryo and a synchronized dialogue between them. Under certain inflammatory conditions, such as diabetes, the window of implantation can be affected preventing normal implantation which could lead to pregnancy loss. The results of our previous study suggest that diabetes-induced pregnancy loss resulted from the death of peri-implantation stage embryos [6, 20]. During diabetes mellitus blastocysts develop normally and the cause of pregnancy loss is unsuccessful implantation in the uterus. Embryo–maternal communication is principally mediated via the action of several cytokines and chemokines and especially via interleukins (ILs), which constitute an essential part of the uterine microenvironment. Interleukins significantly affect the process of embryo implantation, from decidua formation and embryo acceptance to trophoblast invasion and placenta formation [17, 20]. However, abnormal IL production, as it happens during diabetes, may detrimentally affect implantation, despite embryos being of good quality and high developmental dynamic, subsequently leading to pregnancy failure. However, the exact role of ILs on the pathophysiology of diabetes is poorly understood and still debatable. On the assumption of literature data, we hypothesized, that IL-10 has an important role in the implantation processes during diabetes. To improve our hypothesis experiments were conducted on trophoblast cells isolated from placentas of IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$(B6.129P2-*Il10*^{tm1Cgn}/J) pregnant mice. The cells were stimulated by various cytokines and the effect of glucose concentration on their functional activity and expression ability of several markers were determined.

The results of our investigation showed that trophoblasts cells from knockout mice incubated in media with high glucose concentration released higher levels of pro-inflammatory cytokine TNF-α and NO than cells from wild-type mice. During hyperglycemia anti-inflammatory cytokine IL-10 occurs as an inhibitor of TNFα releases and thereby decreases oxidative stress which is an initial key event in the pathogenesis of diabetes-induced pregnancy loss. As known, TNFα induces activation of the transcription factor NF-κB, which has the potential both to stimulate the expression of TNFα and inducible Nitric Oxide

Synthase, which in turn produced NO overexpression. Diabetes mellitus induces alterations of NO production in tissue. NO is able to modulate the activation of MMPs in the fetoplacental unit and provides supportive evidence that increases NOS activity. During diabetes, MMP-9 and Leukemia Inhibitory Factor (LIF) production in the uterus are fully disturbed, and because of that no uterine receptivity for implantation. As our results showed, hyperglycemia reduces LIF production in wild-type and knockout cells; also, under hyperglycemic conditions, there is an overexpression of MMP-9, but its effect is higher on knockout cells. IL-10 stimulation improves LIF expression up to the control level and drops MMP overexpression. As regards LIF stimulation, it also reduces the imbalance of anti and pro-inflammatory markers productivity between normoglycemic and hyperglycemic environments.

Proper invasion of trophoblast into the endometrium is necessary for normal placentation. The results of our investigation establish that IL-10 evolved in the control of invasion and proliferation of trophoblast cells during diabetes. In a normal environment proliferation, invasion and migration of trophoblasts cells from wild-type and knockout mice are similar. D-glucose in doses of 25 mmol/ml significantly decreases cells proliferation, invasion and migration capacity. From our results, we can conclude, that IL-10 has no influence on cell proliferation and invasion, but it's important in a stressful environment. Unclear is the role of IL-10 in cells migration capacity. TNF-α reduces proliferation, invasion and migration of trophoblasts cells from wild-type and knockout mice, but it is necessary to mention, that IL-10 deficient cells are much sensible to this pro-inflammatory cytokine and diabetic environment. From our data we can also conclude, that TNF-α fully downregulates trophoblast functionality, it reduces cell proliferation, invasion and migration capacity. Also, our results carried out on knockout and wild-type cells show that LIF improves the ability of cells to proliferate and invade and IL-10 participates in this process because LIF stimulation works out just in wild-type cells. There is no significant data to understand the role of this cytokine in the migration capacity of trophoblast cells. Also, the results of our investigation have shown, that the stimulation of the cells with anti-inflammatory cytokine IL-10 in the non-diabetic environment affects cells functionality and suggests, that IL-10 is an important cytokine in hyperglycemic, but not in a normal environment; high concentration of this cytokine leads to the imbalance of production of crucial mediators and mediates unsuccessful implantation by this mechanism.

In conclusion, the results of our study suggest that IL-10 and TNF-alpha are crucial cytokines in implantation processes during hyperglycemia. TNFα act as a mediator of diabetes-induced embryotoxic stimuli leading to the death of peri-implantation stage embryos and IL-10 as a suppressor of diabetes-induced abortion in this stage. In addition, they suggest that molecules such as NO, LIF and MMP-9 may be critical players in the mechanisms determining the outcome of diabetes-induced embryopathic stress.

Acknowledgments

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MAKA GONGADZE, NUNU GOGIA, TSITSINO ATAMASHVILI, MAKA MACHAVARIANI, MAIA ENUKIDZE, MANANA IOBADZE

IL10$^{+/+}$ AND IL10$^{-/-}$ TROPHOBLAST CELLS PROLIFERATION, MIGRATION AND INVASION DURING HYPERGLYCEMIA AND EXPRESSION OF CENTRAL MOLECULES

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SUMMARY

Successful embryo implantation requires a functional communication between a blastocyst and a receptive endometrium during a brief period known as the window of implantation. Under certain inflammatory conditions, such as diabetes, the window of implantation can be affected preventing normal implantation which could lead to pregnancy loss. The aim of our current study was to evaluate the role of IL-10, TNFα, LIF, MMP-9, and NO in implantation and to establish their possible impact on the functional ability of trophoblast cells under hyperglycemic conditions. For this reason, the experiments were conducted on trophoblast cells isolated from placentas of IL-10$^{+/+}$ (C57BL/6J) and IL-10 $^{-/-}$(B6.129P2-*Il10*^{tm1Cgn}/J) pregnant mice. The cells were stimulated by IL-10, TNF-alpha and LIF, and the effect of glucose concentration on their functional activity and expression ability of several markers were determined. The results of our investigation showed that during hyperglycemia anti-inflammatory cytokine IL-10 occurs as an inhibitor of TNFα production and decreases oxidative stress. IL-10 regulates LIF and MMP expressions and evolves in the control of invasion and proliferation processes of trophoblast cells. In conclusion, IL-10 and TNF-alpha play a central role in the processes associated with implantation during hyperglycemia. IL-10 is a suppressor of hyperglycemia-induced trophoblast cell dysfunction and LIF, MMP-9, and NO may be critical players in it.

Keywords: Hyperglycemia, IL-10, Trophoblast cell, TNF-alpha, LIF, MMP-9, NO

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IMMUNODEFICIENT STATES IN PRETERM AND TERM INFANTS WITH RECURRENT RESPIRATORY DISEASES

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დარეჯან ხაჭაპურიძე [1], ია ფანცულაია [3]

იმუნოდეფიციტური მდგომარეობები მორეციდივე რესპირატორული დაავადებების დროს დღენაკლ და დროულ ახალშობილებში.

[1] თსსუ პედიატრიის კათედრა, ინგოროყვას მაღალი სამედიცინო ტექნოლოგიების საუნივერსიტეტო კლინიკა, [2] თსსუ პედიატრიის დეპარტამენტი, [3] თსსუ იმუნოლოგიის დეპარტამენტი, ვლ.ბახუტაშვილის სახელობის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი

რეზიუმე

მძიმე რესპირატორული დაავადებები იმუნოდეფიციტის ხშირი გამოვლინებაა. ჩვენი კვლევის მიზანია იმუნოდეფიციტური მდგომარეობების გამოვლენა დღენაკლულ ჩვილებში, ხშირი მძიმე რესპირატორული დაავადებებით, მართვის ტაქტიკის, რეციდივების პროფილაქტიკის და ფილტვების შემდგომი დაზიანების მინიმიზაციის მიზნით. კვლევა შემთხვევა-კონტროლი ტარდებოდა 8 თვის განმავლობაში.

53 დღენაკლული ბავშვი, რომლებმაც გადაიტანეს მწვავე რესპირატორული დისტრესი და ფილტვების ხელოვნური ვენტილაცია ნეონატალურ პერიოდში, რომელთაგან ექვსს, კომპიუტერული ტომოგრაფიით გამოუვლინდათ პათოლოგიური ცვლილებები (ფიბროზული ინფილტრატები, მოზაიკური მილევა, გაუმჭვირვალე მინის ტიპის დაჩრდილვა), შევადარეთ 32 დროულ ახალშობილს, საკონტროლო ჯგუფიდან.

ორივე ჯგუფი ხასიათდებოდა ხშირი რესპირატორული ავადობით. IgG და IgA დეფიციტი გამოუვლინდა ძირითადი ჯგუფის 18 (33,96%) პაციენტს და საკონტროლო ჯგუფის 1 (3,125%) პაციენტს. ფიშერის ტესტის ზუსტი სტატისტიკური მნიშვნელობა P-მნიშვნელობა შეადგენს 0,0009. იმუნოგლობულინის ინტრავენური გადასხმა ჩაუტარდა 18 დღენაკლი ახალშობილიდან 8-ს. მომდევნო 3-7 თვის განმავლობაში რესპირატორული დაავადებების რეციდივი არ დაურეგისტრირდა 6 (75%) პაციენტს. 1 (12,5%) პაციენტს ერთჯერადად აღენიშნა მსუბუქი სიმპტომები, ზედა სასუნთქი გზების მხრივ, 1 (12,5%) პაციენტი განმეორებით იქნა ჰოსპიტალიზებული სუნთქვის უკმარისობით. 3–7 თვის განმავლობაში, სულ მცირე ერთი განმეორებითი ჰოსპიტალიზაცია დაურეგისტრირდა 10-ს, 18 დღენაკლი პაციენტიდან, რომლებსაც არ ჩაუტარდა იმუნოგლობულინის ინტრავენური გადასხმა.

ჩვენი კვლევის მიზანი გულისხმობს შესაძლო მიზეზ-შედეგობრივი კორელაციის გამოავლენას მორეციდივე რესპირაციულ დაავადებებსა და იმუნოდეფიციტურ მდგომარეობებს შორის ნაადრევად დაბადებულ ახალშობილებში. აღნიშნული მოგვცემს საშუალებას გავაუმჯობესოთ პაციენტების მართვა, შევამციროთ ავადობის რეციდივი, ფილტვის შემდგომი დაზიანება და ჰოსპიტალიზაციის სიხშირე.

Pediatric respiratory tract infections are a universal clinical problem across the whole childhood that is associated with significant morbidity and mortality [4,6]. The role of pediatricians is to discriminate among the child with transient increased morbidity and child with increased, complicated respiratory morbidity, which evokes the possible immune defect.

Severe respiratory diseases are common manifestations of immunodeficiencies [4,6]. Prognosis is greatly dependent on infectious and non-infectious respiratory complications. Thus it is extremely important to diagnose PIDs instantly to prevent significant morbidity and mortality [2,8]. Regular examinations by the appropriate tests should reveal the respiratory and immunologic pathologies in the

early stages. Raising awareness of PIDs improves the prognosis of these patients. However, deciding which children to investigate and when is quite challenging for physicians.

The most common clinical manifestation of predominant humoral and/or combined immunodeficiencies are prolonged and recurrent infections involving the respiratory tract, e.g., rhinosinusitis, otitis media, bronchitis, bronchiectasis, and pneumonias [1,4,6]. Respiratory infections in PIDs patients are usually severe, persistent, and recurrent in comparison with infections in non-PID patients. The clinical symptoms in humoral deficiencies, typically tend to occur after the first 6 months of life (after the disappearance of maternal IgG), however, sino-pulmonary infections may occur earlier [3,5]. Clinical history is the most important aspect of suspecting a diagnosis of primary humoral immunodeficiency. Therefore, patients at any age with recurrent upper or lower respiratory infections, where the frequency, severity, course of an isolated pathogen is unusual should be investigated for possible humoral or other type immunodeficiencies [2,8].

Patients with frequent respiratory conditions who are suspected of having immunodeficient states may be evaluated for having:

1. predominantly humoral (antibody) deficiencies,
2. combined T-cell and B-cell immunodeficiencies,
3. other well-defined immunodeficiency syndromes,
4. congenital defects of number and/or function of phagocytes,
5. complement deficiencies,
6. defects of immune dysregulation,
7. autoinflammatory disorders
8. defects in innate immunity.

Among all the immunodeficiencies, antibody deficiencies are the most frequent and comprise approximately 70–75% of all PIDs [1,2,7]. These patients are typically characterized by different respiratory symptoms and complications due to the inherited immune defect. In children, respiratory symptoms are a typical initial presentation of various PIDs. However, also the other groups and classes of PIDs can be associated with significant respiratory morbidity and manifestations Through two simple widely available tests – serum immunoglobulin concentration (IgG, IgA, IgM, and ±IgE) and differential leukocyte cell count – the majority of the PID can be detected and revealed. Therefore, these two simplex tests can be in general recommended as screening tools for PIDs in primary care [8].

Material and methods. The goal of our study is to reveal immunodeficient states in preterm infants with frequent severe respiratory diseases to improve management plans, prevent recurrences and minimize further lung damage.

A case-control study was performed for 8 months.

85 patients who had multiple severe respiratory conditions (bronchiolitis and/or pneumonia) in anamnesis and were hospitalized for respiratory distress syndrome in our clinic were further investigated.

Upon admission to our hospital, all 85 patients expressed increased breathing rate (RR 60-80) (100%), grunting (89%), nasal flaring (72%), retractions (100%), wheezing (100%).

The experimental group contained 53 infants who were born preterm, had ARDS after birth (42%), were exposed to mechanical ventilation in the neonatal period, had multiple (at least twice a month) severe respiratory conditions (bronchiolitis and/or pneumonia) in anamnesis (100%) and were hospitalized for respiratory distress syndrome in our clinic (100%).

6 of the 53 preterm patients showed CT scan abnormalities (fibrotic infiltrates, mosaic attenuation, ground-glass opacity). 32 patients were born term, had frequent severe respiratory conditions in anamnesis (100%), and were hospitalized for respiratory distress syndrome in our clinic (100%).

Blood immunoglobulin tests were performed in all these patients. Both IgG and IgA were deficient in 18 (33.96%) patients of the study group and 1 (3.125%) patient from the control group. IgG and IgM were deficient in 6 (11.3%) preterm infants, IgA was deficient in 4 preterm infants (7.55%). 2 of the patients are diagnosed as having Bruton agammaglobulinemia. Genetic tests were not performed on other patients. The Fisher exact test statistic P-value is 0.0009.

IVIG was transfused in 8 of the 18 preterm infants. During the next 3 to 7 months recurrence of the respiratory conditions was not reported in 6 (75%) patients; 1 (12.5%) patient experienced mild upper respiratory symptoms once, 1 (12.5%) patient was readmitted with respiratory failure.

During 3 to 7 months at least one readmission was reported in 10 of 18 preterm patients who were not transfused IVIG.

None of the term patients were transfused IVIG and 6 of them experienced mild upper respiratory disease once during the next 3 to 7 months.

Results and discussion. In general, a thriving child with recurrent respiratory infections does not suffer from a serious underlying disease. Most of the children do not have an immunodeficiency, but if they do, this often concerns an antibody deficiency [1,7]. The most common clinical manifestation of predominant humoral (and combined immunodeficiencies with associated antibody defects) are recurrent and prolonged infections involving the respiratory tract, either upper airways (e.g., sinusitis and otitis media) or lower respiratory tract [e.g., pneumonia, bronchiectasis, and interstitial lung diseases (ILDs)] [4,5]. The complications from the lower respiratory tract are usually considered to be more important and also more specific for PIDs and they determinate patients' prognosis [4,6]. Predominantly humoral immunodeficiencies represent clinically the most important group of inherited immune defects. The most frequent defects are selective deficiency of IgA, deficiencies of IgG subclasses [2,3,8].

In our study, all the patients had symptoms of respiratory distress upon admission to our hospital, but the course of the disease was far more severe in preterm patients with humoral immunodeficiencies. According to the obtained data, the IgG and IgA deficiency was the most common (33.96%) humoral immunodeficiency in tested patients. IgG and IgM were deficient in 11.3% of preterm infants, IgA was deficient in 7.55%. 2 of the patients were diagnosed as having Bruton agammaglobulinemia. The cause of the immunodeficiency in other patients is still in the research process, it is not determined whether the immunoglobulin deficiency is because of the primary immunodeficiencies or it is transient immunodeficient states in preterm infants whose immune system is not still mature. Repetitive evaluation of blood immunoglobulin levels was done in those patients who were transfused IVIG. 4 of the 8 patients had normal Ig levels on repetitive tests, 4 patients were still IgG deficient, and considering the deficient level of IgG, 3 of them were transfused IVIG again. Frequency and/or severity were decreased in all of those patients. 6 patients were satisfactory on regular follow-ups, 1 patient had a mild respiratory disease and 1 patient had severe respiratory failure once during 3 to 7 months.

The appropriate therapy using the immunoglobulin substitution and antibiotics usually leads to the significant decline of the frequency and severity of infections with a significant impact on the life quality and prognosis of these patients [6,7]. All our patients were treated for their specific infectious diseases, 8 of them were transfused IVIG.

Conclusion. The newborn infant is especially vulnerable to a range of respiratory diseases, that are presenting with signs of respiratory distress. Thorough clinical assessment and appropriate investigation are required for all infants presenting with signs of respiratory distress to ensure accurate diagnosis and correct treatment.The research may reveal a correlation between frequent respiratory diseases and immunodeficiencies as either the cause or the result of the conditions in preterm babies, thus allowing us to improve management techniques to decrease recurrences and reduce further lung damage and readmission rates in the patients.

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IMMUNODEFICIENT STATES IN PRETERM AND TERM INFANTS WITH RECURRENT RESPIRATORY DISEASES

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SUMMARY

Severe respiratory diseases are common manifestations of immunodeficiencies.

The goal of our study is to reveal immunodeficient states in preterm infants with frequent severe respiratory diseases to improve management plans, prevent recurrences and minimize further lung damage.

A case-control study was performed for 8 months. 53 preterm infants who had ARDS and were exposed to mechanical ventilation in the neonatal period, 6 of which showed CT scan abnormalities (fibrotic infiltrates, mosaic attenuation, ground-glass opacity), were compared to 32 term infants from the control group. Both groups presented with frequent severe respiratory diseases. IgG and IgA were deficient in 18 (33.96%) patients of the study group and 1 (3.125%) patient from the control group. The Fisher exact test statistic P-value is 0.0009.

IVIG was transfused in 8 of the 18 preterm infants. During the next 3 to 7 months recurrence of the respiratory conditions was not reported in 6 (75%) patients; 1 (12.5%) patient experienced mild upper respiratory symptoms once, 1 (12.5%) patient was readmitted with respiratory failure. During 3 to 7 months at least one readmission was reported in 10 of 18 preterm patients who were not transfused IVIG.

The research may reveal a correlation between frequent respiratory diseases and immunodeficiencies as either the cause or the result of the conditions in preterm babies, thus allowing us to improve management techniques to decrease recurrences and reduce further lung damage and readmission rates in the patients.

Keywords: respiratory distress. Immunodeficiency, diagnostics, infant, preterm infant

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RETROSPECTIVE STUDY OF NEWBORNS AND PREGNANT WOMEN INFECTED WITH COVID - 19 IN KUTAISI, IMERETI REGION

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მაია შენგელია, ჯანინა აბულაძე, სოფიო გამყრელიძე, სალომე ორმოცაძე

კოვიდ-19-ით ინფიცირებული ორსულებისა და ახალშობილების რეტროსპექტული ანალიზი ქუთაისში, იმერეთის რეგიონში

ქუთაისის უნივერსიტეტი, ა. წერეთლის სახელმწიფო უნივერსიტეტი, ბომონდის საავადმყოფო, ქუთაისი, საქართველო

რეზიუმე

კოვიდ პანდემია მსოფლიოში და მათ შორის საქართველოშიც ტალღებით მიმდინარეობს. ყოველი მომდევნო ტალღა წინაზე უფრო დამაზიანებელია. თუ პირველი და მეორე ტალღების შემთხვევაში ახალშობილთა ავადობა და მოკვდაობა მინიმალური იყო, მომდევნო ტალღების შემთხვევაში გაიზარდა ორსულთა და ახალშობილთა დაინფიცირების მაჩვენებელი. გამოჩნდა დედა-ახალშობილის სიკვდილობის შემთხვევებიც. ზემოაღნიშნულიდან გამომდინარე, კვლევის მიზნად დავისახეთ იმერეთის რეგიონში კოვიდ 19-ით ინფიცირებული ორსულების და ახალშობილების ისტორიების რეტროსპექტული ანალიზი. დასახული მიზნის მისაღწევად, კვლევაში გავაერთიანეთ 27 ორსული/მშობიარე (20 დან - 45 წლის ასაკის) და 31 ახალშობილი (0 დან ერთ თვემდე ასაკის, 18 ქალი და 13 ვაჟი), რომლებმაც გაიარეს სტაციონარული მკურნალობა 01.08.2020 – 01.12.2021 დროის მონაკვეთში, იმერეთის რეგიონის კოვიდ-კლინიკა „ბომონდის" ბაზაზე (ქუთაისი, საქართველო). კვლევის მეთოდს წარმოადგენდა ზემოთაღნიშნული პაციენტების, კერძოდ დედების გამოკითხვა სპეციალურად შემუშავებული ანკეტით და ავადმყოფობის ისტორიების რეტროსპექტული ანალიზი. მიღებული შედეგების ანალიზმა ცხადყო, რომ ორსულთა უმეტესობა - 23 (88%) პაციენტი ფლობდა კოვიდ საწინააღმდეგო ვაქცინაციაზე ექიმისგან ინფორმაციას, კოვიდ ინფექცია გადატანილი ჰქონდა 11 (40%) პაციენტს, კოვიდსაწინაარმდეგო ვაქცინით ვაქცინირებული იყო მხოლოდ 4 (14%) პაციენტი. დანარჩენმა უარი განაცხადა აცრაზე. ყველა კოვიდ დადებითი ორსულის ზოგადი მდგომარეობა იყო საშუალო სიმძიმის. ნაყოფი-დედის რისკების გაანალიზებით, 21 (79%) კოვიდ დადებით ორსულთან საჭირო გახდა საკეისრო კვეთის ჩატარება, ხოლო 6-მა (21%) ორსულმა იმშობიარა ფიზიოლოგიურად, სამეანო გინეკოლოგიური ჩარევების გარეშე.

ორმა ორსულმა იმშობიარა გესტაციის 24-25 კვირაზე. ორივე ნაადრევი მშობიარობის შედეგად მიღებული იყო დღენაკლული, მკვდრადშობილი ახალშობილები. სხვა დანარჩენ შემთხვევაში ახალშობილი დაიბადა კოვიდ უარყოფითი, დროული, შესაბამისი ანთოპომეტრული პარამეტრებით. კოვიდ ინფექციით დაავადებული 27 ორსული მკურნალობდა კოვიდ სტაციონარში, მათ უმეტესობას არ დაჭირვებია რესპირატორული მხარდაჭერა. 7-10 დღიანი მკურნალობის კურსის შემდეგ 25 პაციენტი გაეწერა ბინაზე ჯანმრთელი. მხოლოდ 2 (7%) შემთხვევაში დაფიქსირდა მძიმედ მიმდინარე კოვიდ-ინფექცია, რომელიც დასრულდა ლეტალური გამოსავალით. ასევე, პარალელურად, კოვიდ ინფიცირებული ახალშობილების ისტორიების კვლევამ ჩვენ შემთხვევაში დაადასტურა, რომ კოვიდ ინფექციამ არ გადალახა პლაცენტარული ბარიერი და დადგინდა, რომ ახალშობილები ავადდებიან პოსტნატალურად - პერსონალისგან ან მშობლებისგან. მათი კოვიდ ინფექციის გამოსავალი 31-ვე შემთხვევაში იყო დადებითი. ამდენად, კოვიდ ინფეცირებულ დედათა და ახალშობილთა კოვიდ სტატისტიკა და გამოსავალი იმერეთის რეგიონში ძირითადად კეთილსაიმედოა. თუმცა, ორსულთა მეთვალყურეობა და ხანგრძლივი მონიტორინგი ჯანმრთელი ახალშობილის დაბადების აუცილებელი პირობაა.

Introduction: Corona virus CAPC-CoV-2, (COVID-19) was first reported in December 2019 in Wuhan, China. In a few months, disease spread all over the world and in March of 2020 it was declared a world pandemic by the World Health Organization. Despite the fact that at the early stage of spread of the virus in 2019 was considered rare infection in children, the numbers of infection significantly

increased. Nowadays, Polymerase chain reaction (PT-PCR) is the gold standard for diagnosing COVID-19 infection [12].

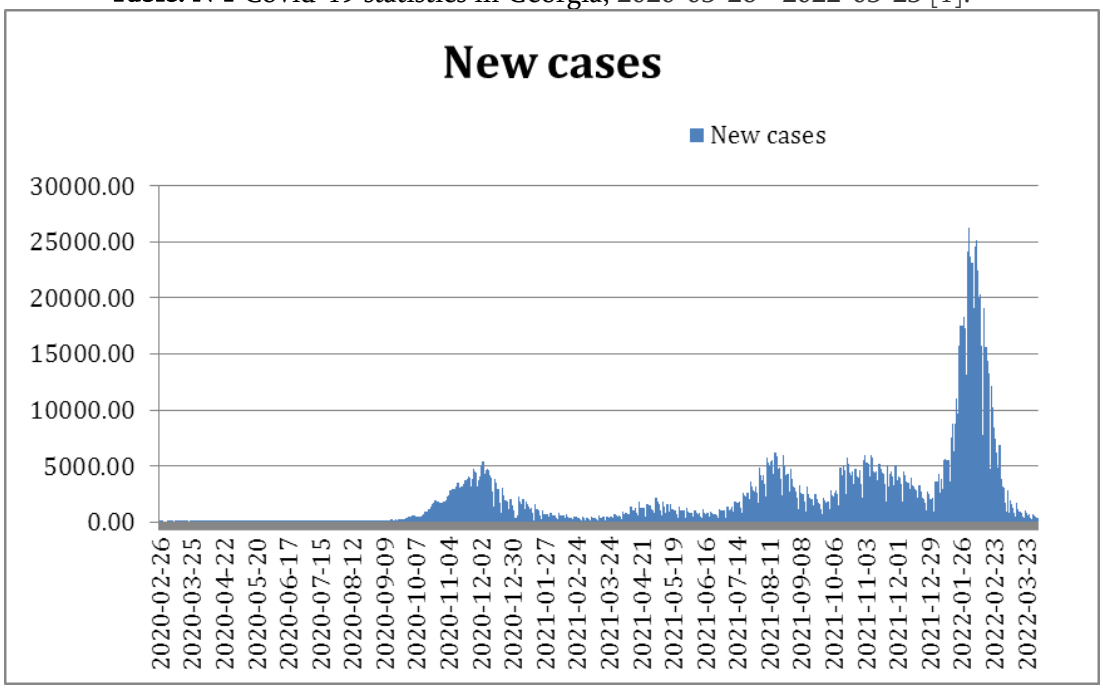
In Georgia the first case of COVID-19 was confirmed on February 26, 2020. In November 2020, a British variant called Alpha was first recorded in Georgia. Alpha variant has almost completely replaced most of the current circulating SARS-CoV-2 variants in the country. At the end of May 2021, a single case of an Indian strain called Delta variant appeared in the country, which has the ability to spread even faster due to mutations in the S gene sequence. By August 2021 this variant was already fully dominant compared to all the other variants previously prevalent. In addition, the so-called Delta + variant soon appeared which is constantly changing and continues to spread in different countries with different mutations.

As of October 1, 2021, among infected 10% are children age 0 - 15. 12% adults and young people age from 15 – 24, 78% people from age 60 and above. Number of deaths from COVID-19 is 8976, with a lethality rate of 1.46%.

From the beginning of the COVID-19 pandemic to October 1, 2021, 85,345 pregnant women were registered in Georgia for antenatal care: 45,516 in 2020 -, 38,345 in 2021.Total number of confirmed cases of COVID-19 in pregnant women was 7 338 (Percentage of infection of pregnant women 8.6%). In 2020 - 3 304 (7.3% from the number of pregnant women registered in 2020)

Of the total number of pregnant women infected with COVID-19, the lethal outcome was recorded in 17 cases, none of which were vaccinated, the lethality rate - 0.23%; in 2020 - 1 case lethality rate - 0.03%. In 2021 - 16 cases, lethality rate - 0.4% [1].

Table: N 1 Covid-19 statistics in Georgia, 2020-03-26 - 2022-03-23 [1].



The COVID-19 pandemic is appearing in waves. Each subsequent wave was more damaging to the previous. If the morbidity and mortality of newborns were minimal in the case of the first and second waves, the rate of infection in pregnant women and newborns increased in the case of subsequent waves. There has also been a case of neonatal death [6].

While physiological, mechanical, and immunological changes in pregnancy may affect a pregnant woman's intake of COVID-19, the solution is complicated by the pregnant woman's older age, obesity and underlying diseases such as, chronic lung disease, cirrhotic hypertension, and pre-gestational diabetes. Severe disease was associated with preeclampsia, preterm labor, gestational diabetes among pregnant women with COVID-19, and childbirth with low-weight gestational infants is more common in individuals with mild disease [11].

Pandemic-related disorders increase neonatal mortality, but caring for sick newborns is relatively new to global health. Being together of mothers and newborns in the postnatal period is a key aspect of comprehensive care and is particularly at risk during pandemics, including for young gestational infants in need of so-called "mother kangaroo" (KMC) care [13].

COVID-19 screenings are performed on mothers and their newborns on the first day of birth and on the day of disease manifestation, the analysis of this information will allow us to determine the ways of disease transmission.

Even if future studies confirm vertical transmission, this will not be an indication for cesarean section as it will increase maternal risk and is unlikely to improve neonatal morbidity rates as COVID-19 infection in infants is mild [9, 5].

Material and Methods: To sum up all the above mentioned, we set the goal to research history of Retrospective analysis in Covid-19 infected pregnancies and newborns in the region of Imereti. In the study we combined 27 pregnant/postpartum (20 to 45 years old) and 31 newborns (0 to one month old, 18 female and 13 male), who have undergone inpatient treatment 01.08.2021-01.12.2021 in Imereti region COVID clinic on the basis of "Bomondi" (Kutaisi, Georgia.) Research methodology consisted of surveys of abovementioned patients, specifically mothers, with specifically developed questionnaires and co-diagnosed maternal and infant morbidity retrospective analysis of stories.

Results and Review. The result of the analysis revealed that the majority of pregnant women 23 (88%) received information about COVID vaccination from doctors. 11 (40%) of patients have recovered from COVID-19 infection, out of them 4 (14%) were vaccinated against COVID infection. Main reason for not vaccinating was personal reasons.

Analysis of histories revealed that 22 (81%) pregnant women, with the disease, gestational age, with mild symptoms from their homes 5 (19%) pregnant women from other clinics were admitted to the clinic by ambulance in "Bomondi" clinic. On the bases of COVID positive test they were placed in the COVID section. Their gestational age of pregnancies ranged from 20 to 40. Analysis of histories revealed that all 27 pregnant women general condition was moderate severity. Fetal analysis of maternal risk, 21 (79%) required cesarean section in the patient and 6 (21%) in the pregnant women gave birth physiologically, without obstetric-gynecological interventions. Out of the above only two of these pregnancies were reported stillbirth due to premature birth at 24-25 weeks. In other cases, newborns were born COVID-19 negative with timely, relevant anthropometric parameters. We also note that out of 27 pregnant women only 2 (7%) were first-time mothers, 16 (59%) were second-time pregnancies and third childbirth was 9 (33%). It is also notable that mother's rhesus group of ABO system examined, group O blood was 15 (56%), group A blood 8 (29%) and group B blood 4 (15%). As for the rhesus factor 23 (85%) gave birth to Rh+ and 4 (15%) to Rh-. Pregnant women had none of the accompanying diseases and only 4 (14%) were vaccinated with 2 doses. These 27 pregnant women were treated for COVID infection. Only 2 (7%) needed resuscitation and in both cases (7%) the case ended with resuscitation. While a CT study confirmed COVID-19 pneumonia in 21 (77%) pregnant women with a CT score of 4-9, and in 6 (23%) patients with a score of 1-17. After 7-10 days of treatment, 25 (92%) patients were discharged home.

Also, the study of COVID-infected newborns, including our cases, also confirmed that COVID infection does not go beyond placental barrier and newborns become infected antenatal or from staff, or parents. Above mentioned is confirmed by the information that the age of newborns brought to the clinic by ambulance was between 6 and 26 days. It is also noteworthy that 5 (92%) patients applied to the hospital from homes and only 6 (18%) were transferred from another clinic with a COVID-positive test. According to the blood rhesus factor in 29 (93%) newborns only 2 were (17%) rhesus negative. Newborns were given blood monitoring analyzes in dynamics and compliance with treatment by the standards. All of them were discharged home healthy.

Studies have shown that infected women in the Imereti region 86% of women were unvaccinated out of which 7% gave birth to a stillborn fetus at the age of 20-25. The rest of 93% were 37-40 weeks pregnant with timely live births. With moderate severity of COVID infection 7% with maternal mortality, thus maternal neonatal covariance statistics in the Imereti region, it is noteworthy, however, that their health is monitored and prolonged monitoring is a necessary condition.

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RETROSPECTIVE STUDY OF NEWBORNS AND PREGNANT WOMEN INFECTED WITH COVID - 19 IN KUTAISI, IMERETI REGION

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SUMMARY

COVID -19 pandemic in the world, as well as Georgia, is progressing through waves and each subsequent wave is more damaging. Maternal-newborn death cases appeared as well. Thus, the study targeted retrospective analysis of neonatal histories of COVID-19 infected pregnant women and newborns. To achieve the goal, we included 27 pregnant women in the study (aged 20 to 45 years old) and 31 newborns (aged 0 to one month old, 18 female and 13 male) who have passed inpatient treatment from 01.08.2021 to 01.12.2021. In the COVID-clinic of the region on the basis of "Bomondi" (Kutaisi, Imereti, Georgia) the research methodology included surveying above mentioned patients, namely mothers with a specially designed questionnaire and medical history retrospective analysis. Analysis of the results revealed that majority of the pregnant women 23 (88%) got information about COVID vaccination from their doctors. COVID infection was transmitted to 11 (40%) patients. Only 4 (14%) patients were vaccinated against COVID -19. The rest were not vaccinated due to personal reasons. Out of all 27 pregnant women tested COVID positive, general condition was of moderate severity. After risk analysis

21 (79%) of patients required C-section and 6 (21%) pregnant women gave birth physiologically, without gynecological interventions. Only two of these women suffered stillbirth due to premature birth at 24-25 weeks. In all other cases the newborns were born COVID negative with timely, appropriate anthropometric parameters with COVID-19 infection these women were being treated in the section of COVID. After 7-10 days of treatment, 25 (93%) of patients were discharged home healthy, only 2 (7) ended lethally. Also, in parallel with the study of newborns histories it was confirmed that COVID infection did not cross the placental barrier and it has been found that infants become ill postnatal or from staff or patients. The solution to their covalent infection in all 31 cases was positive.

Therefore Covid-19 infected mothers-newborns COVID-statistics and solutions in the Imereti region are looking positive, although their health supervision and long-term monitoring is a must.

Keywords: SARS-CoV-2, COVID-19, vertical transmission, fetal death, fetus, maternal death, newborn, postnatal infection, pregnancy

SOPHIO GAMKRELIDZE, NINO JOJUA, MAIA SHENGELIA

THE ROLE OF MONTELUKAST IN MANAGEMENT OF CHILDREN WITH COVID-19 INFECTION

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სოფიო გამყრელიძე, ნინო ჯოჯუა, მაია შენგელია

მონტელუკასტის როლი პოსტკოვიდ-19 რესპირაციული სიმპტომების მქონე ბავშვების მენეჯმენტში

ა. წერეთლის სახელმწიფო უნივერსიტეტი, მედიცინის ფაკულტეტი; ქუთაისის უნივერსიტეტი; შპს N3 ბავშვთა პოლიკლინიკა; ქუთაისი, საქართველო

რეზიუმე

პოსტკოვიდური სიმპტომების მენეჯმენტი მუდმივი განახლების პროცესშია. ახალი კორონავირუსის გავრცელებული რესპირაციული სიმპტომებიდან აღსანიშნავია მშრალი შემაწუხებელი ხველა. სამედიცინო ლიტერატურაში ძნელია მოიპოვო ინფორმაცია მონტელუკასტის ეფექტურობის შესახებ პოსტკოვიდური რესპირაციული სიმპტომების მქონე ბავშვებში. ზემოაღნიშნულმა გამოიწვია ჩვენი დაინტერესებაც და ქ. ქუთაისის შპს ბავშვთა N3 პოლიკლინიკის ბაზაზე ჩავატარეთ კვლევა პაციენტებში კოვიდ და პოსტკოვიდური პერიოდის მშრალი , შემაწუხებელი ხველის ჩივილებით. კვლევაში მონაწილე 76 (2 დან 18 წლის ასაკის, 43 გოგო და 33 ბიჭი) პაციენტს ხველის ჩივილებით, დაენიშნა გლუკოკორტიკოსტეროიდის ადგილობრივი საინჰალიაციო ფორმა-ბუდესონიდი ჯენერიული აქტიური ნივთიერებით და/ან ლეიკოტრიენების რეცეპტორების ანტაგონისტი და/ან ანტიჰისტამინური მედიკამენტები. დაკვირვებამ ცხადყო, რომ მშრალი ხველის კლინიკური ეფექტი უდავოდ გამოვლინდა უფრო მეტად იმ პაციენტებში, რომლებიც ანტიალერგიულ მედიკამენტებს იღებდნენ კომბინაციაში, იგულისხმება საინჰალიაციო კორტიკოსტეროიდები, ლეიკოტრიენების რეცეპტორების ანტაგონისტი და/ან ანტიჰისტამინური მედიკამენტები ერთად. კვლევით მიღებული შედეგების ანალიზმა ასევე ცხადყო, რომ განსაკუთრებული კლინიკური ეფექტი დადასტურებულად გამოვლინდა იმ ბავშვებში, რომლებიც იღებდნენ მონტელუკასტს ასაკობრივი დოზირების გათვალისწინებით, მონო თუ კომპლექსურ თერაპიაში ვიდრე პაციენტები, რომელთა მკურნალობის კურსი არ მოიცავდა მონტელუკასტს. კვლევის მონიტორინგმა ნამდვილად დაადასტურა მონტელუკასტის კლინიკური ეფექტი კოვიდ დადებით ბავშვებში გახანგრძლივებული

მშრალი ხველით. COVID-19-ის მკურნალობის შედეგების რეესტრი დაგვეხმარება გადავჭრათ კლინიკური გამოწვევა, სადაც დღემდე უფრო მეტი კითხვა გვაქვს, ვიდრე პასუხი.

The pandemic, emerged with the new coronavirus 2019 (COVID-19), has not yet been brought under control, despite serious measures taken all over the world and efforts to control and treat the disease. Up till now, a specific treatment for COVID-19 infection is not available. Management of coronavirus infection especially in children is a continuous process of constant updating. The treatment approach of COVID-19 infection may include montelukast, cysteinyl leukotriene (CysLT) receptor antagonist, and the possibility of decrease severe COVID-19 progression will be mentioned [1,3].

Common symptoms of the novel coronavirus, not only in adult in children too, is dry and lingering cough. The cough is caused by increased bradykinin and its bronchoconstrictor effect, and montelukast, a selective LTD4 antagonist, has an inhibitory effect on bradykinin-induced airway hypersensitivity [2,4]. Montelukast is a potent cysteinyl leukotriene (CysLT) receptor antagonist with anti-inflammatory effects and has been proven to significantly suppress oxidative stress. In addition, the use of montelukast is known to have a decreasing effect on the frequency and severity of wheezing in patients with clinical episodic wheezing (wheezing after an upper respiratory tract infection caused by adenovirus, influenza, metapneumovirus, coronavirus). In these patients, montelukast does not prevent these viral infections, but seems to limit the upper respiratory tract [2,4].

It is difficult find the date about effect of montelukast in children with covid-19 infection. According of this, the aim of the present article was to review the role of montelukast that could be beneficial in management of children with covid-19 infection. Based on gathered theoretical evidence, montelukast should be further tested to prevent and treat COVID-19 outcomes.

The above-mentioned aroused interest and the study of the child patients, with the symptoms like a lingering cough was conducted on the basis of the N 3 Children clinic (Kutaisi, Georgia). 76 patients involved in the study (2 to 18 years of age, 43 girls, 33 boys) with the symptoms of dry and lingering cough were prescribed an inhaled form of local glucocorticosteroid – Budesonide with generic active substance and/or leukotriene receptor antagonist, and/or antihistamines. According to treatment design, the patients were divided into treatment groups. One part of the group – 18 patients (23%) was prescribed only inhalers, one part 20 (27%) – leukotriene receptor antagonists, one part -19 (25%) – antihistamines and the last one 19 patients (25%) – all three antihistamines simultaneously, respectively. The patients take montelukast for oral dosage form (chewable tablets): children 2 to 5 years of age - 4 mg once a day in the evening; children 6 to 14 years of age - 5 milligrams (mg) once a day in the evening; adults and children 15 years of age and older - 10 milligrams (mg) once a day in the evening. Duration of treatment was 14 days.

Follow-up showed that the clinical effect of dry cough was more pronounced in children administering montelukast only or in combination in comparison with the patients taking only local glucocorticosteroid and antihistamines, such as desloratadine or levocetirizine separately, on a selective basis. Relatively good clinical efficacy was revealed in children taking agents in combination, that is, the simultaneous administration of inhaled corticosteroids, leukotriene receptor antagonists, and/or antihistamines. Monitoring of clinical results showed reduction in dry cough as a symptom after using montelukast; in addition, XR monitoring has confirmed the rapid and more effective relief of inflammatory foci in children actively and systematically actively and systematically received montelukast, compared with those not taking the mentioned agents to relieve dry cough. It was also noted that the indicator of respiratory distress alleviation degree was not in correlation with treatment using the above medications.

Montelukast works as a cysteinyl leukotriene (CysLT) receptor antagonist. Leukotrienes are inflammatory mediators produced by the immune system. They promote bronchoconstriction, inflammation, microvascular permeability, and mucus secretion. Montelukast has an anti-inflammatory effect with bradykinin and leukotriene antagonist; It suggests that it may be effective to use it, possibly at high doses, in order to reduce its severity during the course of the disease or before the disease occurs fully in people at risk. The healing effects of montelukast on these damages can be seen.

Analysis of the obtained results revealed that 65 (85%) patients, who underwent the above treatment with montelukast, had sooth in cough as a symptom, however, the clinical effect was obtained nearly in 10 days after treatment, while 11 (15%) patients failed to achieve clinical efficacy applying this treatment regimen. The obtained results indicate the efficacy of montelukast, as symptomatic agents, for the treatment of Covid-19 patients with a dry and lingering cough. Research monitoring has also confirmed the ancillary effect of montelukast. The issue of COVID-19 infection management in children still remains open and research in this direction is still active around the world.

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THE ROLE OF MONTELUKAST IN MANAGEMENT OF CHILDREN WITH COVID-19 INFECTION

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SUMMARY

Management of coronavirus infection especially in children is a continuous process of constant updating. It is difficult find the date about effect of montelukast in children with Covid-19 infection. According of this, the aim of the present article was to review the role of montelukast that could be beneficial in management of children with covid-19 infection. 76 patients (2 to 18 years of age, 43 girls, 33 boys) with the symptoms of dry and lingering cough were involved in the study.

According to treatment design, the patients were divided into treatment groups. One part of the group was prescribed only inhalers, one part – leukotriene receptor antagonists, one part – antihistamines and the last one – all three antihistamines simultaneously, respectively. Follow-up showed that the clinical effect of dry cough was more pronounced in patients administering montelukast only or in combination in comparison with the children taking only local glucocorticosteroid and antihistamines, such as desloratadine or levocetirizine separately, on a selective basis.

The obtained results indicate the efficacy of montelukast, as symptomatic agents, for the treatment of Covid-19 patients with a dry and lingering cough. Research monitoring has also confirmed the ancillary effect of montelukast in children with Covid-19 infection.

Keywords: COVID-19, cough, montelukast, children

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TH SUBSETS AND SERUM CYTOKINES IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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ნინო ქიქოძე [1,2], მანანა იობაძე [2], ნინო ცისკარიშვილი [3], ია ფანცულაია [1,2], ნონა ჯანიკაშვილი [1], თინათინ ჩიქოვანი [1]

T ჰელპერების სუბპოპულაციები და შრატის ციტოკინები რევმატოიდული ართრიტის პათოგენეზში

[1]იმუნოლოგიის დეპარტამენტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; [2]ვ.ბახუტაშვილის სახ. სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; [3]ფ. თოდუას სამედიცინო ცენტრი

რეზიუმე

რევმატოიდული ართრიტი სისტემური ანთებითი დაავადებაა. ის ხასიათდება ხრტილის და ძვლის დაზიანებით, რაც სახსრის ფუნქციის დარღვევას იწვევს. დროთა განმავლობაში, შესაძლოა მულტიორგანული: ფილტვის, გულის და თირკმლის დაზიანებები განვითარდეს. დაავადების დაწყებაში, სისტემური აქტიური ფაზის დროს, ასევე მკურნალობის შემდეგ უფრო ლოკალურ არააქტიურ ფაზაში გადასვლის დროს, იმუნური და სტრომის უჯრედების, ამ უჯრედების მიერ წარმოქმნილი ციტოკინებისა და ქემოკინების რთული ქსელი მონაწილეობს. რევმატოიდული არტრიტის მქონე პაციენტების პერიფერიული სისხლის Th1, Th17, Tregs და $CD4^+CD39^+$ უჯრედების და პრო- და ანტიანთებითი ციტოკინების პროფილის შეფასება მოხდა 47 პაციენტსა და 20 ჯანმრთელ ინდივიდში.

რევმატოიდული ართრიტის მქონე პაციენტები დაიყო ორ ჯგუფად: აქტიური და არააქტიური ართრიტი. მოცირკულირე Th1, Th17, Tregs და $CD4^+CD39^+$ უჯრედების სიხშირის განისაზღვრა ციტოფლუორომეტრიის საშუალებით. შრატში ციტოკინების IL-6, IL-10, IL-4, IL-17, TNF-α და TGF-β1 დონე განისაზღვრა იმუნოფერმენტული მეთოდით. აქტიური რევმატოიდული ართრიტის ჯგუფში Th1 და Th17 უჯრედების სიხშირე იზრდებოდა, მაშინ როცა Tregs სიხშირე, უცვლელი იყო ართრიტის ჯგუფებში. CD39 მარკერი სარწმუნოდ დაქვეითდა აქტიურ ჯგუფში, არააქტიურ და კონტროლის ჯგუფებთან შედარებით. თანდაყოლილი იმუნური სისტემის ციტოკინები: IL-6 და TNF-α სარწმუნოდ გაზრდილია აქტიური ართრიტის ჯგუფში, მაშინ როცა შრატში IL-17/IL-21 ციტოკინების კონცენტრაციის მიხედვით ეს ჯგუფი ერთგვაროვანი არ არის. ანტიანთებითი ციტოკინები: TGF-β და IL-4 დაქვეითებულია აქტიური ართრიტის ჯგუფში კონტროლთან შედარებით.

კვლევა აჩვენებს, რომ რევმატოიდული ართრიტის განვითარება ასოცირებულია შრატის პრო- და ანტიანთებითი ციტოკინების ცვლილებებთან, რაც გავლენას ახდენს T ჰელპერების სუბპოპულაციების ბალანსზე და ახალი თერაპიული შესაძლებლობების ფანჯარას ხსნის.

Introduction.

Rheumatoid arthritis (RA) is a systemic inflammatory disease. It is characterized by damage of cartilages and bones which results in destruction of joint function. Multi-organic disorders of lung, heart and kidney can be developed in time. Complex network of immune and stromal cells, cytokines and chemokines produced by these cells participate in onset of the disease, in the systemic active phase and during the transition to more localized inactive disease after the treatment. Recent studies have shown that the balance between T cell subsets plays a crucial role in the development of RA [1,2]. Cytokines produced by dendritic cells and macrophages: IL-1, IL-6, IL-12, IL-15, IL-18, TNF-α determine early onset and development of rheumatoid arthritis. They form pro-inflammatory cytokine milieu which drives differentiation Th1 cells in the synovial tissues of RA patients but not in osteoarthritis patients [3,4,5]. Around 40% of citrulline-reactive $CD4^+$ T cells were found to be CXCR3 (surface marker for Th1 cells) positive [6] in the blood of RA patients [7]. $CD4^+$ T cells, triggered simultaneously by IL-6 and TGF-β differentiated into Th17 cells, which have been reported to play crucial role in the pathogenesis of RA [8,9]: its cytokine IL-17 induces osteoclastogenesis [10], stimulates synovial fibroblasts to produce IL-6 [11] and macrophages to produce TNF-α [12]. Th17 cells produce IL-21, which plays a central role in the amplification of inflammatory processes, the activation and

proliferation of various immune cells including Th17 cells, T follicular helpers, B cells and macrophages facilitating pathogenic role of this cytokine in the development of RA [7,13]. Throughout the course of the disease high quantity of IL-21 is produced by Tfh cells in secondary follicles in joints and lymph nodes, resulting in B cell activation and production of auto-antibodies such as rheumatoid factor, anti-CCP and anti-MCP antibodies [14]. Immune complexes, which deposit in synovium induce activation of neutrophils. Lysosomal enzymes released from neutrophils lead to tissues damage. Pro-inflammatory cytokines activate synovial fibroblasts, cause expansion and differentiation of osteoclasts via RANKL, that lead to bone resorption [15]. Degradation of the collagen matrix is caused by IL-1-induced matrix metalloproteinases (MMPs), membrane-type 1 MMP (MT1-MMP) from synovial cells, that enhance joint injury [16]. Activated autoreactive T helpers can be suppressed by T regulatory cells (Tregs) which are key players in maintenance of immunologic homeostasis and prevention autoimmunity [17]. Presumably microenvironment of chronic inflammation established in RA patients leads to aberrations in Tregs.

It is shown that proportion of Tregs ($CD4^+CD25^+FoxP3^+$) in $CD4^+$ T cells correlates with clinical response in RA patients [18,19]. Kikuchi et al. reported that expression of transcriptional factor Foxp3 positively correlates with expression of CD39 molecules on $CD4^+$ cells [20,21]. The ectonucleotidase CD39 has recently been reported as being responsible for hydrolysis of proinflammatory extracellular ATP, generated adenosine expresses immunosuppressive effects through adenosine A2A receptor (A2AR). This leads to the inhibition of effector T cell activation [22]. Deprivation of ATP, that inhibits differentiation of Th17 cells is additional mechanism of suppression of autoimmune processes [23,24,25,26].

Thus, imbalance between Th17 and Treg cells is an important mechanism which may lead to RA [2]. Influence of cytokines on Th17/Tregs balance is under the intense investigation.

Despite the fact that investigators are mostly focused on the pro-inflammatory cytokines which trigger RA, anti-inflammatory cytokines responsible for the suppression and regulation of the disease are not less important in the pathophysiology of RA [27]. Many authors report lower plasma TGF-β concentration in RA patients. TGF-β leads to Tregs polarization in vitro by increasing FoxP3 and CD39 expression on differentiating and differentiated Tregs and enhances their suppressive activity [28,29].

C.H. QU et al. showed that RA patients had remarkably lower serum IL-10 level [30], main source of which is synovium macrophages. It is worth to note, that data are highly controversial about IL-10 concentration in RA patients. It is reported significant increase in the level of IL-10 in patients having DAS > 3.2 [31]. In animal models IL-10 reduces severity of arthritis [32]. Hui Shen et al. reported, that plasma level of other anti-inflammatory cytokine IL-4 was elevated only in RA patients with interstitial lung disease (ILD), progressive complication of RA compare to RA patients without ILD [33].

The aim was to study Th subsets and pro- and anti-inflammatory cytokines in patients with rheumatoid arthritis.

Methods and materials. Study Design.

47 patients included in the study were recruited at Todua Medical Center and Caraps Medline. They fulfilled the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 classification criteria for RA [34].

Disease activity were assessed by calculating DAS-28 score using an online application. The patients were divided into two groups: active rheumatoid arthritis (active RA group) and low disease activity (inactive RA group). There were 18 females and 9 males in the active group (n = 27) (mean age: 49.6$\pm$9.3). The mean disease duration was 5.2$\pm$4.2 years. In the inactive group (n = 20) 14 patients were females and 6 - males (mean age: 52.7$\pm$6.7). The mean disease duration was 6.1$\pm$5.2 years. 15 females and 5 males healthy individuals (mean age: 50$\pm$4.5) form control group (n = 20).

The patients with active disease: DAS-28$\geq$3.2 ESR>32mm/hour, CRP>9.8mg/l. The patients with inactive disease DAS-28<3.2 ESR<24 mm/hour, CRP<5.6 mg/l. None of the patients had been treated with any corticosteroid during the last three months. All patients were receiving nonsteroidal anti-inflammatory drugs at the time of sampling.

Cellular Subsets.

Peripheral blood samples were collected from each patient in an EDTA anticoagulant-treated tube on day 0 (before starting treatment). The immunophenotypic analysis was accomplished within 24 h of the sample collections. Treg cells - $CD4^+CD25^+FoxP3^+$ and $CD4^+CD39^+$, Th1 cells - $CD4^+$T-bet^+, Th17 cells - $CD4^+$RoR-γt^+ were evaluated in samples.

Flow cytometric immunophenotyping was performed on peripheral venous blood in EDTA-anticoagulated vacutainers. The samples were maintained at room temperature and processed within 24 h of collection. Mononuclear cells were separated on ficol-paque gradient. The cells were added anti-CD3-FITC,

anti-CD4-PE-Cy7, anti-CD39-PE, anti-CD25-PE, anti-FoxP3-APC, anti-T-bet-PE, anti-RoR-γt-PE monoclonal antibodies (eBioscience, USA) and incubated in the dark at $C4^0$C for 30 minutes. The flow cytometric analysis was done on a Facs calibur (BD Bioscience, USA). For the evaluation of the data, the events were gated on the forward side scatter to exclude remaining red blood cell and cellular debris. For each tube, at 10000 gated events were acquired. Data were processed using the Flow Jo v7 software (USA).

Cytokine assay.

Human ELISA kits were used to detect the serum levels of TNF-α, TGF-β1, IL-6, IL-21, IL-17A, IL-10, IL-4 (ebioscience, USA) by following the manufacturer's protocol.

Statistical analysis.

Continuous variables were analyzed with a Student's *t*-test, differences between groups were performed using the Mann–Whitney U test. P-values of <0.05 were regarded as significant.

Ethical Approval.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Results.

Immunophenotyping of T cell subsets demonstrates that the frequency of Th1cells was significantly higher (P <0.005) in active group of patients (19.94±7.24) compare to healthy subjects (8.63±2.02) and inactive group (10.04±0.55) (Figure 1). Remarkable difference (P<0.05) was found between frequencies of Th17cell subset in active RA (10.86±4.18) and healthy subjects (2.00±0.9). The frequency of $CD4^+$RoR-γt^+ cells was significantly (P<0.05) lower in inactive group (2.06±0.51) in comparison with active group. However, there was not statistically significant difference in the frequencies of Th17 cells between inactive and control groups (Figure 1).

The frequencies of circulating Tregs ($Foxp3^+CD4^+CD25^+$) were found unchanged in groups of the patients in comparison with healthy controls (P<0.05) (Figure 1).

On the contrary, significant changes of circulating $CD39^+$ cells in CD4 compartment in any studied groups were revealed. We observed significantly lower frequency in active RA group (1.07±0.80) compare to inactive group of patients (6.19±3.41) and healthy controls (14.60±5.62) (P<0.05) (Figure 1).

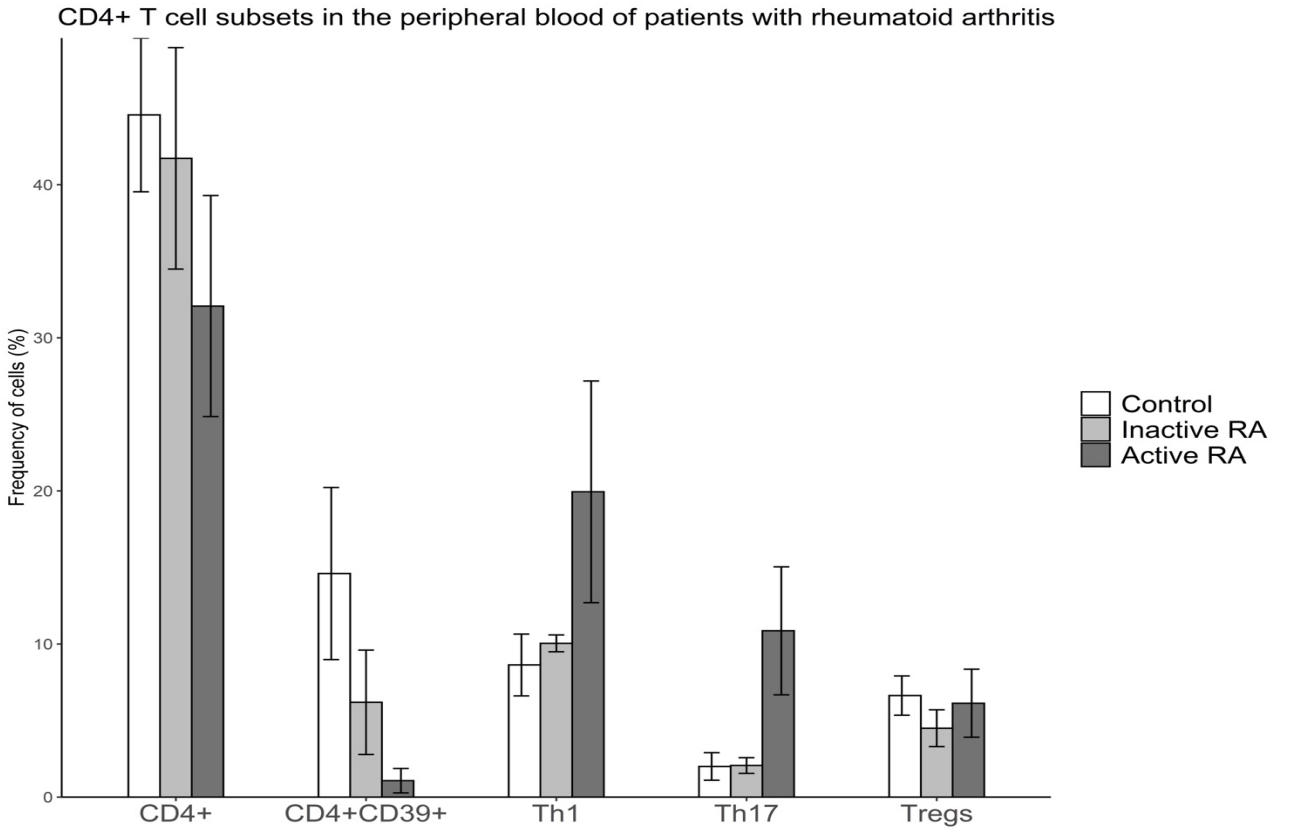

Figure 1. Th1, Th17, Treg cells and the CD4+CD39+/CD4+ cells changes in both study groups and control. A significant decrease was observed in the CD4+CD39+/CD4+ cells in active RA group. A significant increase was shown in Th1 and Th17 cells in active RA group.

Plasma concentrations of IL-17, IL-21, IL-6 and TNF-α in patients with RA are increased compare to healthy controls. Active RA [IL-17: 25.13±10.80 pg/ml; IL-21: 2.55±1.59 ng/ml; IL-6: 30.10±13.35 pg/ml; TNF-α: 120.87±20.02 pg/ml] and inactive RA [IL-17: 22.83±5.63 pg/ml; IL-21: 1.62±0.70 ng/ml; IL-6: 5.24±0.57 pg/ml; TNF-α: 12.56±1.06 pg/ml] in comparison with healthy subjects [IL-17: 13.95±4.28 pg/ml; IL-21: 0.88±0.06 ng/ml; IL-6: 3.25±1.98 pg/ml; TNF-α: 8.11±2.45 pg/ml] (P <0.001), while there was no obvious difference between two RA groups (P<0.05) in plasma concentrations of TGF-β1 and IL-10 in active RA [TGF-β1: 32.63±4.16 ng/ml; IL-10: 3.51±0.86 pg/ml] and inactive RA groups [TGF-β1: 32.76+3.94 ng/ml; IL-10: 4.29±1.20 pg/ml]. TGF-β1 significantly decreased when compared with healthy control group [78.48±6.34ng/ml] (P<0.001). Other anti-inflammatory cytokine IL-4 significantly decreased in RA patients (active RA: 3.27±2.13pg/ml; inactive RA: 19.05±4.71pg/ml) compare to healthy control group (34.30±8.92 pg/ml) (Figure 2).

In addition, frequencies of Th17 cells were positively correlated with plasma concentrations of IL-21 (r = 0.338, P<0.01), IL-17 (r = 0.398, P<0.05) and negatively correlated with plasma concentrations of TGF-β1 (r = -0.402, P<0.05).

Discussion.

Disturbed peripheral balances of Th1, Th17, Treg cells and serum pro- and anti-inflammatory cytokines were shown in the present study. Frequencies of Th1 and Th17 cells in the peripheral blood of active RA group are significantly increased in comparison with healthy controls and inactive RA group as it is reported by many authors [1,3]. That confirms suggestion about crucial role of these helper cells in the development of RA. Higher frequency of Th17 cells along with activated synovial fibroblasts and macrophages leads to the enhance production of cytokines: IL-17, TNF-α and IL-6 and sustains an inflammation of joint tissues in RA [35,36,37].

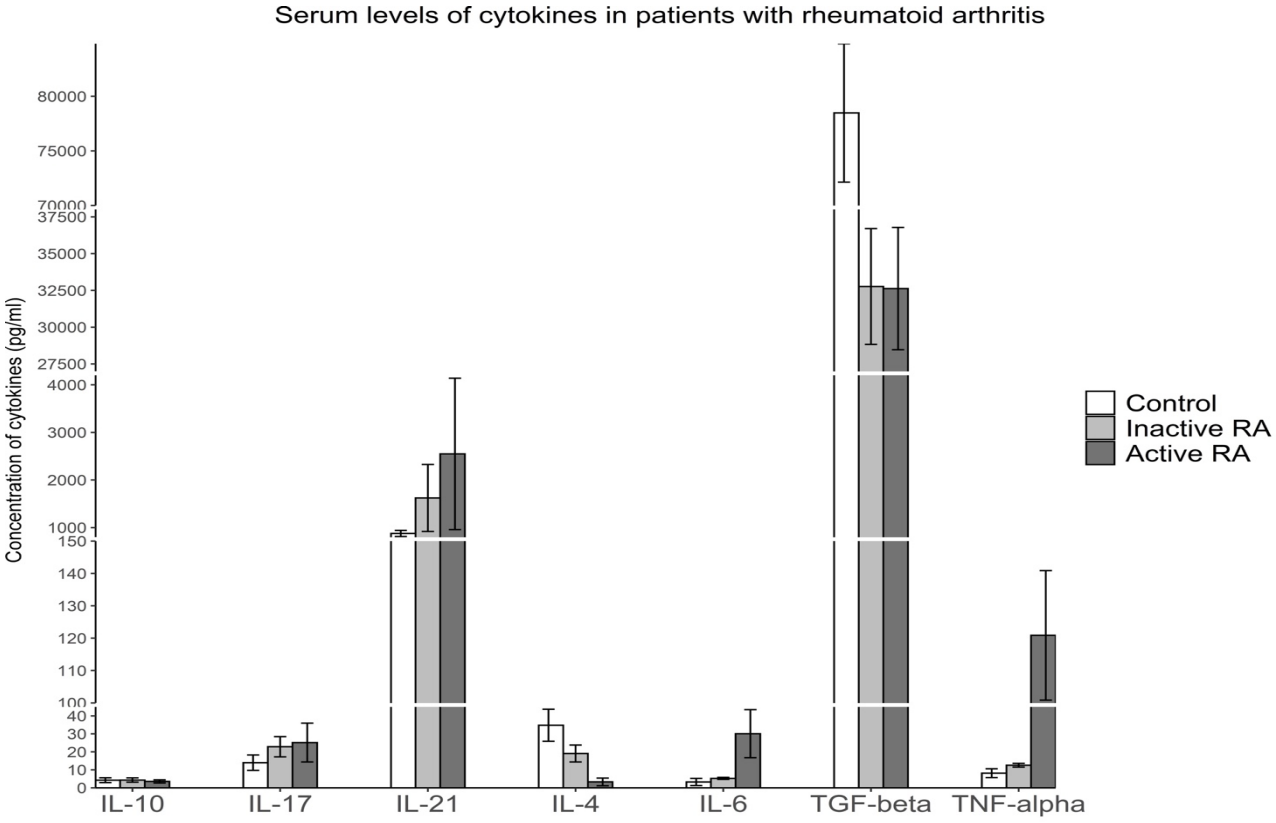

Figure 2. Serum IL-6, IL-10, IL-4, IL-17, IL-21, TNF-α and TGF-β1 concentration changes in both study groups and control. A significant increase was noted in TNF-α and IL-6 in active RA group. A significant decrease was noted in TGF-β and IL-4 in the both RA groups.

Hierarchical importance of IL-6 and TNF-α in the pathogenesis of RA is already well established [38]. Present study confirms significant difference between active and inactive RA groups and healthy controls according to the peripheral levels of these cytokines. Importance of identification of other cytokines (besides IL-6 and TNF-α) which could present additional therapeutic targets of treatment of RA is obvious. In active RA group of patients two subgroups are identified according to two correlated cytokines IL-17/IL-21: with high and low level of serum IL-17 and IL-21. It is supposed that in patients with higher serum concentration

of IL-17 and IL-21 remission is difficult to achieve. It is suggested that in this group of refractory disease IL-17, IL-21 can play role of therapeutic targets for biologics.

T regulatory cells (Tregs) express reciprocal developmental pathway of generation from pathogenic Th17 cells. Normal amount and function of Tregs prevent breach of self-tolerance at the periphery and avoid development of autoimmune diseases [38]. Expression of CD39 ectonucleotidase on Tregs intensifies their suppressive capacity on autoreactive T cells [39,40,41]. We revealed that frequency of Treg cells in the peripheral blood of RA patients did not significantly differ from that of in healthy individuals. Whereas, frequency of $CD39^{+}CD4^{+}$ cells in active RA group were significantly decreased than in inactive RA patients and healthy controls. We suppose, CD39 stabilizes suppressive activity of Tregs. Reduced expression of CD39 leads to the functional disorder of Tregs. Therefore, despite normal frequency of these cells in the peripheral blood breach of tolerance takes place. Enhance of CD39 expression on Tregs via activation of TGFβRII, TGFβRI and other molecules can be induced by TGF-β stimulation [29]. We suppose, that reduced serum TGF-β concentration in our RA patients explains decreased suppressive activity of Tregs in these patients. Reduced TGF-β and dramatically increased IL-6 at the same time form cytokine milieu beneficial for Th17 differentiation. Drastically increased frequency of Th1 and Th17 cells in active RA in combination with decreased suppressive activity of Tregs shifts balance to the side of autoreactive cells and triggers autoimmune process. Th17 cell cytokine - IL-17 along with IL-9 promotes the secretion of chemokine and cytokines which in turn accelerates the infiltration of neutrophils in the tissues thereby aggravates inflammation and tissue injury [42].

IL-10 is an anti-inflammatory and immunoregulatory cytokine which suppresses the formation of pro-inflammatory cytokines as well as down regulates the functioning of antigen-presenting cells [43]. It also inhibits the production of proteases and stimulates the formation of tissue inhibitor of metalloproteinases-1 (TIMP-1) by monocytes [32]. We did not find out significant changes in the serum level of IL-10 between groups unlike studies which detected higher level of IL-10 in response to higher inflammatory state of RA patients [31].

The role of Th2 cell cytokine - IL-4 is not clearly understood in the pathogenesis of RA. The study results indicate that anti-inflammatory and modulatory cytokine IL-4 seems to play an important role in the pathogenesis of disease. This cytokine is not detected in synovium and synovial fluid of patients and lack of IL-4 is likely to contribute to the uneven Th1/Th2 balance and to the chronic nature of RA. IL-4 gene therapy reduced IL-17 and RANKL expression in the synovium and prevents bone erosion [44]. Serum IL-4 concentration was significantly decreased in RA groups in comparison with healthy control. Therapeutic strategies that enhance local IL-4 production may protect against cartilage and bone destruction in RA.

Substantial plasticity between T cell subsets, different cytokine milieu during discrete phases of disease development provides various therapeutic opportunities to meet clinical needs of patients.

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TH SUBSETS AND SERUM CYTOKINES IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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SUMMARY

Rheumatoid arthritis (RA) is a systemic inflammatory disease. It is characterized by damage of cartilages and bones which results in destruction of joint function. Multi-organic disorders of lung, heart and kidney can be developed in time. Complex network of immune and stromal cells, cytokines and chemokines produced by these cells participate in onset of the disease, in the systemic active phase and during the transition to more localized inactive disease after the treatment. To evaluate the Th1, Th17, Tregs and $CD4^+CD39^+$ cells pattern and pro- and anti-inflammatory cytokines in peripheral blood of patients with RA, 47 RA patients and 20 healthy individuals were included in the study. RA patients were divided into active and inactive RA groups. Frequencies of circulating Th1, Th17, Tregs and $CD4^+CD39^+$ cells were analyzed by flow cytometry. Serum levels of cytokines: IL-6, IL-10, IL-4, IL-17, TNF-α and TGF-β1 were detected by ELISA. The results demonstrated an increase of Th1, Th17 cell frequencies in active RA patients, whereas Tregs remain unchanged in RA groups. CD39 marker expression shows significant decline in active RA patients in comparison with inactive RA group and controls. Concentrations of innate cytokines IL-6, TNF-α in the peripheral blood was significantly increased in active RA while according to IL-17/IL-21 serum concentration this group was divided into two subgroups. Anti-inflammatory cytokines TGF-β, IL-4 shows decrease in active RA group compared to healthy controls.

Study demonstrated that development of RA is associated with changes of serum pro- and anti-inflammatory cytokines which influence balance of T helpers subsets and could provide therapeutic opportunities.

Keywords: Rheumatoid arthritis, Th1, Th1, Treg, $CD4^+CD39^+$ cells, IL-6, IL-10, IL-4, IL-17, IL-21, TNF-α and TGF-β1.

TINATIN KITUASHVILI, TAMAR URUSHADZE

CASE STUDY - ALLERGIC CONTACT DERMATITIS IN RELATION TO TATTOOS

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რეზიუმე

ტატუირების პოპულარიზაციის პარალელურად, სამედიცინო პრაქტიკაში მატულობს ტატუირების შედეგად კანზე განვითარებული რეაქციების შემთხვევათა რიცხვი. ჩვენს ნაშრომში აღწერილია ტატუთი გამოწვეული კანის ალერგიული რეაქციის შემთხვევა და განხილულია მისი იმუნოლოგიური მექანიზმი. ასევე აღწერილია ტატუს მელნის შემადგენელი კომპონენტები, მათი ალერგენული პოტენციალი და განვითარებული ალერგიული რეაქციების მკურნალობის შესაძლო ვარიანტები.

ტატუს მელანი შესაძლოა შეიცავდეს სხვადასხვა ტიპის ალერგენს. გამოყოფენ ბიოდეგრადირებად და არაბიოდეგრადირებად კომპონენტებს. პირველ ჯგუფში შემავალი ნივთიერებები, როგორებიცაა ბუნებრივი საღებავები და კონსერვანტები, განაპირობებენ კანზე ისეთი ალერგიული პროცესის განვითარებას, რომელიც კარგად ემორჩილება კონსერვატიულ თერაპიას. რაც შეეხება არაბიოდეგრადირებად კომპონენტებს, მათ მიერ განვითარებული ალერგიული რეაქციები, როგორც წესი, საჭიროებენ ინვაზიური მეთოდების გამოყენებას, როგორებიცაა ქირურგიული ჩარევა, დერმის ე.წ. "გაპარსვა" და ლაზერული თერაპია, რომელიც ყველაზე ხშირად გამოიყენება პრაქტიკაში. ამ მხრივ ყველაზე საყურადღებოა წითელი ფერის პიგმენტი.

ტატუს შემადგენელ ნივთიერებებთან დაკავშირებით მოქმედი რეგულაციები არ არსებობს, რაც უფრო მეტად ზრდის გვერდითი მოვლენების განვითარების რისკს. არსებობს ალერგიული რეაქციების თავიდან აცილების გარკვეული პრევენციული საშუალებები, როგორებიცაა კანის ალერგიული სინჯები. მსგავსი ტესტების სპეციფიკურობა საეჭვოა, რამდენადაც უარყოფითი პასუხი სრულებით არ გამორიცხავს ალერგიული რეაქციის განვითარების შესაძლებლობას მოგვიანებით, რამოდენიმე კვირის და თვის შემდეგ. აღსანიშნავია, რომ არ არსებობს მკურნალობის მკაცრად განსაზღვრული გაიდლაინი, ამიტომ თითოეული შემთხვევა ფასდება ინდივიდუალურად და ამავე პრინციპით ხდება მკურნალობის შერჩევაც. ჩვენი პაციენტი იყო ახალგაზრდა ქალი, რომელსაც ტატუს არეში, კერძოდ, წითელი პიგმენტის ადგილას, განუვითარდა მწვავე ალერგიული რეაქცია. ამ კონკრეტულ შემთხვევაში, საწყისი მკურნალობა მიზნად ისახავდა ანთებითი რეაქციის შემცირებას, რათა შემდგომში შესაძლებელი ყოფილიყო ალერგენის, წითელი პიგმენტის, სრულად მოცილება დერმიდან. როგორც ცნობილია, სწორედ ეს უკანასკნელი წარმოადგენს ალერგიული კონტაქტური დერმატიტის ალაგების აუცილებელ პირობას. პაციენტს ჩაუტარდა ადგილობრივი, ანთების საწინააღმდეგო თერაპია. ირიტაციისა და ანთებითი მოვლენების უკუგანვითარების შემდეგ, ჩატარდა ლაზეროთერაპია Q-switched Nd 532 nm ლაზერით. პირველი პროცედურის შემდეგ ტატუს არეში განვითარდა ირიტაცია და ექსუდაცია, რის გამოც ჩატარდა ადგილობრივად ანთების საწინააღმდეგო მკურნალობა. ლოკალური და ლაზერული თერაპიის უშედეგობის გამო, პაციენტს დაენიშნა სისტემური კორტიკოსტეროიდი კლებადი დოზით და დაავადების კონტროლით. ორთვიანი მკურნალობის ფონზე პაციენტის მდგომარეობა გაუმჯობესდა. იგი კვლავ ავრძელებს სისტემურ სტეროიდულ თერაპიას.

Introduction. With the growing popularity of tattooing, allergic reactions caused by inks are becoming a serious health concern. This paper, along with a single case study, gives a general overview of causes, results and treatment options for such allergic reactions. It is known from several investigations that tattoo inks may contain contact allergens such as metals, colorants and preservatives [6]. Cutaneous injection of these agents can elicit different types of immunological reactions. Reactions from inks can

range from mild local symptoms to systemic ones affecting patient's quality of life. The situation is complicated since there are no strict regulations on dyes used in inks. More new products are appearing in tattoo shops, the quality of which may be questionable and in certain cases may be contaminated with different carcinogenic agents [21]. This, along with already present dangers from dyes themselves, puts tattoo enthusiasts at a higher risk of immunological complications. Most notable in this regard are red inks with the highest overall incidence. Unfortunately, research in this field is lacking and there are no set guidelines for treatment or prevention. Topical or intralesional corticosteroids can be used as the first line of treatment. However, their effectiveness varies and may be temporary or insufficient. It is well understood that allergic reactions will persist as long as an irritant is present. Thus, more drastic measures must be taken. This includes surgical interventions and laser therapy. The effectiveness and safety of laser removal have been disputed in the past [2]. However, a recent study (in regard to red ink) [1] has shown that complications such as anaphylactic reactions are unlikely and all outcomes in this study have been positive. Regarding prevention, professional tattoo artists do perform patch and dot tests prior to tattooing. Unfortunately, such measures are insufficient [1] since allergic reactions may occur weeks or months later.

Case report. We present a case of a 25-year-old woman with severe tattoo irritation and itching on the flexor surface of her right forearm. The patient got a multicolored tattoo (black, yellow, green and red) (Fig. 1) with permanent ink 6 months ago with skin irritation forming 10 days after and worsening ever since. Itching and pain were very severe. The patient had one other old permanent colored tattoo (black and blue) on the same hand, on the extensor surface, more proximally. Information regarding this tattoo is limited due to them not causing allergic reactions.

Fig. 1. Flexor surface of right forearm. Erosions, exudation, infiltration and scaling on red-colored parts.

She has previously visited a dermatologist and was prescribed: for local application - Betamethasone dipropionate, Zn-hyaluronate, Terbinafine, Triamcinolone, Tetracycline, Ethacridine lactate; and per os – Chloropyramine, Ceftriaxone, Dexamethasone, Fexofenadine. The treatment slightly improved symptoms, but the patient still had recurrent episodes of strong itching and pain. On examination skin over the tattoo was hyperemic with slight erosions on red-colored parts (Fig. 1). Scaling, exudation, infiltration and multiple crusts were also present.

Diagnosis of allergic contact dermatitis was made. The patient was prescribed oral Bilastine - 1 tab. BID, for local application - Mometasone furoate - in the evenings; Microdacyn hydrogel - BID, in the morning and afternoon. The patient was advised to undergo laser tattoo removal therapy once inflammation would subside. One month later the patient returned with decreased swelling, infiltration and erosions (Fig.2). Because of this, laser therapy removal session was arranged.

Fig. 2. One month after treatment. Decreased swelling, infiltration and erosions.

Three days after the first sessions of laser tattoo removal therapy with Q-switched neodymium (Nd) 532 nm laser, the patient consulted with us regarding pain in the tattooed area. Skin irritation was present with exudation (Fig.3) Ethacridine lactate pads and Microdacyn hydrogel were prescribed for local application; to alleviate pain Ibuprofen was added.

Fig. 3. After laser removal treatment. Skin irritation with exudation. Erosions over the tattooed skin.

After one week, patient visited us again. Acute inflammation and exudation had subsided. On examination parts of the skin treated with laser removal had developed deep erosions and hypertrophic scarring (Fig. 4). The itchiness remained while the pain was slightly reduced.

Fig. 4. One week after treatment (and laser removal therapy). Deep erosions and hypertrophic scarring.

The patient was prescribed Mometasone furoate and Dexpanthenol/Chlorhexidine for topical application. To reduce the pain, Meloxicam per os QD was prescribed. Due to the ineffectiveness of topical and laser removal therapy, patient was prescribed systemic treatment with Methylprednisolone (16mg) with an indication to gradually decrease the dosage. After two months of systemic therapy, patient's condition improved (Fig.5). She still continues the treatment.

Fig. 5. Two months after treatment with systemic corticosteroids.

Discussion.

Complications of tattooing are not a novel problem. Different types of reactions have been recorded throughout the history. With the growing prevalence of tattooing, more research and regulations

are required to eliminate possible adverse reactions. These effects include acute allergy directly after tattooing or delayed hypersensitivity after long-term exposure to the chemicals in the inks [18,19,31]. According to the literature, the most frequent tattoo reactions concern allergic contact dermatitis due to delayed hypersensitivity reaction to different pigments contained in the tattoos [29]. There are two types of contact dermatitis: Irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). ICD arises in response to the obligate irritant, while ACD is caused by a facultative agent (also called contact allergens). ICD accounts for ~80% of all contact dermatitis, with the rest being ACD [5]. These processes cause inflammation of the skin manifested by varying degrees of erythema, edema and vesiculation [10].

Mechanism of Allergic contact dermatitis.

In ACD, a distinction should be made between induction (sensitization) and effector (elicitation) phases [20]. The induction phase includes the events following first contact with the allergen. This is also called contact allergy [20].

Contact allergy is a T-cell mediated reaction induced by haptens. These are low molecular weight (<500 Daltons) chemicals (such as metal salts, dyes, preservatives and fragrances), which are not immunogenic by themselves but can be efficiently recognized by the immune system after binding to the skin components and penetrate the stratum corneum barrier of the skin. The allergen penetrating the skin readily associates with all kinds of skin components, including major histocompatibility complex (MHC) proteins. These molecules, in humans encoded for by histocompatibility antigen (HLA) genes, are abundantly present on epidermal Langerhans cells (LC) [6,15]. Hapten-Protein binding is the initial step in the development of allergic contact dermatitis [6]. During the induction phase, skin contact with a hapten triggers the migration of epidermal Langerhans cells (LC) via the afferent lymphatic vessels to the skin-draining lymph nodes. Haptenized LC home into the T-cell-rich paracortical areas. Here, conditions are optimal for encountering naive T cells ($CD8^+$ and $CD4^+$) that specifically recognize allergen–MHC molecule complexes. Hapten-specific T-cells now expand abundantly and generate effector and memory cells, which are released via the efferent lymphatics into the circulation. With their newly acquired homing receptors, these cells can easily extravasate peripheral tissues. The induction phase ends here and begins the second, effector phase [6,17,21,30].

The second phase is also called the elicitation or provocation phase [6] and results in the clinical manifestation of ACD. It occurs after re-exposure to the allergen and is mediated by CD8+ T cells, which are primed in lymphoid organs during the sensitization phase and are recruited in the skin upon re-exposure to the haptens. By renewed allergen contact, the effector phase is initiated, which depends not only on the increased frequency of specific T-cells, and their altered migratory capacities, but also on their low activation threshold. Due to their lowered activation threshold, hapten-specific effector T-cells are triggered by various haptenized cells, including LC and keratinocytes (KC), to produce proinflammatory cytokines and chemokines. Thereby, more inflammatory cells are recruited further amplifying local inflammatory mediator release. This leads to a gradually developing eczematous reaction.

Tattoos as allergens.

Tattooing involves injection of tattoo ink into the dermis to a depth of 1-2 mm using a tattoo machine. Tattoo inks are complex formulations containing several ingredients, both organic and inorganic, by-products and impurities. The inks are usually ready-to-use-products, which consist of insoluble pigments (responsible for the color) in a liquid made of binder(s) and solvent(s). Preservatives are often added to the mixture to avoid microbiological contamination of the often water-based mixture. Besides intentional ingredients, other substances may be present as impurities such as metals from inorganic and organometallic pigments. Colorants are by far the major ingredient of tattoo inks and may reach high concentrations. The colorants can be classified into dyes and pigments. Dyes along with other biological ingredients and preservatives are soluble and biodegradable. In case of adverse immunological reactions, no long-term treatment will be needed since they will be metabolized and cleared naturally. Pigments, on the other hand, are insoluble, chemically resistant and the preferred choice of tattoo inks. Therefore, sensitization caused by them will be last as long as pigment remains in the dermis [6]. Allergic contact dermatitis in tattoos has been reported regularly since the 1950s [6]. The severity of allergic reaction is mainly determined by the composition of tattoo ink. The components of tattoo ink are difficult

to determine and undergo changes with time. There are also no regulations for tattoo inks or color additives, which contain potentially allergic substances and in some cases these pigments used in the formulation of tattoo inks are not even produced for this purpose [28]. The main pigment causing allergic reactions historically is the red one, due to the presence of mercury and its sulfides. Other common etiological factors include chrome and cobalt representing the different dyes (Table 1).

Table 1. Types of dyes and their composition

Color	Composition
Red	• Mercury sulfide (cinnabar) • Ferric hydrate (sienna) • Sandalwood • Brazilwood • Iron oxide
Black	• Carbon (India ink) • Iron oxide • Logwood
Brown	• Ferric oxide
Blue	• Cobalt aluminate • Azure blue • Cobalt blue
Green	• Chromic oxide • Lead chromate • Phthalocyanine dyes • Ferrocyanides and ferricyanides
Yellow	• Cadmium sulfide
Purple	• Manganese • Aluminum
White	• Titanium oxide • Zinc oxide • Lead carbonate

However, not all reactions are due to the traditional presence of mercury sulfides and other metal-derived colors, but due to new organic pigments (e.g., Pigment Red 181 and Pigment Red 170) [3,6]. Studies have shown, that people with different colors in their tattoos are at higher risk of developing chronic reactions than the ones with single-colored tattoos. The two ink colors most commonly involved in chronic color-associated reactions were red (8/18) and black (6/18), although other colors were also reported [7]. Red tattoo pigment can be either organic or inorganic. Inorganic red pigment includes mercury, cadmium selenide and sienna (ferric hydrate - iron oxide and manganese oxide) [13]. Organic red pigment includes sandalwood and brazilwood, both organic vegetable dyes [8]. The red pigment can also be made with cinnabar (a mercury derivative) and this is the one that is thought to cause the cell-mediated delayed hypersensitivity reaction [13].

Prevention.

We've made it abundantly clear that tattooing comes with its risks which should not be underestimated. Unfortunately, things are not quite clear in terms of harm reduction and allergy prevention. In general, contact allergy can be demonstrated by an allergy test, called a patch test, which is an internationally accepted tool to diagnose contact allergy. The methodology has been in use for over 100 years and is in use worldwide. At patch testing, small amounts of the suspected allergens are applied in aluminum chambers to the upper back of the person under investigation. The patches are left in place for two days and then removed. The skin is inspected for allergic reactions several times over the following days. The diagnosis of allergic contact dermatitis requires typical clinical symptoms, as described, and positive results from patch testing to substances, to which the person is exposed to. Alternatively, in professional tattoo shops, dot tests are performed. This means that a section of skin is tattooed with a single dot of ink. This area is monitored over the course of 24 hours to see what happens. Any swelling or redness

could indicate an allergic reaction. These methods however don't guarantee anything. For example, studies [1] have shown that patch and dot testing do not correlate to tattoo reactions caused by mercury (red ink) and understanding of the mechanisms behind the reactions observed in red tattoos is still lacking with type I–III hypersensitivity reactions playing a role [2]. There is no conclusive evidence to date. In light of all these new steps are being taken by world healthcare organizations to prevent unnecessary harm from tattooing. New bills have passed this year in EU that will limit and regulate ingredients in inks. The consequences of such actions remain to be seen. Since outright banning some colors might force tattoo enthusiasts to seek their desired designs from unlicensed professionals further increasing their risks [2].

Treatment.

Treatment of allergic reactions to tattoos is difficult, as tattoo pigments are permanently stored in the dermis. Topical, oral and/or intralesional corticosteroids are indicated as first-line treatment. Conservative therapy options also include calcineurin inhibitors and oral antihistamines, but these methods are often insufficient [4,12]. The best treatment option to remove the responsible allergen is unknown. Surgical excision, dermatome shaving or lasers (Q-switched nanosecond laser, ablative CO_2 lasers, picosecond laser) are reported as treatment options with permanent results [12,23]. Though, each treatment option has its disadvantages, such as possible scarring, infection, risk of generalized allergic reactions and treatment imprecision [4]. Laser removal of tattoos has been reported to stimulate an allergic response itself, as it causes fragmentation of the pigment-containing cells, exposing the pigment to the extracellular environment, where it can be recognized as foreign by the immune system [12].

Dermatome shaving is the surgical removal of pigment (hapten) concentrated in the outer dermis. This method is proposed as a first-line treatment of chronic tattoo reactions.

A study from 2015 examined the treatment of 50 patients, with chronic tattoos reactions, with dermatome shaving. Tattoos with red/red nuances dominated the study material. Shaving was performed to the level of the dermis which was free of tattoo pigment as assessed visually by the surgeon. After surgery severity rating of patient's symptoms declined from 3,2 pre-operatively to 1,0, 0,8 and 0,7 after 3,6 and 12 months, respectively [9,24]. With Q-switched laser technology, tattoo removal can be achieved through permanent pigmentary alteration. They are considered the standard method for removing both regular and traumatic tattoos [22]. There are three types of Q-switched nanosecond lasers, that are currently used for tattoo removal: Q-switched ruby laser (694 nm), Q-switched Nd:YAG laser (532 nm,1064 nm) and Q-switched alexandrite laser (755 nm). The Q-switched ruby and alexandrite lasers are useful for removing black, blue and green pigments. The Q-switched 532 nm Nd:YAG laser can be used to remove red, brown and green pigments and the 1064 nm Nd:YAG laser is used for the removal of black and blue pigment. The most common adverse effects following laser tattoo treatment with the Q-switched ruby laser include textural change, scarring, and pigmentary alteration. Other types of Q-switched nanosecond lasers have less risk of scarring or hyperpigmentation [16]. Ultra-pulse CO_2 lasers remain a second-line treatment option. This is a microsurgical laser beam, which removes both the ink and the skin, layer by layer. Complete removal of a tattoo is determined by the size, ink pigment and localization of the tattoo. Several sessions may be needed to reach the desired goal. With this method, entire tattoo can be removed but is replaced by scar tissue. According to studies, in the initial stages of traumatic tattoo removal, the ablative fractional laser treatment appears to be as effective as the standard ruby laser therapy. However, from the 6th session onwards, ruby laser therapy becomes more effective [22]. Picosecond laser, associated with minimal risk of scarring, was recently introduced. It selectively destroys the target pigment without damaging healthy, normal tissue. These lasers use pulse durations of less than 1 nanosecond, which causes predominantly photoacoustic, rather than photothermal destruction of pigment or ink particles (in the case of Q-switched nanosecond lasers). This allows rapid clearing of the abnormal pigmentation with minimal collateral damage to surrounding tissue. Although potential side effects from picosecond laser treatment include pain, erythema, edema, pinpoint bleeding, crusting, blistering, scarring and post-inflammatory hyperpigmentation. [14]

Conclusion.

The case presented in this paper is one of the most common ways patients may present with a tattoo caused allergic reaction. As was described, the allergic reaction was severe enough to affect the quality of life of the patient. A diagnosis of ACD was made. Topical treatment with corticosteroids was

initiated. Allergic reaction couldn't be fully cleared, since pigments of ink remained in the skin. The patient underwent one session of tattoo removal therapy with Q-switched Nd 532 nm laser. This, however, caused local irritation, for which she was prescribed topical anti-inflammatory medications. Due to the ineffectiveness of topical and laser removal therapy, the patient was prescribed systemic treatment with corticosteroids. After two months of this therapy, the patient's condition improved. She is still undergoing this treatment.

ACD, the most common reaction caused by tattoos, is a delayed-type hypersensitivity reaction. This process may be broken down into two parts. First being sensitization and second, caused by re-exposure, immune reaction. In cases of ACD's caused by tattoos, we have continued exposure to such allergens. Since most dyes used in inks are not bio-degradable, patient's condition won't be resolved unless pigments are fully removed. Due to their components, red and black inks are the most common cause of tattoo-induced allergic reactions. Modern prevention methods that are widely used in tattoo shops are patch and dot allergy tests. They are performed 24 hours before the actual tattoo is applied to check for possible allergies. Such measures may not be enough, since allergic reactions may not appear acutely. Topical or intralesional corticosteroids can be used as conservative therapy. If the allergen is bio-degradable material and can be easily cleared, this may be enough. In cases where it's not, laser-assisted tattoo removal remains the gold standard for treatment. Since many wavelengths are needed to treat multicolored tattoos, not single laser will be enough to remove all variety of inks and their combinations [16]. In case laser removal therapy proves to be ineffective, systemic treatment with corticosteroids may be initiated with scheduled monitoring of patient's condition.

New measures are just now being put in place to regulate the quality and content of inks to reduce the incidence of such allergic reactions.

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TINATIN KITUASHVILI, TAMAR URUSHADZE

CASE STUDY - ALLERGIC CONTACT DERMATITIS IN RELATION TO TATTOOS

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SUMMARY

As tattooing becomes more and more popular, growing numbers of skin reactions caused by tattoos are also becoming frequently encountered by medical professionals. We present a generic case of a tattoo-induced allergic reaction and explore its' immunological mechanism. This paper also highlights components of tattoo inks, their allergenic potential, and possible options for treatment. There can be different types of allergens in tattoo inks. Some are biodegradable, while others are not. Examples of biodegradable components include natural dyes and preservatives. Allergic reactions caused by such agents may resolve with simple therapy since after a short period they will be cleared from the skin. On the other hand, synthetic molecules and other non-degradable dyes will need invasive therapy, such as surgery, dermatome shaving and most commonly used - laser removal therapy. Most notable in this regard is red ink with the highest incidence. There are no current regulations on tattoo inks, which puts tattoo enthusiasts at a higher risk of developing allergic reactions. There are certain preventive measures, such as patch and dot tests. Because the specificity of these tests is mediocre, despite negative results, an allergic reaction may develop weeks or months later. There are no strict treatment guidelines and each case must

be assessed individually. Our patient was a young woman, who developed a local allergic reaction due to the red pigment used in her tattoo. Initial treatment, in this case, was anti-inflammatory to reduce inflammation. The only way to get full resolution in such cases is to remove the allergen (red pigment) from the dermis. The patient was prescribed topical treatment with corticosteroids. Once irritation subsided tattoo removal therapy with Q-switched Nd 532 nm laser was initiated. The inflammation returned after the first session, for which local anti-inflammatory medications were started. Due to the ineffectiveness of laser removal and local treatments systemic therapy with corticosteroids was prescribed with gradually decreasing the dosage and controlling the disease. After two months of this treatment, the patient's condition improved. She is still undergoing therapy with systemic corticosteroids.

Keywords: Allergic contact dermatitis, Tattoo ink, red pigment, Laser removal therapy, Q-switched Nd laser, Treatment with systemic corticosteroids.

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PROSPECTS FOR IMMUNOTHERAPY TREATMENT AGAINST COVID-19 INFECTION

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ნინო კუკულაძე, ალექსანდრე ბახუტაშვილი

COVID-19 ინფექციის საწინაღმდეგო იმუნოთერაპიული მკურნალობის პერსპექტივები

ვლ.ბახუტაშვილის სახელობის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, თბილისი, საქართველო

რეზიუმე

COVID-19 პანდემია დღევანდელი მსოფლიოს მნიშვნელოვან პრობლემას წარმოადგენს. დღესდღეობით არ არსებობს მკურნალობა SARS-CoV2 წინააღმდეგ. ჩვენს ნაშრომში აღვწერთ და ვაანალიზებთ რამდენიმე თერაპიულ პრეპარატს, მოქმედების მექანიზმით, მიმართული იმუნურ სისტემაზე, გამოყენებული COVID-19 სამკურნალოდ, კლინიკურ კვლევებში.

განვიხილავთ იმუნური სისტემის მოდულაციას, 2 ძირითად ასპექტში:

- *პასიური იმუნიტეტის მიღწევა, როგორც პროფილაქტიკა მძიმე და კრიტიკულ პაციენტებში. თანმდევი რისკებით.*
- *ციტოკინების შტორმის დათრგუნვა, რომელიც დაავადების მძიმე და კრიტიკული ფორმის მთავარი გამომწვევი მიზეზია.*

საბოლოოდ აშშ წამლის სააგენტოს თანახმად, განიხილავენ მონონუკლეარულ ანტისხეულებს პასიური იმუნური პასუხის მისაღებად და დექსამეტაზონს ციტოკინების შტორმის დასათრგუნად, პრეპარატის შეყვანის დროის გაკონტროლებით, დაავადების სტადიის მიუხედავად.

COVID-19 pandemic is a real threat for the people worldwide. Currently there is no specific treatment to eliminate SARS-CoV2 from the infected humans. In this review we describe and analyze several therapeutical preparations, which target immune system, used in the clinical trials to treat COVID-19. We discuss immune system modulation in two main aspects:

- first, achievement of passive immunity as prophylactic of severe and critical disease in SARS-CoV-2 infected people with concomitant risks;
- second, inhibition of cytokine storm, which is main cause of severe and critical disease.

Finally, according to the USA NIH guidelines, monoclonal antibodies for passive immunity and dexamethasone for cytokine storm are discussed with emphasis on the timing of administration concerning COVID-19 patients' stage of disease.

Passive Immunity

Neutralizing antibody production by the immune system is triggered to interrupt initial interaction between the SARS-CoV-2 spike protein and the human ACE2 receptor and subsequent cellular uptake of the virus (1,2). The immune response kinetics, magnitude, and its casuality with disease severity during acute-phase response have been defined extensively. SARS-CoV-2 elicits humoral and cellular immune responses; within 7 days of infection, virus-specific memory CD4+ and CD8+ T cells emerge, peaking within 2 weeks but remaining detectable at comparatively lower levels for ≥100 days. Simultaneously, there are strong B-cell responses with immunoglobulin M (IgM) and IgA antibodies detected by days 5–7 and IgG antibodies by days 7–10(3). The magnitude of both antibody and T-cell responses is not uniform among individuals with COVID-19 and appears to be influenced by disease severity (4,5). In patients with first contact with the new viral antigen enhancing concentration of antibodies in blood by infusion of plasma from convalescent individuals or synthesized monoclonal antibodies could benefit the course of disease. These opportunities were tested in several clinical trials.

Convalescent Plasma (CP)

Plasma from donors who have recovered from COVID-19 may contain antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that may help suppress the virus and modify the inflammatory response (6). The use of CP involves transfusing plasma collected from patients who have already recovered from an illness, in an attempt to transfer neutralizing antibodies and confer passive immunity (7,8). The potential efficacy of CP was first described during the Spanish influenza pandemic of the early 1900s (9). Since then, CP has been used to attempt to treat a wide range of viral infections, including measles, parvovirus B19, H1N1, Ebola and some coronaviruses (10).

In the Pandemic of Covid-19, plasma therapy has been used for treating COVID-9 patients (11). In an initial study, five patients with COVID-19 with ARDS[1] underwent plasma therapy and clinical outcomes were compared before and after CP transfusion. The results showed improvement in the patients' clinical condition. In a study by Duan et al. in ten severe adult cases, the results showed that a dose of 200 mL CP was well tolerated and could significantly increase or maintain neutralizing antibodies at a desirable level. This treatment was capable of reducing viremia within 7 days. After the application of this treatment method, clinical and paraclinical symptoms improved rapidly within 3 days. Radiological studies also showed varying degrees of absorption of lung lesions within 7 days. According to these observations, CP can be expected as a life-saving option in patients with severe COVID-19 (12).

On 24 March 2020, the US FDA announced the approval of convalescent plasma therapy for critically ill individuals with COVID-19 as an emergency investigational new drug (13). Obviously, CP should be used on the initial stages of COVID-19 to help eliminate viral particles from the patients and prevent massive invasion of virus in the tissue. During the severe and terminal stages CP will not benefit patients as the course of disease is driven by mechanisms which involve immune overreaction and hyper-inflammation, which are not prevented by specific antiviral antibodies from CP.

Anti SARS-CoV-2 monoclonal antibodies (MaB)

Bamlanivimab (Elly Lilly) and the combination of casirivimab plus imdevimab (Regeneron Pharmaceuticals) are anti-SARS-CoV-2 monoclonal antibodies (anti Spike virus protein) available through US FDA Emergency Use Authorizations (EUAs) for the treatment of outpatients with mild to moderate COVID-19 who are high risk for progressing to severe disease and/or hospitalization.

Bamlanivimab was authorized for patients with positive results of direct SARS-CoV-2 viral testing who are 12 years of age and older weighing at least 40 kilograms, and who are at high risk for progressing to severe COVID-19 and/or hospitalization. This includes those who are 65 years of age or older, or who have certain chronic medical conditions.

Clinical trials are continued but there are promising positive clinical results for Banlavinimab and Casirivimab plus Imdevimab (14,15). An interim analysis of this study suggested a potential clinical benefit of bamlanivimab for outpatients with mild to moderate COVID-19 who received the antibody infusion a median of 4 days after symptom onset (16).

[1] ARDS - acute respiratory distress syndrome

Casirivimab plus imdevimab for outpatients with mild to moderate COVID-19 who received an infusion of the drug combination a median of 3 days after symptom onset (17).

Inhibition of immune response

Cytokine storm as the hallmark of ARDS, is an uncontrolled systemic inflammatory response triggered by some immune system cells due to the release of proinflammatory cytokines and chemokines. In this regard, high expression levels of cytokines and chemokines, including IL1-β, IL1RA, IL7, IL8, IL9, IL10, FGF2, GC-SF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGFB, TNF-α, and VEGFA are observed in the serum of patients with COVID-1(18). Inability of the immune system to control this condition has led to the death of many patients with COVID-19(19). Consequently, attenuating cytokine storm in patients with COVID-19 is one of the goal of hospital therapy.

Therapeutic Management of Patients with COVID-19 for the COVID-19 Treatment Guidelines Panel's (the Panel's) recommendations on the use of the following:

- Dexamethasone (or other corticosteroids) with or without remdesivir
- Baricitinib with remdesivir.

Corticosteroids

Dexamethasone have been studied in critically ill patients with ARDS with conflicting results (20,21,22). Seven randomized controlled trials that included a total of 851 patients evaluated use of corticosteroids in patients with ARDS. A meta-analysis of these trial results demonstrated that, compared with placebo, corticosteroid therapy reduced the risk of all-cause mortality (risk ratio 0.75; 95% CI, 0.59–0.95) and duration of mechanical ventilation (mean difference, -4.93 days; 95% CI, -7.81 to -2.06 days) (23,24).

Finally, Recommendations on the use of corticosteroids for COVID-19 are largely based on data from the "RECOVERY" trial, a large, multicenter, randomized, open-label trial performed in the United Kingdom (25). This trial compared hospitalized patients who received up to 10 days of dexamethasone to those who received the standard of care. Mortality at 28 days was lower among patients who were randomized to receive dexamethasone than among those who received the standard of care. This benefit was observed in patients who were mechanically ventilated or required supplemental oxygen at enrollment. It should be emphasized that this study also found no benefit of dexamethasone was seen in patients who did not require supplemental oxygen at enrollment.

Due to wide availability and low-price dexamethasone is the best recommended and effective choice of COVID-19 patient treatment of (Table 1).

Baricitinib (Br)

The kinase inhibitors are proposed as treatments for COVID-19 because they can prevent phosphorylation of key proteins involved in the signal transduction that leads to immune activation and inflammation (26).

Baricitinib, sold under the brand name Olumiant, is a drug for the treatment of rheumatoid arthritis (RA) in adults whose disease was not well controlled using RA medications called tumor necrosis factor (TNF) antagonists, Br is an inhibitor of Janus kinase an important enzyme in the mechanics of inflammation (27,28).

The Panel's recommendations for the use of baricitinib are based on data from the Adaptive COVID-19 Treatment Trial 2 (ACTT-2), a multinational, randomized, placebo-controlled trial of baricitinib use in hospitalized patients with COVID-19 pneumonia. Participants (n = 1,033) were randomized 1:1 to oral baricitinib 4 mg or placebo, for up to 14 days, in combination with intravenous (IV) remdesivir, for up to 10 days. Participants who received baricitinib had a shorter time to clinical recovery than those who received placebo (median recovery time of 7 vs. 8 days, respectively). This treatment effect was most pronounced among those who required high-flow oxygen or non-invasive ventilation but were not on invasive mechanical ventilation. The difference in mortality between the treatment groups was not statistically significant (29).

Other Janus kinase (Ruxolitinib (30), Tofacitinib[Noclinical trials to date]) as well as Bruton's Tyrosine kinase inhibitors were used or proposed to do so to treat cytokine storm in COVID-19 patients, but only Br has some recommendations from US NIH as others have very limited clinical data.

Interleukin inhibitors

Interleukin (IL)-6 is a pleiotropic, pro-inflammatory cytokine produced by a variety of cell types, including lymphocytes, monocytes, and fibroblasts. Infection by the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) induces a dose-dependent production of IL-6 from bronchial epithelial cells (31).

Preliminary, unpublished data from randomized, controlled trials failed to demonstrate efficacy of sarilumab or tocilizumab in patients with COVID-19. There are only limited, unpublished data describing the efficacy of siltuximab in patients with COVID-19(32).

Small one center published trial is available for tocilizumab (33) Treatment of patients with COVID-19 through tocilizumab therapy, some laboratory parameters including C-reactive protein (CRP) and IL-6 concentrations should be assessed before and after tocilizumab therapy. In addition, tocilizumab was used along with methylprednisolone in some patients with COVID-19. The studies have shown that the level of IL-6 decreased in patients after taking tocilizumab, while the level of IL-6 increased significantly in patients who were not treated with tocilizumab. TCZ appears to be an effective treatment option in patients with COVID-19 at high risk of cytokine storm. More studies needed to evaluate definite clinical benefits of the drug.

Vitamin D

Immunoregulatory role of Vitamin D (VD) is well known (34). VD was evaluated as a supplement to COVID-19 patients' diet, especially in obese cohort (35,36). Combined to obesity VD deficiency hypothesized as a cause of severe symptoms of COVID-19 (37). Indeed, VD deficiency or insufficiency, defined as 25(OH) D below 20 ng/mL and 30 ng/mL respectively were associated with an increased risk of SARS-CoV-2 (38). Moreover, very low vitamin D levels appear to be associated with greater risk for admission to an intensive care unit (ICU) and consequent mortality (50%) (39,40,41). Few randomized clinical studies are published up to date with positive (42) and negative results (43) and further studies are needed to evaluate VD's effect in COVID-19.

There is some evidence that VD deficiency is associated with hypocalcemia, which is observed in COVID-19 patients and could serve as a potentially useful biomarker for disease severity and outcome in patients with SARS-CoV-2 infection (44,45,46). Therefore, vitamin D supplementation might have a therapeutic role in these patients.

In our opinion would be reasonable to recommend a goal of VD blood levels >30 ng/mL. at least to those who are at particularly high risk such as older men with co-morbidities such as diabetes and obesity.

Finally, recommendations and treatment guidelines from USA NIH are most balanced guide for the treatment of COVID 19 patients (Table 1).

Table 1. NIH basic COVID-19 treatment guidelines

Disease severity	NIH Panel's treatment recommendation
Not hospitalized. Mild to Moderate COVID-19.	SARS-CoV-2 neutralizing antibodies for the patients with high risk of disease progression. Bamlanivimab (Elly Lilly) and the combination of casirivimab plus imdevimab (Regeneron Pharmaceuticals).
Hospitalized but does not require Oxygen supplementation.	Remdesevir could be appropriate. Pannel recommends against dexamethasone or other corticosteroids administration at this stage.
Hospitalized and required noninvasive Oxygen supplementation.	Use one of the following options: • Remdesevir for patient who require minimal oxygen dose • Dexamethasine + Remdesevir for patients with increasing amounts of oxygen • Dexamethasone alone when Remdesevir is not available or couldn't be used for patients with increasing amounts of oxygen
Hospitalized and required invasive Oxygen supplementation.	Dexamethasone only

Further clinical and experimental studies are necessary to improve clinical care of COVID-19 patients.

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PROSPECTS FOR IMMUNOTHERAPY TREATMENT AGAINST COVID-19 INFECTION

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SUMMARY

COVID-19 pandemic is a real threat for the people worldwide. Currently there is no specific treatment to eliminate SARS-CoV2 from the infected humans. In this review we describe and analyze several therapeutical preparations, which target immune system, used in the clinical trials to treat COVID-19. We discuss immune system modulation in two main aspects: first, achievement of passive immunity as prophylactic of severe and critical disease in SARS-CoV-2 infected people with concomitant risks; and, second, inhibition of cytokine storm, which is main cause of severe and critical disease. Finally, according to the USA NIH guidelines, monoclonal antibodies for passive immunity and dexamethasone for cytokine storm are discussed with emphasis on the timing of administration concerning COVID-19 patients' stage of disease.

Keywords: Covid-19, immunotherapy, prospects

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THYROID DYSFUNCTIONS INDUCED BY IMMUNE CHECK-POINT INHIBITORS

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იმუნური მაკონტროლებელი მოლეკულების ინჰიბიტორებით გამოწვეული ფარისებრი ჯირკვლის დისფუნქციები

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რეზიუმე

იმუნური სისტემა კიბოს განვითარებისა და პროგრესირებისაგან იცავს ორგანიზმს. იმუნოთერაპიული მიდგომები, რომელიც სიმსივნის მიკროგარემოში დათრგუნული იმუნური უჯრედების ფუნქციას გააქტიურებს, ბოლო ათეული წელია ინტენსიურად ინერგება კლინიკურ პრაქტიკაში. სამედიცინო საზოგადოების განსაკუთრებული ყურადღება მიიპყრო ავთვისებიანი სიმსივნეების იმუნოთერაპიამ ე.წ. Immune checkpoints inhibitors - იმუნური მაკონტროლებელი მოლეკულების ინჰიბიტორებით (ი.მ.მ.ი). ი.მ.მ.ი-ს ძირითადად წარმოადგენს ციტოტოქსიური T ლიმფოციტის ანტიგენ 4-ის (CTLA-4), უჯრედის დაპროგრამებული სიკვდილის პროტეინ 1-ის (PD-1), უჯრედის დაპროგრამებული სიკვდილის პროტეინის ლიგანდ 1-ის (PD-L1) საწინააღმდეგო მონოკლონური ანტისხეულები. ისინი T უჯრედებს სიმსივნური უჯრედების წინააღმდეგ ააქტიურებენ, თუმცა მთელ რიგ აუტოიმუნურ გვერდით ეფექტებს აღძრავენ, რომლებიც სამედიცინო ლიტერატურაში ცნობილია როგორც იმუნური გვერდითი მოვლენები. იმუნოთერაპიით გამოწვეული გვერდითი მოვლენები, ქიმიოთერაპიასთან ასოცირებული გვერდითი მოვლენებისგან განსხვავებით, ხასიათდებიან დაყოვნებული რეაქციით და გახანგრძლივებული მოქმედებით, რომელთა ეფექტური მართვა და აღმოფხვრა დამოკიდებულია მათ ადრეულ ამოცნობაზე.

წინამდებარე მიმოხილვა მიზნად ისახავს გაზარდოს ონკოლოგებისა და ენდოკრინოლოგების თეორიული და პრაქტიკული ცოდნა იმუნური მაკონტროლებელი მოლეკულების ინჰიბიტორებით გამოწვეული თირეოიდული დისფუნქციების შესახებ, კრიტიკულად გადაამუშაოს ამ პაციენტთა მართვის მიდგომები, რათა უზრუნველყოფილი იქნას ონკოპაციენტების სიცოცხლის ხარისხის გაუმჯობესება.

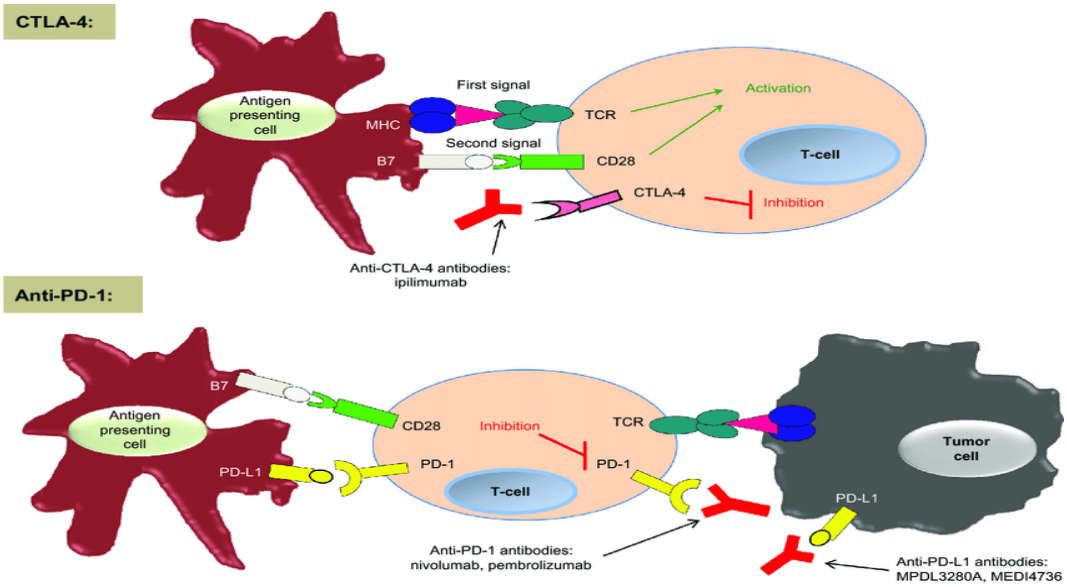
Introduction. Until recently, chemotherapy, radiation and surgery were considered the cornerstones of cancer treatment [1]. Advances in immunotherapy have revolutionized tumors treatment. Currently, the most widely used approach is the administration of targeted monoclonal antibodies (mAbs) directed against T cell activation [2].

Under homeostatic conditions, there is a balance between pro-inflammatory and anti-inflammatory signaling maintained by immune checkpoints [1]. These immune checkpoints are a set of inhibitory and stimulatory pathways that directly affect the function of immune cells. Malignant cells disrupt this balance by promoting an immunosuppressive state that favors immune evasion and tumor growth [1].

Cancer cells recruit or induce development of regulatory T cells (Tregs), downregulate tumor antigen expression, induce T cell tolerance and/or apoptosis and produce immune suppressive cytokines that stimulate inhibitory immune check-points. This leads to a unique and highly immunosuppressive tumor microenvironment (TME) [1]. In an attempt to overcome these immunosuppressive conditions, immune check-point inhibitors act by blocking the effects of selected inhibitory pathways [1]. The best described immune checkpoints are CTLA-4, PD-1, PD-L-1[1].

CTLA-4 is constitutively expressed by regulatory T cells and upregulated after T cells activation, acting as an "OFF" switch. CTLA-4 binds the B7 ligand on antigen presenting cell (APC). Binding CTLA-4, immune check-point inhibitor prevents it from binding with B7, and allows B7 to bind with CD28, in this way inducing the immune system to attack tumor cells [3].

PD-1 is present on T, B, and NK cells, and binds to PD-L-1, expressed by tumor cells, preventing apoptosis of the cell expressing PD-L-1 by the immune system. ICIs, that bind either PD-L-1 or PD-1, prevent this process [3].



Simplified concept of CTLA-4 and PD-1 immune checkpoints. Notes: In the priming phase, antigen-presenting cells present antigens to the T-cell. Two signals are required to initiate a T-cell response. CTLA-4 is upregulated after T-cell activation and inhibits the T-cell response. Anti-CTLA-4 antibodies bind to CTLA-4, turning off the 'inhibitory signal', thus resulting in an enhancement of T-cell function. In the effector phase, the PD-1 inhibitory receptor is expressed by the T-cell and, when it is engaged by its ligands PD-L1 and PD-L2, it serves to inhibit the T-cell response. Anti-PD-1 antibodies bind to PD-1, turning off the 'inhibitory signal' in the peripheral tissues and enhancing T-cell function. PD-1/PD-L1 interactions are complex, and this interaction is also involved in the priming phase. We have chosen to portray the main concepts for both of these immunologic checkpoints in this figure for simplicity. Abbreviations: CD, cluster of differentiation; CTLA, cytotoxic T-lymphocyte-associated antigen; MHC, major histocompatibility complex; PD, programmed cell death; TCR, T-cell receptor [4].

Currently FDA approved seven ICIs for the treatment of different solid tumors: CTLA-4 inhibitor – ipilimumab, PD-1 inhibitors – pembrolizumab, nivolumab and cemiplimab and PD-L-1 inhibitors – atezolizumab, avelumab and durvalumab [5,6]. Table-1 summarizes the different ICIs and their main clinical indications [1,5,7].

Table-1. different ICIs and their main clinical indications

TUMOR	ANTI-PD-1			ANTI-PD-L1			ANTI-CTLA4
	PEMBROLIZUMAB	NIVOLUMAB	CEMIPLIMAB	ATEZOLIZUMAB	AVELUMAB	DURVALUMAB	IPILIMUMAB
MELANOMA				V	V	V	
NSCLC					V	V	V
HNSCC				V	V	V	V
RCC	V			V	V	V	V
UROTHELIAL							V
cHL		V		V	V	V	V
MSI-H		V		V	V	V	V
MCC	V	V		V		V	
CSCC			V				

NSCLC-non-small cell lung cancer, HNSCC- head and neck squamous cell carcinoma, RCC-renal cell carcinoma, cHL- classical Hodgkin's lymphoma, MSI-H - high microsatellite instability tumors, MCC-Merkel cell carcinoma, CSCC- cutaneous squamous cell carcinoma.

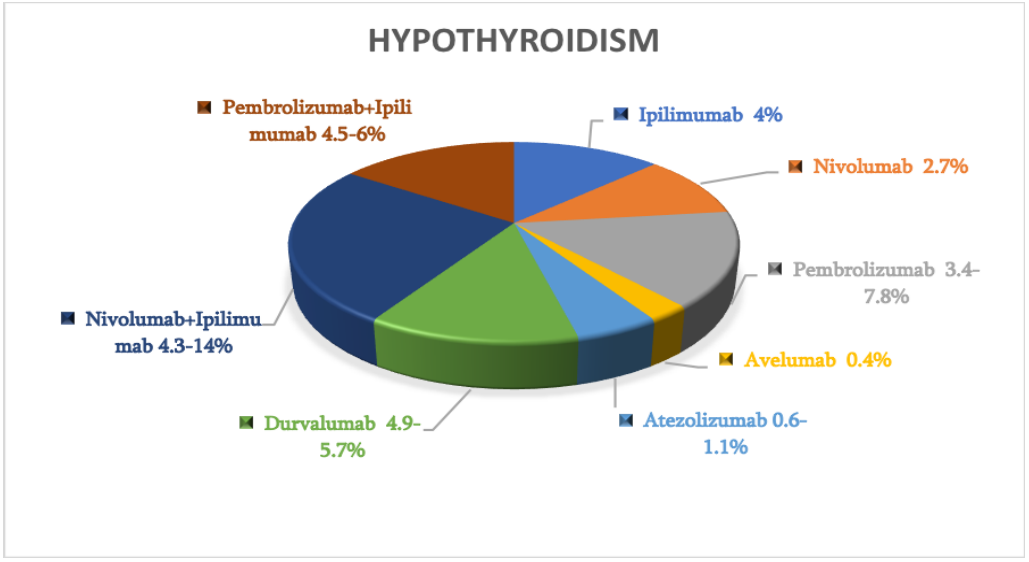
Immune inhibitory pathways have significant role in the maintenance of self-tolerance, therapeutic targeting of these pathways can lead to imbalances in immunologic tolerance, that manifest as immune-related adverse events (irAEs) [7]. A broad range of autoimmune toxicities have been reported [4]. Endocrine diseases are among the most common associated irAEs, especially immune related thyroid dysfunctions.

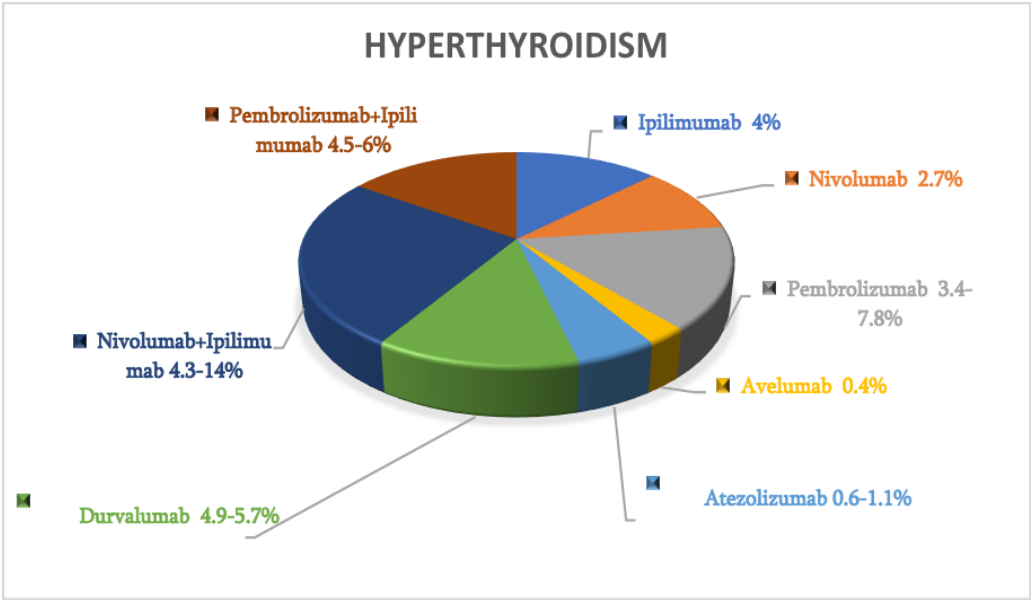
The main goal of this article is to describe and analyze the incidence, pathogenesis, clinical manifestations and guidelines on the management and screening of thyroid disorders associated with ICIs [5]. Thus, physicians and specialists involved in treating patients can easily identify and manage immune-related side effects.

Thyroid Disorders. The spectrum of thyroid disturbances under ICIs can present as thyrotoxicosis, hypothyroidism, painless thyroiditis, thyroid eye disease and occasionally sever form such as thyroid storm [3,5,8].

The incidence of thyroid disorders differs between different ICI classes [5]. Chart-1 represents reported frequencies of hypothyroidism (%) and hyperthyroidism (%) [7,9]. Thyroid dysfunctions are mostly provoked by anti-PD-1 or anti-PD-L-1 mAbs and incidence ranges from 4 to 19.5% [7].

Chart-1. Immune-related Hypothyroidism and Hyperthyroidism frequencies (%) under different ICIs.





The median time to onset of hyperthyroidism is reported to be around 21 days in combination therapy (CTLA-4 +PD-1/PD-L-1 inhibitors) and 75 days in monotherapy with PD-1 inhibitors [5, 10]. The predicted incidence of hypothyroidism was higher with combination therapy approximately 13% and 7% for PD-1 inhibitors alone [5,10]. However, immunotherapy is a "living drug" and the modulation of the adaptive immune response might persist for years, resulting in immune-related thyroid dysfunctions after cessation of treatment [11].

Although the etiology of immune-related thyroid disorders remains elusive, the knowledge that the antitumor immunity and the autoimmunity represents indistinguishable models of attack by T cells rationalizes the assumption that ICIs manipulating the T cells signaling toward unleashing the antitumor response, can exacerbate the autoimmunity [11]. But there are some data that provide support for a distinct pattern of immune-mediated thyroid destruction in autoimmune patients compared with PD-1 inhibitor (pembrolizumab) induced thyroiditis patients. In pembrolizumab-induced thyroiditis patients there was no detectable surface expression of PD-1 on T cells. On the contrary, PD-1 expression on T cells from autoimmune patients was not different than healthy volunteer controls. As such, whereas the role of PD-1 dependent T cell activation may contribute to T cell mediated destruction of the thyroid in pembrolizumab treated patients, the role of PD-1 in the autoimmune setting seems less likely, consistent with known antibody-mediated mechanism for the latter [12].

The clinical manifestations of either hypothyroidism (bradycardia, fatigue, weight gain, constipation, dry skin, cold intolerance) or thyrotoxicosis (tachycardia, fatigue, weight loss, palpitations, new onset atrial fibrillation, diarrhea, heat intolerance, excessive diaphoresis) may be misinterpreted as symptoms of the underlying malignancy [5,7,13]. The diagnosis of thyroid dysfunction due to ICI is based on TSH and FT4 levels to differentiate primary from central thyroid disorders [5] In case of thyrotoxicosis additionally total T3 is necessary to be measured [5] and complementary investigations are useful, in the case of severe thyrotoxicosis or an unfavorable evolution: TRAb assay, Ultrasound-Doppler, and iodine/99mTc scintigraphy should then be performed to distinguish thyroid disruption from hyperfunction [5,14].

The handling of immune-related thyroid disorders depends on the level of TSH and the severity of symptoms. In the absence of prospective data, these patients should be managed as per established guidelines based upon pooled clinical experience [15]. Table-2 depicts current guidelines for management of immune-related thyroid disorders [16,17].

Table-2. Management of immunotherapy-related hyperthyroidism and hypothyroidism.

ir Adverse Event	ASCO Clinical Practice Guidelines		SITC Clinical Practice Guidelines
Hypothyroidism	Grade1: TSH, 10 mIU/L and asymptomatic	Should continue ICI with close follow-up and monitoring of TSH, FT4	► Thyroid function (TSH, fT4) should be tested every 4–6 weeks during ICI treatment and should continue to be tested every 6–12 months following the conclusion of ICI treatment. ► Patients with elevated TSH and normal fT4 should receive repeat TSH and fT4 testing routinely, and if this pattern persists without
	Grade2: Moderate symptoms; able to perform ADL; TSH persistently. 10 mIU/L	May hold ICI until symptoms resolve to baseline Consider endocrine consultation Prescribe thyroid hormone supplementation in symptomatic patients with any degree of TSH elevation or in asymptomatic patients with TSH levels that persist. 10 mIU/L (measured 4 weeks apart) Monitor TSH every 6-8 weeks while titrating hormone replacement to normal TSH FT4 can be used in the short term (2 weeks) to ensure adequacy of therapy in those with frank hypothyroidism where the FT4 was initially low Once adequately treated, should	

		monitor thyroid function (at least TSH) every 6 weeks while on active ICI therapy or as needed for symptoms to ensure appropriate replacement; repeat testing annually or as indicated by symptoms once stable	hypothyroidism symptoms, then levothyroxine treatment should be considered. Levothyroxine should be administered to patients with hypothyroidism at 1.5–1.6 μg/kg/day for young, healthy patients, and should be administered at 25 or 50 μg/day for patients >65 years of age or with heart disease. ► Patients with symptoms of hypothyroidism and/ or with elevated TSH and low fT4 should be tested for morning cortisol to identify possible concurrent adrenal insufficiency. ► Patients with low TSH and normal fT4 should receive repeat TSH and fT4 testing routinely, and if symptoms of hyperthyroidism or high fT4 develop patients should be treated with beta-blockers. Patients with asthma or chronic obstructive pulmonary disease should be treated with cardio selective beta-blockers such as atenolol or metoprolol. ► Patients with persistently low TSH and high fT4 should be evaluated for hyperthyroidism and Graves' disease etiology
	Grade3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICI until symptoms resolve to baseline with appropriate supplementation Endocrine consultation May admit for IV therapy if signs of myxedema (bradycardia, hypothermia) Thyroid supplementation and reassessment as in G2	
	Additional considerations For patients without risk factors, full replacement can be estimated with an ideal body weight–based dose of approximately 1.6 mg/kg/d For elderly or fragile patients with multiple comorbidities, consider titrating up from low dose, starting at 25-50 mg Extreme elevations of TSH can be seen in the recovery phase of thyroiditis and can be watched in asymptomatic patients to determine whether there is recovery to normal within 3-4 weeks Under guidance of endocrinology, consider tapering hormone replacement and retesting in patients with a history of thyroiditis (initial thyrotoxic phase) Adrenal dysfunction, if present, must always be replaced before thyroid hormone therapy is initiated		
Hyperthyroidism	Grade1: Asymptomatic or mild symptoms	Can continue ICI with close follow-up and monitoring of TSH, FT4 every 2-3 weeks until it is clear whether there will be persistent hyperthyroidism or hypothyroidism	
	Grade2: Moderate symptoms, able to perform ADL	Consider holding ICI until symptoms return to baseline Consider endocrine consultation b-Blocker (e.g., atenolol, propranolol) for symptomatic relief Hydration and supportive care Corticosteroids are not usually required to shorten duration for persistent hyperthyroidism (. 6 weeks) or clinical suspicion, work-up for Graves' disease (TSI or TRAb) and consider thionamide (methimazole or PTU) Refer to endocrinology for Graves' disease	
	Grade3-4: Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Hold ICI until symptoms resolve to baseline with appropriate therapy Endocrine consultation b-Blocker (e.g., atenolol, propranolol) for symptomatic relief for severe symptoms or concern for thyroid storm, hospitalize patient and initiate prednisone 1-2 mg/kg/d or equivalent tapered over 1-2 weeks; consider also use of SSKI or thionamide (methimazole or PTU)	

Conclusion. As check-point blockade is becoming a standard of care and several combination therapy strategies enter clinical practice [9], increased awareness is imperative at any time during treatment with immune check-point inhibitors and long after treatment cessation. Severe symptoms indicative of thyroid dysfunction requires prompt intervention, while in case of non-severe and non-specific symptoms close monitoring is advocated [11]. The precise mechanism is not well understood why some patients are more prone than others to develop endocrinopathies by ICIs [5,18]. Therefore, further research and investigation are needed to identify the patients who are at risk for immune-related thyroid toxicity. A further step that should be followed is the identification of biomarkers which can indicate when is best to use the checkpoint inhibitors [19].

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THYROID DYSFUNCTIONS INDUCED BY IMMUNE CHECK-POINT INHIBITORS

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SUMMARY

The immune system is the core defense against cancer development and progression. Failure of the immune system to recognize and eliminate malignant cells plays an immense role in the pathogenesis of cancer. The paramount achievement in immunotherapy particularly – Immune Check-point Inhibitors (ICI) over the recent decade has brought about major paradigm shift in cancer treatment. ICIs, represented mainly by inhibitory monoclonal antibodies – anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) (ipilimumab), anti-programmed cell death protein 1 (PD-1-pembrolizumab/nivolumab/cemiplimab), Anti-PD-1 Ligand molecules (PD-L-1- atezolizumab/avelumab/durvalumab) reactivate the immune system against tumor cells but can also trigger a myriad of autoimmune side effects, termed immune-related adverse events (irAEs). Immunotherapy related adverse events typically have a delayed onset and prolonged duration compared to adverse events from chemotherapy, and its effective management depends on early recognition and prompt intervention with immune suppression and/or immunomodulatory strategies.

The present review aims to raise awareness about thyroid side effects of immune check-point inhibitors to physicians who are taking care of cancer patients and to specialists - mainly oncologists and endocrinologists who are urged to cooperate for the management of thyroid immunotoxicity.

Keywords: immune check-point inhibitors, thyroid side effects, anti-CTLA-4, anti-PD-1, anti-PD-L-1 monoclonal antibodies.

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CD4+CD39 T CELLS IN THE PERIPHERAL BLOOD AND SPLEEN OF PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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CD4+CD39+ T უჯრედები პერიფერიულ სისხლსა და ელენთაში იმუნური თრომბოციტოპენიის მქონე პაციენტებში

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რეზიუმე

პირველადი იმუნური თრომბოციტოპენია (ითპ) ხასიათდება თრომბოციტების რაოდენობის შემცირებით და სისხლდენის მაღალი რისკით. ითპ-ის პათოგენეზი ჯერ კიდევ შესწავლის საგანია, თუმცა ლიმფოციტების მიერ აუტოანტისხეულების წარმოქმნას T-ჰელპერული უჯრედები მართავენ. ითპ პათოგენეზში აქტიურად შეისწავლება მარეგულირებელი T-უჯრედების როლიც. უკანასკნელი კვლევებით, ითპ პაციენტების პერიფერიულ სისხლში ნანახია (Treg) უჯრედების კლება, თუმცა ასევე აღსანიშნავია პუბლიკაციები სადაც (Treg) უჯრედები არ განსხვავდება საკონტროლო ჯგუფისგან. (Treg) უჯრედებზე ექსპრესირებული CD39 მნიშვნელოვნად განსაზღვრავს (Treg) უჯრედების იმუნოსუპრესორულ პოტენციალს. აღნიშნულ კვლევაში ჩვენს მიზანს წარმოადგენდა შეგვესწავლა CD4+CD39+ T-ლიმფოციტები იმ ითპ პაციენტთა სისხლსა და ელენთაში, რომლთაც პირველი რიგის მკურნალობაზე პასუხი ვერ მიიღეს და ჩაუტარდათ სპლენექტომია, როგორც მეორე რიგის თერაპია. სპლენექტომირებული პაციენტები რომელთაც არ ჰქონდათ ითპ წარმოდგენილი იყვნენ საკონტროლო ჯგუფში. ჩვენი შედეგების მიხედვით CD4+CD39+ T-უჯრედების პროცენტული მაჩვენებელი სარწმუნოდ დაბალია ითპ პაციენტების ელენთაში საკონტროლო ჯგუფთან შედარებით. აღსანიშნავია რომ ეს სხვაობა არ აღინიშნება სისხლში. ასევე აღსანიშნავია, რომ CD4+CD39+ T-უჯრედების სიხშირე უფრო სტაბილურია ითპ პაციენტების ელენთაში, ვიდრე სისხლში. ჩვენი კვლევა მიუთითებს CD39+-ის, როგორც ითპ-ის პოტენციური ბიომარკერის მნიშვნელობაზე და ადასტურებს ელენთის ქსოვილში იმუნური მარკერების კლინიკურ და სამეცნიერო ღირებულებას აღნიშნულ პათოლოგიაში.

Introduction. Immune thrombocytopenia (ITP, also called idiopathic thrombocytopenic purpura) is an acquired thrombocytopenia characterized by a reduced platelet lifespan (<100 G/L) due to antibody mediated destruction [1, 2]. The review of published reports determined an annual ITP incidence of approximately 2,6-6,6 per 100,000 in adults [3-5]. The pathogenesis of ITP is incompletely understood. The principal mechanism is thought to involve specific autoantibodies produced by the patients B cells (IgG) most often directed against platelet membrane glycoproteins such as GPIIb/IIIa. The involvement of helper T cells in ITP pathogenesis is crucial [6,7]. The T follicular helper cells (TFH) support B cell differentiation and antiplatelet production [8,9]. However, other mechanisms are important, including cytotoxic T cells, [10-12] as well as humoral and cellular autoimmunity directed at megakaryocytes, causing impaired platelet production [2,13-16].

Recent evidence proves the significance of Regulatory T cells (T cell subset with a CD4+CD25-high+Foxp3 phenotype) in ITP patients. The low frequency of circulating Tregs has been observed in most of studies, [16-18] however similar levels with controls have also been reported [19]. Audia et al demonstrated that the percentage of circulating regulatory T cells (Tregs) was similar to that in controls, however splenic Tregs were reduced in ITP patients. Interestingly, the ratio of proinflammatory Th1 cells to suppressive Tregs was increased in the spleens of patients who failed RTX therapy [20].

The ectoenzyme CD39 within CD4+CD25+ cells highly suggest the immunosuppressive function of Tregs and might be considered as a biomarker in autoimmune disease [21]. In the recent study where patients with newly diagnosed primary Immune thrombocytopenia (ITP) were enrolled, the expression of CD39 in CD4+CD25+ Treg cells was initially decreased compared to normal controls. After high dose of dexamethasone therapy, the response group showed elevated CD39 expression within Treg cells, while non-responder group did not show any difference in compression to that before treatment [22]. In these studies, the readouts of CD39 in ITP patients are associated with the first line treatment.

Splenectomy is a second line therapy of ITP [23]. This therapeutic approach presents a very rare clinical opportunity to study splenic cells in patients as spleen is the primary site of the autoimmune response during ITP.

We aimed to study frequency of CD4+CD39+ T lymphocytes in the spleen and blood of patients with ITP and in control group that might further reveal new targets for therapeutic intervention.

Materials and Method. Patients. This study was carried out in accordance with the principles of the 1975 Declaration of Helsinki and its later amendments or comparable ethical standards, and was approved by Tbilisi State Medical University Biomedical Research Ethics Committee. Formal consent was not required for this retrospective study, while all data were kept confidential.

The main inclusion criterion was thrombocytopenia (platelet count<100×109/L). Familial, viral, or drug-induced etiologies were excluded. Patients suffering from other autoimmune diseases (eg, systemic lupus erythematosus and antiphospholipid syndrome) were also excluded. Most of the patients were treated with steroids and, if necessary, with IVIg as first-line therapies. All of these patients were refractory to first line treatment and had splenectomy as a second line therapy.

Blood and spleen samples were obtained from ITP and nonITP patients as controls. None of control group individuals had other hematologic disease, cancer, acute or chronic infections, liver or kidney disease. Blood was collected (from ITP patients and Controls) 1 hour before splenectomy.

Spleen preparation. The spleen samples were obtained from patients during scheduled surgery within the hour of splenectomy. Sterile spleen tissues were mechanically disrupted using a syringe plunger. After the cells were dissociated, they were filtered through a 100-μm nylon strainer, BD Bioscience. Cell suspension was incubated for 10 minutes in a hemolytic solution (150mM ammonium chloride, 10mM potassium bicarbonate, and 0.1mM EDTA) at room temperature to remove RBCs. Cells were then washed in medium (RPMI with 10% FBS) and filtered again. Samples were then divided for Flow cytometry (FCM) analysis; remaining cells were prepared for cryopreservation.

Blood samples. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll gradient centrifugation and prepared for analysis as described in spleen preparation.

Flow cytometry (FCM). CD4-APC/CD39-PE staining was performed following the manufacturer's instructions (eBioscience). Data were acquired on FacsCalibur flow cytometer and analyzed using FlowJo® v10 software.

Statistical analysis. Statistical analysis was performed using GraphPad Prism. Two-tailed unpaired t-test was used to compare patients and controls.

Results and discussion. ITP patients and Control individuals included in our study were characterized according to gender and age. No differences were found between the groups (Table 1).

Table 1 Characteristics of splenectomized ITP patients

Patient	Sex	Age	Previous treatments
1	M	20	Steroids
2	F	24	Steroids;Thrombopoietin agonist (TPO-RA).
3	F	47	Steroids
4	M	60	Steroids
5	F	67	Steroids
6	M	76	Steroids
7	M	25	Steroids
8	F	66	Steroids
9	F	74	Steroids

Recent immunotherapeutic approaches aim to trigger the functional state of immunosuppressive cells in autoimmune pathologies. Controversial findings are available regarding immunosuppressive cells frequencies are functions in ITP patients.

In our study the frequencies of CD4+CD39+ T cells were comparable in ITP patient's blood and spleen (Figure 1). There was no difference in the blood levels of these cells between controls and ITP patients (Figure 2). The expression of ectoenzyme CD39 was diminished in CD4+ T cells in spleen of the patients with immune thrombocytopenia compared to controls (15.80%±2.205% to 37.15% ± 6.482%, P=0.0325) (Figure 3).

Figure 1. Spleen vs. blood levels of CD39 in CD4+ T lymphocytes in ITP

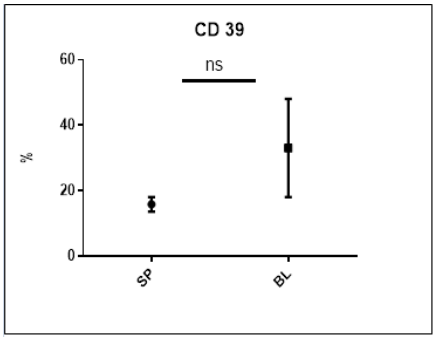


Figure 2. Blood levels of CD39 in CD4+ T lymphocytes in ITP vs. Control

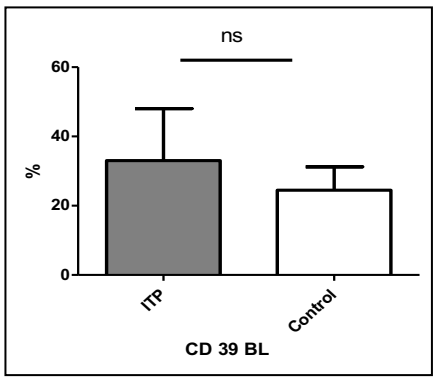
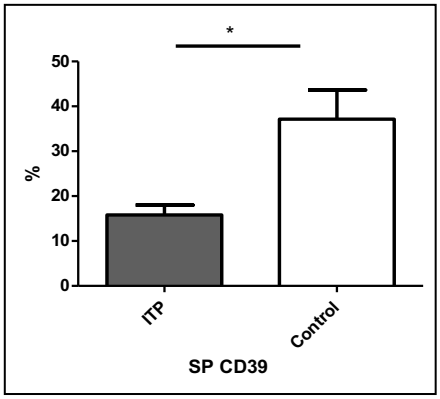


Figure 3. Splenic levels of CD39 in CD4+ T lymphocytes in ITP vs. Control



Pathogenesis of ITP still remains complex and its biomarkers uncovered. These complicate disease management and reflect in the frequent unresponsiveness of patients to the first line treatment. [24-26]. Splenectomy as a second line therapy of ITP removes the major site of phagocytosis of antibody-coated platelets, as well as lymphocytes that resides in the spleen that might be responsible for producing antiplatelet antibodies [23,27,28]. This therapeutic approach presents a very rare clinical opportunity to study splenic cells in patients as spleen is the primary site of the autoimmune response during ITP [20].

Increasing evidence suggest the importance of measuring CD39 expression in T lymphocytes to speculate on their suppressive functional state. CD39 and CD73 are coexpressed on the surface of murine and human Treg cells and generate extracellular adenosine, contributing to Treg immunosuppressive activity [21,29]. A high level of CD39+ within CD4+CD25+Treg cells highly suggests their immunosuppressive function [21]. Lu et al have demonstrated decreased expression of CD39 in circulating

CD4+ CD25+ Treg cells of ITP patients compared to normal controls. CD39 expression was elevated within Treg cells after high dose of dexamethasone therapy in the response group, while non-responder group did not show any difference in compression to that before treatment [22]. Another study has demonstrated that methotrexate (MTX) unresponsiveness in rheumatoid arthritis (RA) is associated with low expression of CD39 in peripheral blood CD4+CD25+FoxP3+ Tregs and the decreases suppressive activity of these cells [30,31].

Precedent findings suggest that the expression of CD39 on Tregs can predict the response on treatment, however they all report the results based on blood Tregs in humans. In our study, we show for the first time in ITP patients that CD39 expression is diminished in splenic CD4+ T lymphocytes. Therefore, our findings have important scientific and clinical value for understanding ITP pathogenesis and open new avenues for further investigations in this field.

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CD4+CD39 T CELLS IN THE PERIPHERAL BLOOD AND SPLEEN OF PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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SUMMARY

Primary immune thrombocytopenia (ITP) is characterized with decreased platelet count and increased risk of bleeding. The mechanism of thrombocytopenia in ITP is incompletely understood but thought to involve autoantibodies which are produced by the B cells and are stimulated by helper T cells. Regulatory T cells (Treg) have been seen to be significant in ITP pathogenesis. Recent studies have reported reduction of circulating Treg cells in ITP patients but similar levels with controls have also been observed. The ectoenzyme CD39 is highly expressed on the surface of Treg cells and can suggest its immunosuppressive function.

In this study we aimed to analyze CD4+CD39+ T lymphocytes both in the blood and spleen of patients with ITP who did not respond to the first line treatment and underwent splenectomy as a second line therapy. Non-ITP patients undergoing splenectomy were involved in the control group. Our data demonstrates significant diminution of in splenic but not circulating CD4+CD39+ T cells in ITP patients compared to controls. Of note, the comparison of spleen and peripheral CD4+CD39+T lymphocytes indicates that the frequency of CD39+ Treg cells is more stable in spleen compared to blood in ITP patients. Our data suggests the potential of CD39 as an important biomarker for ITP and underlines the clinical and scientific value of spleen immune analyzes in this pathology.

Keywords: Primary immune thrombocytopenia, CD4+CD39+ T cells, blood, spleen

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FIRST CASE REPORT OF PAPILLON-LEFÈVRE SYNDROME FROM GEORGIA

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პაპილონ-ლეფევრის სინდრომის პირველი შემთხვევა საქართველოდან

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის მოლეკულური და სამედიცინო გენეტიკის დეპარტამენტი, თბილისი, საქართველო

რეზიუმე

პაპილონ-ლეფევრეს სინდრომი (PLS) ძალიან იშვიათი აუტოსომურ-რეცესიული დაავადებაა, რომელიც პალმოპლანტარული ჰიპერკერატოზითა და მძიმე, ადრეულ ასაკში გამოვლინებული პერიოდონტიტით ვლინდება, რაც ორივე, სარძევე და მუდმივი კბილების ნაადრევ დაკარგვას იწვევს. PLS-ის ეტიოლოგის კომპლექსურია და მოიცავს გენეტიკურ, იმუნოლოგიურ და მიკრობიოლოგიურ ფაქტორებს. მუტაციები 11q14-q21 გენში, რომელიც სხვადასხვა ანთებით და იმუნოლოგიურ პროცეს მონაწილე გენს - კათეფსინ C-ს მოიცავს, წარმოადგენს წამყვან გენეტიკურ დეფექტს, რომელიც PLS-ს იწვევს. PLS-ის მკურნალობა კომპლექსურია და მოითხოვს მულტიდისციპლინურ მიდგომას. წარმოდგენილ სტატიაში ჩვენ აღვწერთ PLS-ის პირველ შემთხვევას საქართველოდან, აქამდე აღუწერელ ვარიანტს CTSC გენში და ყურადღებას გავამახვილებთ მთლიანი ეგზომის სექვენსირების (WES) მნიშვნელობაზე საბოლოო დიაგნოზის დასმაში.

Introduction

Papillon-Lefèvre syndrome (PLS) is an exceedingly rare genetic condition with around 250 reported cases in the literature [1]. It's characterized by palmoplantar hyperkeratosis and severe prepubertal periodontitis, which results in premature loss of both deciduous and permanent teeth. PLS is inherited in an autosomal recessive condition and results from mutations of the *CTSC* gene that regulates production of lysosomal protease cathepsin C. The exact etiology of disease is still not fully elucidated, and it seems that environmental, genetic, and immunologic factors influence its onset [2]. Cathepsin C is expressed at high levels in immunogenic responses that activate serine proteases, as well as in epithelial tissue [3]. This could explain the main clinical features of PLS that include an increased risk of infectious diseases, progressive periodontitis, gingivostomatitis and palmoplantar keratoderma. Here, we report the first case of PLS described in Georgia.

Case Report

We describe a case of 10-year-old otherwise healthy girl who presented to the Givi Zhvania Pediatric Academic Clinic at Tbilisi State Medical University, Georgia with a chief complaint of loosening of permanent teeth, loss of mandibular central incisor and diffuse mild transgradient palmoplantar keratosis and erythema. The condition started at the age of 7 with a periodontal disease. She was born with a hemangioma on the upper lateral arm which was surgically treated. She was a second child in non-consanguineous family. The parents and other family members were not affected. Pregnancy and delivery were normal. Her birth height was 51 cm, and his weight was 3000 gr. Treatment included various antiseptic oral rinses and topical steroid medications for palmoplantar keratosis previously diagnosed as psoriasis with a little improvement. Intraoral examination showed loosening of permanent teeth and loss of mandibular central incisor (Fig. 1). Dermatologic examination revealed diffuse erythema and mild hyperkeratosis of palms and soles (Fig. 2). Dental panoramic radiograph displayed several floating teeth with generalized horizontal and vertical bone loss (Fig. 3). Physical and cognitive development was normal. Reminder of the physical examination was unremarkable. Routine hematological and biochemical tests were normal. Whole exome sequencing (WES) was performed, which revealed two variants in *CTSC* gene: a previously described c.415G>A p. (Gly139Arg) and a novel c.1220T>A p. (Val407Asp) variants. The patient was prescribed topical tretinoin 0.1% cream once a day and emollients which resulted in complete clearing of palmoplantar hyperkeratosis within one month, treatment with retinoid

discontinued and the effect is long-lasting (6 months posttreatment). The enforcement of oral hygiene habits was advocated, and she was referred to dental care team for the management of periodontal disease.

Figure 1 A and B: Intraoral photograph showing missing in lower arch.

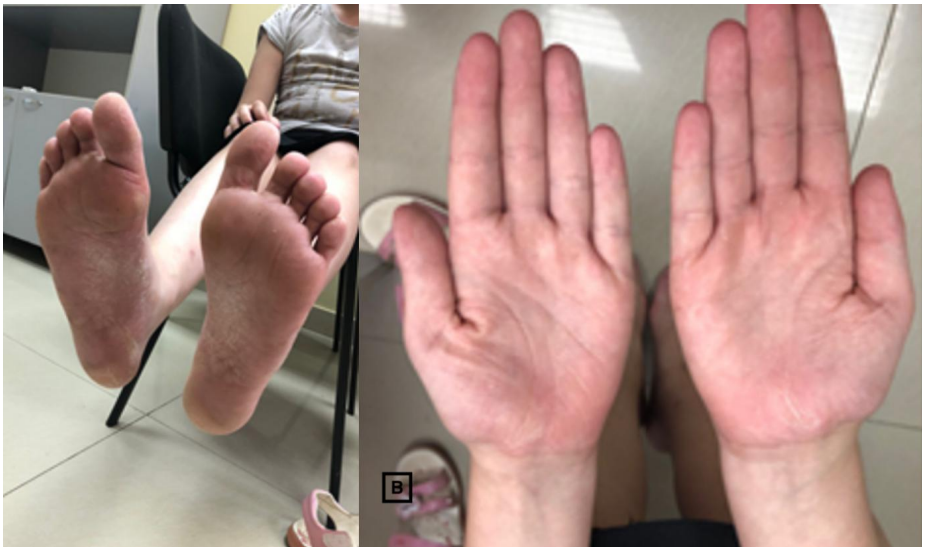

Figure 2 A and B: Diffuse erythema and mild hyperkeratosis of palms and soles.

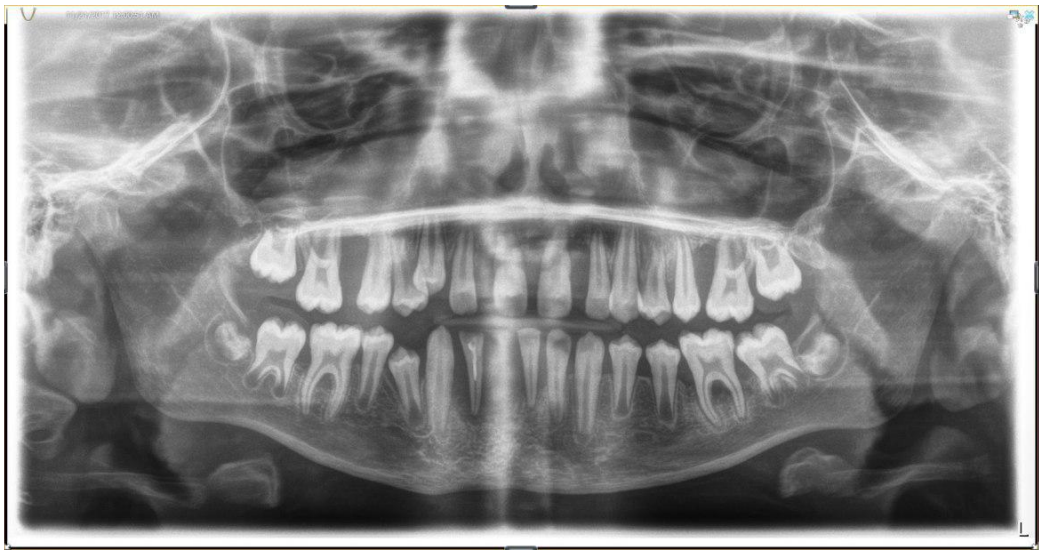

Figure 3: Panoramic radiograph of the dental arch.

Discussion

Papillon-Lefèvre syndrome (PLS), also known as hyperkeratosis palmoplantaris with periodontosis or keratoris palmoplantaris with periodontopathia, is a rare ectodermal dysplasia characterized by palmoplantar keratoderma with early-onset periodontitis. It was first described by French physicians

M. M. Papillon and Paul Lefèvre in 1924 [4]. The prevalence is estimated between 1/250,000 and 1/1,000,000 individuals with no sex or racial differences. The onset of the symptoms starts early during childhood and typically becomes apparent from approximately one to five years of age. PLS is an autosomal recessive genetic disorder that is caused by a mutation in the *CTSC* gene encoding lysosomal exo-cysteine protease cathepsin C [5]. Cathepsin C appears to be a critical coordinator for activation of several serine proteases in immune and inflammatory cells. It also regulates the formation of the enveloped corneocytes [6]. Clinically PLS is characterized by symmetric palmoplantar hyperkeratosis and severe, early-onset periodontitis, leading to the premature loss of teeth. Cases with mild and/or late-onset periodontal disease have been reported occasionally. Other manifestations include increased susceptibility to skin and systemic infections, hyperhidrosis, follicular hyperkeratosis, nail dystrophy, mild mental retardation, or dural calcifications [7]. PLS affects psychological, social, and esthetic well-being of the patient and requires multidisciplinary approach to improve the quality of life. Dental team involvement is needed for the treatment of periodontal disease and prevention of its complications. A dermatologist can manage the skin manifestations. Emollients, salicylic acid, urea, and topical steroids are used. Retinoids can also be used, and it showed excellent results in our patient.

The presented case is significant in various ways. First, there was a diagnostic delay and multiple physician visits before the final diagnosis of PLS was made highlighting the importance to include the PLS in differential diagnosis in every patient with palmoplantar hyperkeratosis and periodontitis. Diseases with oral features as seen in PLS periodontitis are acrodynia, hypophosphatasia, severe congenital neutropenia, histiocytosis X, cyclic neutropenia, and Takahara syndrome. Dermatologic conditions with similar features of PLS but not associated with periodontopathy are Unna Thost syndrome, Mal de Meleda, Howel-Evans syndrome, Vörner's syndrome, Vohwinkel's syndrome, and Greither's syndrome [8]. Second, WES offers clear diagnostic benefits for patients with rare disease and impact management, and genetic counseling options. Inability to identify potential therapies, failure to detect the risk of recurrence in later pregnancies, and failure to provide anticipatory counseling and prognosis are all consequences of a lack of diagnosis for patients and their families. WES approaches have greatly facilitated the discovery of candidate genes or gene variants and it is increasingly applied to genetic testing for undiagnosed patients [9]. Third, our patient has a novel previously undescribed c.1220T>A p. (Val407Asp) variant in *CTSC* gene, expanding the mutation spectrum of this rare condition. Finally, the presented case shows that retinoids can offer benefits for patients with PLS.

Conclusion

Hyperkeratosis of the palms and soles and generalized severe periodontitis characterize Papillon-Lefèvre syndrome. A mutation in the gene that codes for CTSC causes this autosomal recessive genetic disease. Furthermore, unless genetic testing is performed, the diagnosis of PLS may delayed for years. To improve the quality of patients' lives and improve results, early detection and adequate management by a multidisciplinary team is critical.

Conflict of interest statement - The authors declare that they have no conflict of interest.

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FIRST CASE REPORT OF PAPILLON-LEFÈVRE SYNDROME FROM GEORGIA

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SUMMARY

Papillon-Lefévre syndrome (PLS) is a highly rare autosomal recessive condition characterized by palmoplantar hyperkeratosis and severe early-onset widespread periodontitis, resulting in the premature loss of both primary and permanent teeth. PLS has a complex etiology, with genetic, immunological, and microbiological factors being the main causes. Mutations in the gene 11q14-q21, which codes for cathepsin C, an enzyme implicated in a range of inflammatory and immunological processes, are the leading genetic abnormalities that cause PLS. Treatment of PLS is challenging and requires a multidisciplinary approach. Here, we report the first case of PLS described in Georgia, describe a novel previously undescribed variant in the *CTSC* gene, and highlight the importance of whole exome sequencing (WES) in making a definitive diagnosis.

Keywords: Papillon-Lefévre, PLS, CTSC, WES, Georgia

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DESTRUCTION OF TUMOR MICROENVIRONMENT AS A PROMISING TREATMENT APPROACH IN PANCREATIC CANCER

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სიმსივნის მიკროგარემოს დესტრუქცია - პანკრეასის კიბოს მკურნალობის პერსპექტიული სტრატეგია

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რეზიუმე

პანკრეასის კიბოს 90%-ზე მეტს პანკრეასის სადინრის ადენოკარცინომა წარმოადგენს. ყოველწლიურად მისი დაახლოებით 500,000 ახალი შემთხვევა და თითქმის ამავე რაოდენობის პაციენტის გარდაცვალება აღირიცხება როგორც ქალებში, ასევე მამაკაცებში.

დაავადების ცუდი პროგნოზი სიმსივნის მიკროგარემოს იმუნოსუპრესიული შემადგენლობით შეიძლება აიხსნას. ეს უკანასკნელი სიმსივნის საწინააღმდეგო იმუნური პასუხის ინჰიბირებას უზრუნველყოფს, რაც იწვევს კიბოს წარმოქმნის ინიცირებას, მის შეუმჩნეველ, სწრაფ პროგრესირებას და გავრცელებას. სიმსივნური მიკროგარემოს მნიშვნელოვან კომპონენტს მკვრივი დესმოპლასტიკური სტრომა წარმოადგენს. ის იმ ფიზიკურ ბარიერად გვევლინება, რაც პანკრეასის კიბოს მკურნალობის მიმართ რეზისტენტულს ხდის. აბლაციური და სხივური თერაპიის გამოყენებით აღნიშნული მკვრივი და იმუნოსუპრესიული სტრომის დესტრუქციამ, როგორც სიმსივნის საწინააღმდეგო იმუნური პასუხის შესაძლო ჩამრთველმა და გამაძლიერებელმა საშუალებამ, შესაძლოა, დამაიმედებელი შედეგები მოგვცეს. აღნიშნულ სტატიაში ჩვენ მომივიხილავთ სიმსივნის სტრომაზე დამიზნებით აბლაციურ და რადიო თერაპიას, როგორც სიმსივნის საწინააღმდეგო იმუნიტეტის პოტენციურ გამააქტიურებლებს.

Introduction. Pancreatic cancer death rates continue to increase, with a very low survival for all stages combined [1]. Its incidence varies greatly across regions, which suggests that lifestyle factors play an important role in its etiology. Pancreatic cancer risk is associated with more than 50 specific risk factors with two-thirds of them potentially modifiable, such as smoking, alcohol intake, sedentary lifestyle, etc. affording a unique opportunity for preventing one of the deadliest cancers [2]. Unfortunately, pancreatic cancer was anticipated to move from the fourth to the second leading cause of cancer death in the United States by 2020. Management of pancreatic cancer depends on tumor staging. While detected early, cancer can be treated with surgery, neoadjuvant therapy, chemotherapy, radiotherapy, immunotherapy, and combination treatments [3-7]. Unfortunately, a vast amount of currently available therapeutic options almost always fails to cure PDAC patients. The insidious and quick progression of PDAC is attributed to its characteristic "Immunologically cold" environment, making pancreatic cancer very difficult to diagnose until the disease has reached an advanced stage. PDAC is infiltrated with pro-tumorigenic cells causing impaired antitumor immune response resulting in rapid disease progression and poor survival even when treated with all currently available methods. Due to no effective antitumor response tumor has an insidious course and rapidly disseminates, resulting in metastatic pancreatic ductal adenocarcinoma (mPDAC) found in most patients at the time of diagnosis. The prognosis is poorest for mPDAC regardless of innovative treatment approaches and combination therapies with a 5-year survival rate below 5%. A poor performance status, which is very common in patients with metastatic disease, leaves these patients with no effective treatment [5,8]. Currently available chemotherapies have a difficult regimen that is best reserved for fit patients who are rare exceptions at this stage of disease [9]. Dysregulation during PDAC carcinogenesis, broad heterogeneity of genetic mutations and dense stromal environment precisely fibroblasts in desmoplastic stroma compressing the blood vessels, reducing blood perfusion into the tumor makes PDAC one of the most chemoresistant cancers [10-14]. Considering the rapid upregulation of antitumor pathways, even the therapies targeting cancer-associated molecular pathways have not given satisfactory results [8]. Immunotherapy has only limited efficacy against PDAC because of an immunosuppressive tumor-associated stroma [14,15]. Disruption of the protumorigenic immunosuppressive stroma has been described as a promising approach to treat cancer patients. Disruption of the dense and immunosuppressive stroma with ablative therapies can give us promising results in pancreatic cancer treatment. Despite the ongoing active medical effort, unfortunately, nothing effective can be suggested for mPDAC patients with poorly controlled comorbid conditions [6]. However, if left untreated, median survival in patients with metastatic disease is only 3 months [8,16]. Palliative treatment may be the only option in many cases. Furthermore, most suggested palliative treatments for metastatic disease are not effective even with the aim of prolonging survival and relieving disease-related symptoms. Thus, the development and implementation of novel PDAC treatment approaches is a crucial emergency for patients [17,18]. The scope of this review is to summarize the current concepts and newest advances in research of the tumor microenvironment destruction with RT and ATs and following immunomodulatory changes.

Immunology of PDAC. Approximately 50% of the cell mass of pancreatic cancer is made up of immune cells, most of which are immunosuppressive. Therefore, PDAC is also considered an "immunologically cold" tumor [19]. Immunosuppressive microenvironment includes tumor-associated

macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs). These cells are responsible for the downregulation of immune response against pancreatic cancer. Macrophages are polarized to the M2 subtype that secretes IL-10 and carbon monoxide and, therefore, suppresses cytotoxic T cells [20]. MDSCs are immature monocytes and granulocytes. Cancer-derived molecular signals block the maturation of these cells into their mature counterparts. MDSCs then suppress both innate and adaptive immunity and are associated with a poorer prognosis. In most cases, pancreatic cancer is surrounded by dense connective tissue called desmoplasia [14]. Additionally, desmoplastic stroma contains pancreatic stellate cells (PSCs), which keep $CD8^+$-T cells away from the tumor microenvironment [21]. High infiltration of Tregs and Th17 cells causes the consequent impairment of effector, CD4+ and CD8+ cell-mediated antitumor responses [22,23]. Due to no effector response, metastases are more frequently observed in patients with decreased immune effector cells [24]. The presence of CD4+ and CD8+ T cells in the tumor has been showed to correlate with better prognosis because anti-tumor immunity cannot be activated without them [25].

Some of the immune escape mechanisms in PDAC include elevated levels of immune-suppressive tumor necrosis factor-alpha, TGF-β1, IL-10, and IL-1β [26].

Taking these premises into account, destruction of stroma to disrupt Treg-mediated immunosuppression and to block the inhibitory pathways on effector T cells seems to be potentially effective in PDAC treatment.

Immunological aspect of RT and Ats. Use of RT and ATs to expose these antigens and induce the immune response against the tumor has been studied over a period of time. It has been known for a long time that tumor cells release a large number of antigens, referred to as tumor-associated antigens in the form of necrotic and apoptotic tumor cells and debris [27-29].

The major goal of immune stimulation is to create immune memory and systemic response against these tumor-associated antigens, also known as an abscopal effect [30].

RT. The effect of radiation on immune response remains controversial with a number of studies suggesting immunosuppression, significant modulation of, or no effect at all.

Generally, immunomodulatory changes following RT include enhanced antigen presentation and tumor immunogenicity, increased production of cytokines and altered tumor microenvironment, enabling the destruction of the tumor by the immune system [31].

Later studies suggest that lower doses of radiation have a greater potential to enhance immune responses [32]. This reflects the capability of moderate-dose radiation to enhance tumor antigen presentation, resulting in a greater diversity of antigen recognition by the antitumor T-cell response [33].

The substantial increase in the number and diversity of tumor-associated antigens can enable antigen-presenting cells and dendritic cells to stimulate a tumor-specific immune response. In addition to tumor cells acting as the trigger, the destruction of the tumor-supporting stroma that often results from radiotherapy can also potentiate immune recognition [34].

RT is also able to induce a local antitumor immune response, potentially leading to systemic antitumor immunity having an “abscopal effect” in many tumors [35,36].

RT triggers immunogenic cell death by DNA damage and releasing damage-associated molecular patterns (DAMPs) from tumor cells. This turns the tumor cells into an “in situ vaccine” [37]. These effects promote modulation of the peptide repertoire, antigen-processing machinery components. Enhancement of MHC I expression and DC antigen presentation induces differentiation of naïve T-cells towards an effector phenotype [38,39]. Release of ’danger’ signals was also seen following radiotherapy, which stimulates the transition from nonspecific immune responses to adaptive immunity [40,41]. Radiation induces molecular alterations in the biology of the cancer cell that make the tumor more susceptible to cytotoxic-T-lymphocyte-mediated destruction [42].

These radiation-induced changes have not been well researched in pancreatic cancer.

The optimal role of radiation in immunomodulation of pancreatic cancer is unknown. Only several studies have suggested its effectiveness [43-46]. Lee et al. found that ablative RT is more effective than fractionated RT at recruiting T cells in a murine orthotopic pancreatic tumor model. Fractionated RT induced more myeloid-derived suppressor cell infiltration than ablative RT [47].

RFA. RFA has been successfully used to treat many tumors. As RFA is a relatively newer treatment option the definitive role of RFA for pancreatic cancer remains under investigation. Although its use in the management of unresectable pancreatic cancer is increasing [48].

RFA is a safe and effective procedure and may improve survival in patients with advanced stage pancreatic cancer. The co-author of this article (Mizandari et al.) performed percutaneous RFA in 134 patients with malignant obstructions of bile and pancreatic ducts (32 patients with pancreatic adenocarcinoma) and reported a 97% success rate of the procedure with only two patients experienced procedural technique related adverse events (contrast extravasation) following RFA [49].

RFA does not remove the tumor from the body. Instead, RFA causes tumor destruction and cytoreduction through multiple mechanisms such as coagulative necrosis, protein denaturation and generates an intense immune response. While local immune response removes the necrotic debris, and activates anticancer immunity [50-52].

Although the precise mechanism of RFA-induced immune response is not yet well established, dendritic cells (DCs) are thought to play a major role as the cells of innate immunity. RFA induces hyperthermia causing protein denaturation, vessel disruption, and cellular necrosis. Exposing antigens and activation DCs is one mechanism RFA can induce antitumor immunity [53]. Hyperthermia upregulates heat shock protein 70 (HSP-70) which then activates DCs [54]. Dendritic cells are the most potent antigen-presenting cells that recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [14].

Researchers reported the production of DAMP ingested by DC as an effect of cancer cells necrosis via RF in mice. Subsequently, matured DCs stimulate effector CD4+ T cells [55].

Brok *et. al* showed that RFA-induced DC infiltration is a more potent immune stimulator than ex-vivo generated antigen-loaded DC-based vaccines. The data revealed that upon tumor destruction by RFA, up to 7% of the total draining lymph node DC contained antigen, whereas only a few DCs from the conventional vaccine were able to reach the lymph nodes [53]. Dromi *et. al* investigated the RFA-induced immune response while treating murine urothelial tumors in mice. Their experiment revealed that RFA of the urothelial tumor produced systemic CD4+ and CD8+ T-cell responses, which were measurable in the spleens of female mice. Additionally, it was found that there are higher numbers of DCs in RFA treated tumors compared to untreated tumors. It was also shown that the nonablated tumor portion regressed in several mice due to the systemic T-cell antitumor immune response. Furthermore, primary tumor eradication was accompanied by rejection towards a second tumor implant without additional RFA. This was explained by immune memory generated by initial RFA [55].

Another research showed promising results regarding RFA-induced immune response in the management of lung cancer. Intense infiltrations of CD4+ and CD8+ T lymphocytes were found on the periphery of the RFA-treated tumor tissue, whereas the central area remained almost free of lymphocytes in 8 days after RFA treatment. In the peripheral blood, the concentration of proinflammatory and immunostimulatory DCs was increased after RFA. Additionally, a significant rise in T-cell proliferation was detected in T-cell assays after RFA. In other words, they found that RFA-induced necrotic debris may serve as an antigen source to induce an antitumor immune response [56].

Qinglin et al. established a PDAC mouse model with tumor bilateral implants. Upregulation of PD-1 (*Pdcd1*) in CD4+ and CD8+ T cells, as well as upregulated LAG3 in T cells after RFA treatment, indicated the prevalence of T-cell exhaustion at distant tumors. It was found that RFA treatment reduced the proportions of immunosuppressive cells, including regulatory T cells, tumor-associated macrophages, and tumor-associated neutrophils, whereas increased the percentages of functional T cells in distant non-RFA tumors [51].

Faraoni et al. reported Endoscopic ultrasound guided radiofrequency ablation (EUS-RFA) immunomodulatory effect on preclinical mouse models. Reduction in tumor growth rate was seen 4 days after RFA treatment in RFA and non-RFA side contralateral tumors in mice. This reduction in size was accompanied by significant upregulation of cleaved Caspase3 expression in RFA-treated tumors and significant remodeling of the stroma. Granzyme B was significantly increased in RFA treated tumors, compared to controls. These results led to hypothesize that RFA initially promoted a strong local and

systemic anti-tumor response which might have generated sustained Th1 responses and reduced secondary systemic immunosuppression [50].

Giardino et al. compared the concentration of CD4+ and CD8+ T cells before and after RFA in 10 PDAC patients. Study revealed an increase of above-mentioned T cells from the third day after treatment suggesting the activation of the adaptive response. Immunosuppressive Treg cells were not increased after the procedure despite laparotomy and heating. Myeloid DCs, that present tumor-associated antigens, increased at day 30. Circulating IL-6 was increased at day 3 after RFA but this decreased to baseline by day 30, consistent with the supposed anti-tumor effect. There was no increased concentration of essential chemokines, such as CCL-5 and SDF1, VEGF, TGF-β and TNF-α, that might be involved in tumor-growth by sustaining cancer angiogenesis and promoting tumor-associated inflammation. These immunological changes suggested general activation of adaptive response along with a decrease of immunosuppression. Furthermore, most cells showed prolonged activation some weeks after the procedure, suggesting immunomodulation rather than an inflammatory response [57].

IRE. IRE has been demonstrated to be a safe and effective method for locally advanced pancreatic cancer (LAPC). This procedure may be used as a tool to overcome the immunosuppressive "cold" tumor microenvironment in LAPC.

Chaobin et al. retrospectively studied 34 patients after IRE treatment. It was shown that there was a transitory decrease followed by a steady increase for CD4+ T cell, CD8+ T cell, NK cell, IL-2, C3, C4, and IgG while a reverse trend was observed for Treg cell, IL-6, and IL10 after IRE. Elevation of CD8+ T cell was associated with favorable overall survival and progression-free survival in LAPC patients after IRE. This can be explained by a stimulated host immune response which might limit the progression and invasion of the tumor, and therefore, better survival was achieved [58].

Furthermore, Pandit et al. studied immune regulatory T cells (Tregs) which induce immunosuppression of tumors by inhibiting patients' anti-tumor adaptive immune response. They reported that IRE induced an obvious decrease in the absolute number of Treg cell in 11 patients with LAPC. IRE treatment influenced all three Tregs (CD4+CD25+, CD4+CD25+FoxP3+, and CD4+CD25+FoxP3–) compared with pancreatectomy (4 patients) [59].

Histotripsy. Histotripsy is a novel, non-thermal, image guided ablation modality using pulsing regimens to generate cavitation bubble clouds that lead to precise non-thermal tumor ablation that can rapidly kill cells in a targeted region with millimeter precision [60]. Several works established that there is an immune response to histotripsy ablation in many tumors, however a definite mechanism behind this response has not been established [61-63].

Hendricks et al. detected robust immunomodulatory response to histotripsy tumor ablation utilizing an *in vitro* model of pancreatic adenocarcinoma. DAMP and antigen release was demonstrated after ablation. Systemic immune response involving CD4+ and CD8+ T cell activation was confirmed with IL-2 and INFg ELISAs as well as flow cytometry. Further, changes in immune cell populations within the tumor were consistent, there was an increase in macrophages and dendritic cells 24 hours and 7 days and in T cell populations at 7 and 14 days after treatment [64].

Summary

As described above, the pancreatic cancer microenvironment is insensitive to treatment, characterized by a desmoplastic stroma, acting as a physical barrier filled with immunosuppressive immune cells and molecules. Destruction of this stroma and exposition of tumor antigens might be the cause of prolonged survival of the mPDAC patients treated with ablation techniques discussed above.

Use of tumor microenvironment destruction in the management of unresectable pancreatic cancer as a potential inductor and stimulator of antitumor immunity is a relatively new field of research that may one day build a bridge between local and systemic cancer treatments and could be combined with other treatments in a multimodality approach to treating cancer.

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DESTRUCTION OF TUMOR MICROENVIRONMENT AS A PROMISING TREATMENT APPROACH IN PANCREATIC CANCER

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SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of pancreatic cancers. Every year, we face approximately 500,000 new PDAC patients and almost the same number of deaths from this devastating disease both in men and women. The dismal prognosis can be attributed to the immunosuppressive composition of the tumor microenvironment, causing antitumor immune response inhibition, resulting in pancreatic cancer initiation, insidious and rapid progression, and dissemination. The dense desmoplastic stroma, an essential component of the cancer microenvironment, is acting as a physical barrier manifesting in treatment-insensitive pancreatic cancer. Disruption of the dense and immunosuppressive stroma with radio and ablative therapies gives us promising results as the possible inductor and enhancer of an antitumor immune response. In this review, we discuss stromal-targeting ablation methods along with radiotherapy as a dense stromal environment destruction tool and activator of antitumor immune response in pancreatic cancer patients.

Keywords: Pancreatic ductal adenocarcinoma (PDAC), immunomodulation, ablative therapy (AT), radiofrequency ablation (RFA), irreversible electroporation (IRE), histotripsy, radiotherapy (RT)

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PROFESSIONAL ACTIVITY PECULIARITIES OF THE FAMILY PHYSICIANS IN THE PRIMARY HEALTH CARE SYSTEM, CONSIDERING THE COVID-19 PANDEMIC SITUATION

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მაია ციმაკურიძე [1], ნინო ლობჟანიძე [1], მარინა ციმაკურიძე [1], რუსუდან ჯავახაძე [3], ეკატერინე მირველაშვილი [2], ნატო ხუნაშვილი [1]

ოჯახის ექიმის პროფესიული საქმიანობის თვისებურებები პირველადი ჯანდაცვის სისტემაში, COVID-19 -ით გამოწვეული პანდემიის გათვალისწინებით

თსსუ, [1]ნუტრიციოლოგიისა და ასაკობრივი მედიცინის, გარემოსა და პროფესიული ჯანმრთელობის დეპარტამენტი, [2]საზოგადოებრივი ჯანდაცვის, მენეჯმენტის, პოლიტიკისა და ეკონომიკის დეპარტამენტი, [3]ნ. მახვილაძის სახ. შრომის მედიცინისა და ეკოლოგიის ს/კ ინსტიტუტი

რეზიუმე

პირველადი ჯანდაცვის ქვაკუთხედს წარმოადგენს ზოგადი პრაქტიკა, ანუ ოჯახის ექიმის ინსტიტუტი. ოჯახის ექიმის პრაქტიკული საქმიანობა გულისხმობს არა მხოლოდ წმინდა თეორიულ, არამედ გარკვეულ ჩვევებსა და ტექნიკას (5,10,15), რომელიც, თავის მხრივ, გარკვეულ სპეციფიკას იძენს ქვეყნის სოციალური და ეკონომიკური შესაძლებლობებიდან გამომდინარე.

***კვლევის მიზანი** იყო პირველადი ჯანდაცვის სისტემაში ოჯახის ექიმის პროფესიული საქმიანობის თავისებურებების დადგენა COVID-19-ით პანდემიიდან გამომდინარე რეალობის გათვალისწინებით. კვლევის ამოცანებად ჩამოყალიბდა: 1. კითხვარის დახმარებით სტატისტიკური მასალის მოპოვება-დამუშავება; 2. ოჯახის ექიმის საქმიანობისთვის დამახასიათებელი თავისებურებების გამოვლენა, მათ შორის COVID-19 -ით გამოწვეული პანდემიის პერიოდში; 3. პრევენციული ღონისძიებების შემუშავება. კვლევით დადგინდა, რომ ოჯახის ექიმის სამუშაო განეკუთვნება დაძაბული და სტრესული პროფესიების ჯგუფს; ოჯახის ექიმის სტრესული სამუშაო გარემო უარყოფითად მოქმედებს ჯანმრთელობაზე და განაპირობებს ისეთი დაავადებების განვითარების რისკს, როგორიცაა არტერიული ჰიპერტენზია და შაქრიანი დიაბეტი, რასაც ხელს უწყობს აგრეთვე, კვების არასწორი რეჟიმი. ოჯახის ექიმებს შორის გამოვლენილი დაავადებების (შაქრიანი დიაბეტისა და არტერიული ჰიპერტენზიის) შემთხვევები შეიძლება განხილულ იქნეს როგორც პროფესიით განპირობებული დაავადებები.*

The cornerstone of primary health care is General Practice, i.e. the discipline of a family medicine, as it can provide effective, realistic, and high-quality primary health care [1,2]. The family physicians do not deal with only part of the health problems; they serve the entire population, regardless of age and gender [13,16,6].

The practical work of a family doctor involves not only purely theoretical but also certain skills and techniques [5,10,15] which, in turn, acquire certain specificity depending on the social and economic capabilities of the country. For very poor countries and populations, different and much more basic needs are to be met than is required by Family Medicine. Basic public health measures remain a top priority for many countries, although this is not the only necessity. However, the solution to other, quite important problems is possible only with an individual and family-oriented approach [3,9].

Nowadays, The difficulties in the family doctor activities and the importance of extensive theoretical knowledge and practical skills of the medical staff working in this field determine the peculiarities of their professional work [4]. The basic principles of family medicine are based on the doctor-patient relationship, and it is of great importance to ensure and maintain the continuity of this relationship [12], which should be based on the mutual obligations of the doctor-patient relationship. Prerequisite for

the effectiveness of medical services is the availability of the service, the competence of the doctor, good communication skills, and an appropriate mechanism for connecting one consultation with another.

The study [7,8] found that the data of patient information, in 40% of cases, reduce the duration of consultations, reduce the number of laboratory tests, physicians are more likely to use waiting practices, fewer prescriptions are written, and more often referral letters to hospitals and different specialists are given out. Throughout the existence of modern medicine, the problem of the family doctor/specialist has always been relevant.

The family doctor's practice is greatly influenced by local factors such as population structure, economic status, physician/population ratio, accessibility to various primary health care services, and administrative constraints. The COVID-19 pandemic of recent years has significantly increased the role of the family physician in providing comprehensive and effective services to the population. At the same time, the workload of the family doctor and his / her responsibilities have been increased, as the online service of the patient / infected person is different and adds additional aspects to the family physician's work [14,11].

The study aimed to determine the specificity of a family physician's professional practice in the primary health care system under the COVID-19 pandemic conditions. The objectives of the research were: 1. Obtaining and processing statistical materials with the help of a questionnaire; 2. Identification of family physician characteristics, including the characteristics of the COVID-19 pandemic period; 3. Development of preventive measures. The study was conducted at the Family Medicine Center.

A questionnaire developed by us, which was distributed to the family physicians participating in the study, was used in the study. The questionnaire consisted of both closed and open-ended questions. During the research process, the respondent was explained that the survey was anonymous and confidential. 300 family physicians participated in the study. The material was processed using biostatistical methods.

The distribution of the respondents in the survey by age and gender are presented in diagrams №1 and №2.

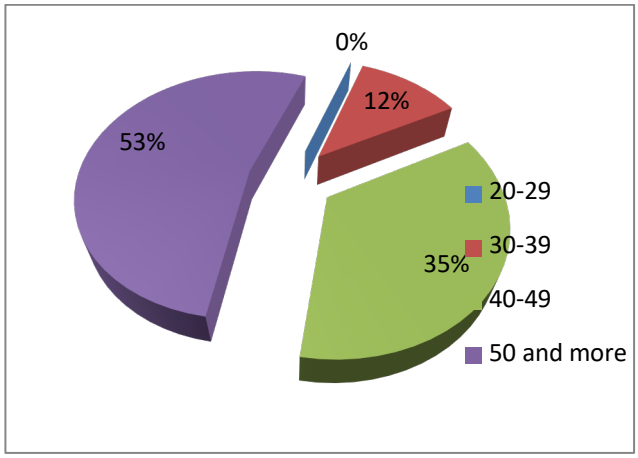

Diagram 1. Distribution of respondents by age

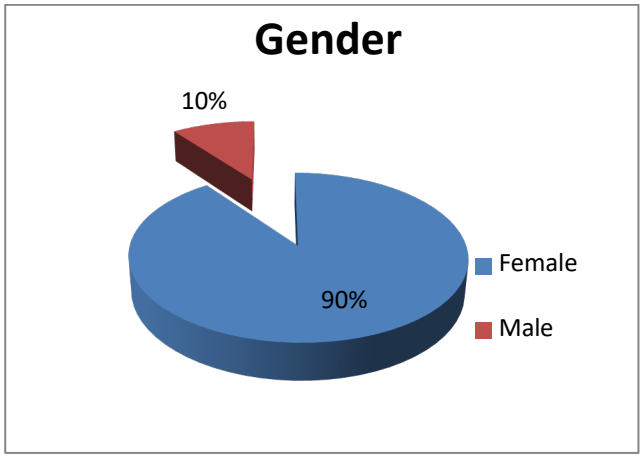

Diagram 2. Distribution of respondents by gender

90% of the respondents were women and 10% were men. The majority of respondents were physicians aged 50 and over (53%). 12% of the respondents had 1-5 years of work experience, 42% had 6 -10 years of work experience, while the remaining 46% had 10 years or more of work experience. Depending on the situation in the country (Covid-19 pandemic), all of them (100%) have to work at only one full-time job, which includes a 12-16 hour workday. However, in the period before the pandemic, the work schedule of the respondents was 6-8 hours. The survey of respondents found that if the patient is elderly or if the patient has any chronic disease, their examination time appears to be approximately 30 minutes or more. In other cases, 20% of GPs spend 10 -15 minutes on communication with patients, 47% spend 20-25 minutes, and the remaining 33% spend 30 minutes or more (Figure 3).

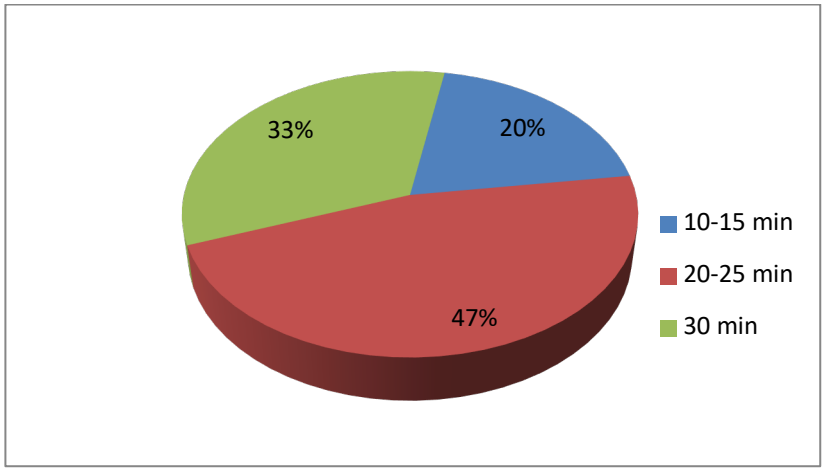

Diagram 3. Data on time spent on patient examination

Based on the results of the conducted survey to find out the main reasons for patient visits to primary care physicians, a list of the 25 most common problems, complaints, and symptoms that a family doctor encounters in his / her practical work, has been created (Table 1). This list includes the major problems that patients primarily face. The symptoms are quite varied and can reflect the pathology of any system. This once again confirms the immeasurably wider nature and diversity of the family physicians' work. The reasons for family doctor visits in Georgia are as follows (Table 1):

Table 1

Reasons for the visits to primary care physicians in Georgia

Reasons to visit family doctors	(%)
Complaints caused by arterial blood pressure (headache, ringing in the ears ...)	20%
Cough (with a history of bronchial asthma)	16%
Problems with the lower extremities	15%
colds and sore throat	13%
Nausea, diarrhea	13%
Pain in the joints	10%
Skin lesions	8%
Gynecological problems	5%

In Georgia, according to our data, the symptoms developed as a result of chronic diseases occupy the leading places.

The analysis of the family physician survey data revealed how much time doctors spend on speaking about disease prevention during patient consultations, including the information on COVID-19 prevention regulations and vaccinations; it appeared that 27% of the respondent physicians spend 20-25 minutes, 50% - 30 minutes, and the remaining 23% - 30 minutes or more (the data are presented in Diagram 4).

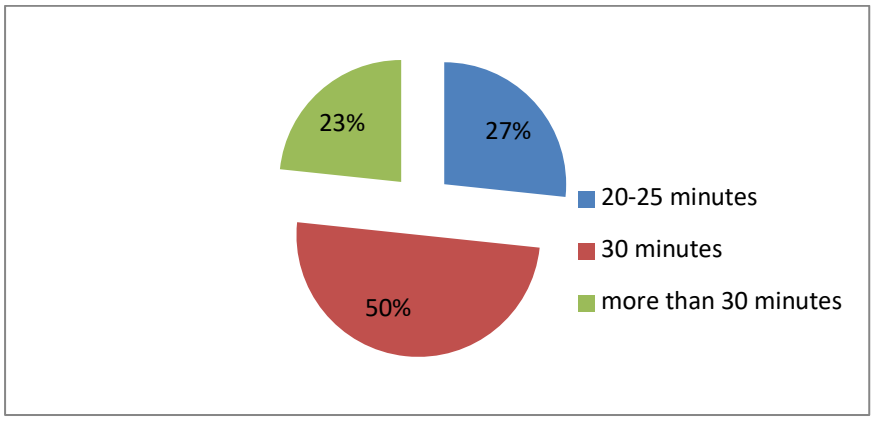

Diagram 4. Time spent on talking about disease prevention issues by the physicians

The questionnaire was focused on the conditions of service continuity. It was found that 13% of respondents think that the prerequisite for the continuity of medical services is depended on the competence and good communication skills of the doctor, 20% - the competence of the doctor and a proper mechanism to connect one consultation to another, 7% think that the competence of the doctor is sufficient, the rest 60% believe that the best precondition for continuity is the physician competence, good communication skills and proper mechanism to have a connection between consultations (Diagram 5).

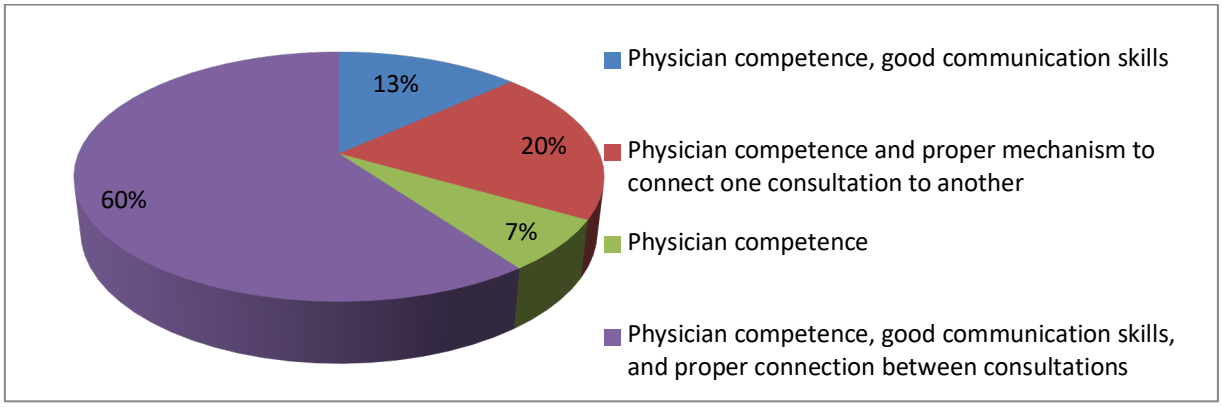

Diagram 5. Important Prerequisites for the Continuity of Medical Care

According to the survey of respondents, due to the epidemiological situation in the country, family physicians consult patients remotely - by phone. The online doctor consultations reduce the length of consultation time and doctors do not have to work overtime. When asked "how long does the consultation with each patient take, whether it is the first or the second consultation", respondents said that if in a normal situation this time length normally ranged from 20 minutes to 30 minutes, the pandemic reduced the time to 15 minutes due to a large number of patient services.

In addition, data on the prevalence of various diseases among respondent physicians were also analyzed (Diagram 6). The respondents mainly identified two chronic diseases - diabetes mellitus and arterial hypertension. 27% of the family physicians surveyed had diabetes, 40% had arterial hypertension, and 33% did not have any health disorders.

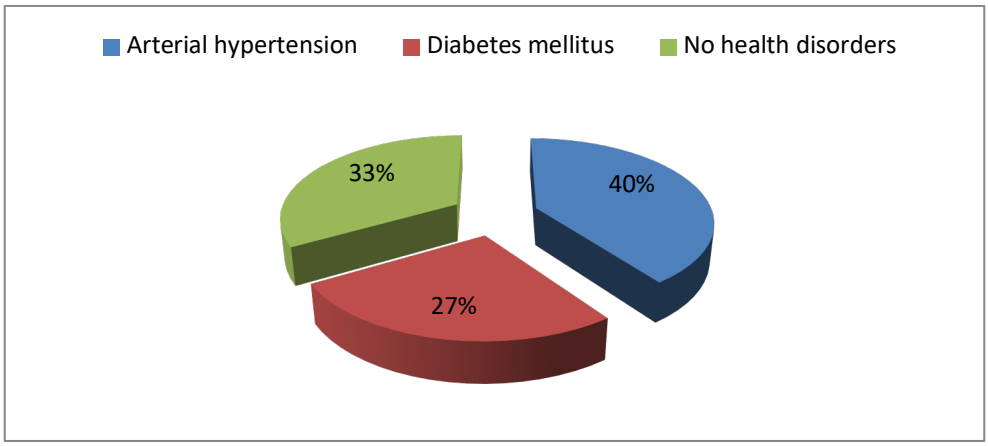

Diagram 6. Data of the Diseases Detected in the Respondents

In addition, 20% of respondents consumed tobacco, 83% used tobacco and caffeinated beverages; 27% used moderate amounts of alcohol, tobacco, and caffeinated beverages; And 6% of respondents did not consume any of them. It was also mentioned that 33% of the respondents were able to have nutrition and follow eating regimens at work, 50% of them could rarely follow the regimen and 17% of them could not follow the eating regimen at all.

The study found that the variety of communications with patients, the wide range of problems that the physician has to solve in a short period of time and especially in times of pandemic due to time constraints, the increased responsibility and the increasing number of patients create a stressful work

schedule for family physicians, which, in turn, contributes to occupational stress and increases workload and tension.

The study found that:

- The job of a family physician belongs to a group of tense and stressful professions;
- Qualifications of a family doctor can be considered the following:
  - Busy work schedule;
  - A large number of patient visits during the working day;
  - Variety of problems to be solved;
  - Personal and professional responsibility;
- A family doctor's stressful working environment has a negative impact on their health and increases the risk of developing diseases such as high blood pressure and diabetes, which is also facilitated by bad eating time schedule.
- Cases of diseases (diabetes and arterial hypertension) detected among family physicians can be considered as work-related diseases.

Based on the results of the research, we think that taking into account the specificity of the family physician's work, to reduce the impact of stressful factors of an unfavorable working environment, it is necessary to reduce the family physician's working time and adjust the rest regime; It is also necessary a family physician regulate/reduce the number of patient consultations during their working day; And the management of the administration should consider resolving the issues of nutrition and rest (holidays, part-time work, etc.) of the family physicians.

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PROFESSIONAL ACTIVITY PECULIARITIES OF THE FAMILY PHYSICIANS IN THE PRIMARY HEALTH CARE SYSTEM, CONSIDERING THE COVID-19 PANDEMIC SITUATION

TSMU, [1]Department of Nutrition and Aging Medicine, Environmental and Occupational Health, [2]Department Of Public Health, Health Care Management, Policy and Economics, [3]Makhviladze Scientific Research Institute of Labor Medicine and Ecology

SUMMARY

The study aimed to determine the specificity of a family physician's professional practice in the primary health care system under the COVID-19 pandemic conditions.

The study found that: the job of a family physician belongs to a group of tense and stressful professions; qualifications of a family doctor can be considered the following: busy work schedule, a large number of patient visits during the working day, variety of problems to be solved; personal and professional responsibility; A family doctor's stressful working environment has a negative impact on their health and increases the risk of developing diseases such as high blood pressure and diabetes, which is also facilitated by bad eating time schedule. Cases of diseases (diabetes and arterial hypertension) detected among family physicians can be considered as work-related diseases.

Keywords: Family medicine, working environment, risk factors, COVID-19 pandemic conditions.

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DEMENTIA IN PATIENTS WITH EPILEPSY

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დემენცია ეპილეფსიით დაავადებულებში

[1] ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტი; [2] თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი [3] მაღალტექნოლოგიური კლინიკა მედცენტრი

რეზიუმე

თემის აქტუალობა. *დღეს მსოფლიოში, დემენციით დაავადებულთა რიცხვი მკვეთრად იზრდება, მაღალია ეპილეფსიით დაავადებულთა რაოდენობაც. 2012 წელს ჩატარებული კვლევის მიხედვით საქართველოში ყოველი 1000 ადამიანიდან ეპილეფსია აქვს 8.8-ს. ქვეყანაში აქტიური ეპილეფსიით დაავადებული უნდა იყოს 35 000-მდე ფიზიკური პირი.*

თემის მიზანია, *დავადგინოთ დემენციის გავრცელება ეპილეფსიით დაავადებულ პაციენტებში, რომელთაც აქამდე დემენციის დიაგნოზი დადგენილი არ ჰქონდათ.*

გამოყენებული მასალა და მეთოდები. *კვლევა ჩატარდა ბათუმში, მაღალტექნოლოგიურ კლინიკა მედცენტრში. 2018-2020 წ.წ. ჩატარებულ კვლევაში ჩართული იყო 82 ეპილეფსიით დაავადებული (დადასტურებული) პირი 20 წლიდან 80 წლამდე. აქედან ქალი იყო - 40, მამაკაცი- 42, საკონტროლო ჯგუფში გამოკვლეულ იქნა- 20 პაციენტი არაეპილეფსიური დაავადებით.*

დემენციის დიაგნოზის დასადგენად ვსწავლობდით *პაციენტის ანამნეზს, ფიზიკურ მონაცენებს, ვახდენდით მათ ფსიქიკურ და კოგნიტური ფუნქციების შეფასებას - Mini-*

MentalStateExamination (MMSE). გამოკვლეულ პაციენტებს ჩაუტარდა თავის ტვინის მაგნიტურ-ბირთვული რეზონანსული ტომოგრაფია.

ჩვენს მიერ, ეპილეფსიის მინიმუმ ერთი წლის ანამნეზით, გამოკვლეულ იქნა 82 პაციენტი. პარციალური ეპილეფსიით გამოკვლეულ იქნა-35 (42,68%) პაციენტი. გენერალიზებული ეპილეფსიით - 47 (57,31%) პაციენტი. მონოთერაპიას იტარებდა (პირველი რიგის პრეპარატებს) 64 (78,04%) პაციენტი. ორ პრეპარატს იღებდა 18(21,95%) პაციენტი. პირველ ჯგუფში (A)- გაერთიანდნენ პაციენტები, რომელთა გულყრები აღენიშნებათ წელიწადში 5-10 ჯერ. 2-ეჯგუფში (B)- 2-3 ჯერ, ეპილეფსიური გულყრით წელიწადში - 3-ჯგუფში (C)-ერთი წლის ეპილეფსიის გულყრის ანამნეზით. საკონტროლო ჯგუფში გაერთიანდა 20 პაციენტი-არა ეპილეფსიური დაავადებით - 20 იდან - 80-წლის ჩათვლით.

ჩვენი კვლევებით, გამოკვლეულ ეპილეფსიით დაავადებულ. პირველი ჯგუფის პაციენტებს მძიმე ხარისხის კოგნიტური დარღვევები აღენიშნებათ - 41(50%) %-ს, მეორე ჯგუფის პაციენტებს - 34 (41,46%). მესამე ჯგუფის პაციენტებს კი - 7(8,53%)

კვლევით დადგინდა, რომ ქცევითი დარღვევები შედარებით მეტადაა გამოვლენილი ფრონტო-ტემპორალური პათოლოგიური რადიოლოგიური ცვლილებების ფონზე. საფეთქლის მარცხნივი წილის ეპილეფსიისას ვერბალური მეხსიერების დარღვევებია გამოხატული, ხოლო მარჯვენა მხრივის დროს გამოხატულია მეხსიერების არავერბალური (სივრცითი) დაზიანების ნიშნები. საყურადღებოა საფეთქლის წილის მარცხენა მხრივი დაზიანება, რომლის ფონზეც ვითარდება ფსიქოზური სიმპტომები. ჩვენი კვლევების თანახმად ვერბალური მეხსიერების დეფიციტი - მარცხენა, ხოლო არავერბალური - მარჯვენა სფეროს დაზიანებასთანაა დაკავშირებული. რადიოლოგიური კვლევებით ვლინდება, რომ ჰიპოკამპის დაზიანებისას ვითარდება მკვეთრად გამოხატული მეხსიერების დეფიციტი, აფექტური სინდრომები დეპრესიის სახით. კორძიანი სხეულის დაზიანებისას სწრაფად ვითარდება ინტელექტუალური დეფიციტი, თალამუსის და ბაზალური განგლიის დაზიანება კოგნიტური და ფსიქიკური ფუნქციების დარღვევას იწვევს, მათ შორის: მეხსიერების, მეტყველების, გუნებ-განწყობის ცვლილებებს.

საკონტროლო ჯგუფში, სადაც გაერთიანებულია არაეპილეფსიით დაავადებული პაციენტები- კოგნიტური დარღვევები აღენიშნებათ -15 (75%) პაციენტს.

ჩვენი კვლევებით, კოგნიტური პრობლემები ვლინდება რეგისტრაციის (ფრონტო-ტემპორალური კავშირების), კოდირების (მედიალური საფეთქლის წილის) ან რემინესცენციის - (შუბლის წილის) უნარის დაქვეითების სახით. საკმაოდ მაღალია დემენციის მაჩვენებელი როგორც ეპილეფსიით, ისე არა ეპილეფსიით დაავადებულ პაციენტებში. ეპილეფსიით დაავადებულ პაციენტებში თვალსაჩინოა ახალი მასალის ათვისება უწყვეტი ადეკვატური ანტიეპილეფსიური მკურნალობისა და სამედიცინო მეთვალყურეობისას, პაციენტის მიერ მკურნალობის რეჟიმის დაცვის შემთხვევაში და ადეკვატურ ფსიქო-სოციალურ გარემო პირობებში დაავადებულთა 70-75%-ში მიღწევადია ეპილეფსიური გულყრების სრული კუპირება, თუმცა გასათვალისწინებელია კოგნიტური დარღვევების მკურნალობა.

Introduction

In today's world, the number of dementia patients is dramatically increasing. It is due to several factors [4,5,6]. The number of patients with epilepsy is also high. According to a study conducted in 2012, 8.8 out of every 1000 people in Georgia have epilepsy. There should be up to 35,000 individuals with active epilepsy in the country [1,2,3].

The aim of the study is to determine the prevalence of dementia in patients with epilepsy who have not previously been diagnosed with dementia.

Materials and methods.

The study was conducted in Batumi, Georgia at a high-technology clinic in the MED center. During 2018-2020, the study included 82 people with epilepsy (confirmed) aged from 20-80 years. 40 people were women, 42 people were men, and 20 age-matched patients with non-epileptic disease, were studied in the control group: 12 patients with arterial hypertension and circulatory encephalopathy, 6 patients with endocrine pathologies and arterial hypertension, 2 patients with brain injury. From the patients with epilepsy, 45 patients with arterial hypertension and circulatory encephalopathy, 15 of them with a diagnosis of ischemic stroke, 14 patients with endocrine pathology. To diagnose dementia, we

studied the patients medical history, physical examination, and assessed their mental and cognitive functions, conducted Mini-Mental State Examination, Surveyed family members since often the patient found it difficult to accurately describe the symptoms. We paid special attention to the starting point of the disease, duration, and progress of the worsening of the symptoms. The examined patients underwent magnetic resonance imaging of the brain.

Results

We examined 82 patients with a history of epilepsy of at least one year. Of these, posttraumatic epilepsy - detected -14 (17.07%) patients. 5 (6.09%) of patients - disease started after covid-infection, 17 (20.73%) patients - after cerebral stroke. 10 (12.19%) patients were examined for partial epilepsy. 5 (6.09%) of patients were examined for attack - disturbances of consciousness; In the study were involved patients with generalized epilepsy - 31 (37.80%), with tonic seizures -7 (8.53%) with tonic-clonic seizures - 24 (29, 26%) patients.

The examined patients were taking antiepileptic drugs. On monotherapy was (first-line drugs) 64 (78.04%), (52 of them (63.41%). - Carbamazepine, 8 (9,75%). - Valproate acid- DepakineCrono, 4 (4.87%). - Lamictal.) Two drugs were taken by 18 (21.95%) Patients. (11 (13.41%) Valproate acid and topiramate. 7 (8.53%). - Patients of Kepra and Lamictal.)

Patients were divided into three groups: Group A - unite patients with epilepsy who experience seizures 5-10 times a year, Group B - 2-3 times, Group 3 - with a history of one-year epilepsy and no seizures. The control group includes 20 patients with non-epileptic disease - from 20 to 50 years old.

According to our study severe cognitive impairment was found in group A (seizures 5-10 times a year) in 41(50%). In the patients of the second B group (seizures 2-3 times a year) - 34 (41,46%), in the third C group (no seizures during one year) of patients – 7 (8,53%). Patients experienced memory impairment (11%), speech impairment (5%), personal traits were erased - critical thinking, exchange of thoughts, ideas or information, communication impairment was found in those examined. Most of the respondents follow the established way of life.

In patients with severe epilepsy with dementia, the intellectual capacity is reduced (55%), the person becomes incontinent, engages in socially unacceptable behaviors, is attracted to alcohol (15%). Urinary incontinence is expressed in (25%), of patients. In this group, patients have difficulty completing tasks.

Table 1 cognitive impairment found in different subgroups.

	GROUP A (seizures 5-10 times a year)	GROUP B (seizures 5-10 times a year)	GROUP C (no seizures during one year)
Severe cognitive impairment	N41 (50%)	N34 (42%)	N7 (8%)

Attention is reduced, which is manifested by a decrease in focusing on information, disturbed perception, reasoning, logical thinking, which, in turn, is associated with the disability to solve problems, altered orientation in space and time. The patient's practical skills, purposefulness, amnesia, partial or complete memory loss are developed, Which, in turn, prevents the individual from finding or storing information.

Getting into an unfamiliar environment often causes the patient’s anger for no reason. Along with decreased cognitive functions, the person becomes less able to maintain personal hygiene and neglects social norms. The patient's thinking process slows down, erroneous thoughts are revived, ideas of a persecution scenario are frequent. At a later stage of the disease, thinking becomes fragmentary. Speech skills are impaired.

The study found that behavioral disorders were relatively more expressed in correspondence to fronto-temporal radiological changes. In left-side epilepsy, there are verbal memory disorders, and in right-side epilepsy, there are signs of non-verbal memory impairment.

Radiological studies reveal that a lesion of the hippocampus develops a memory deficit, affective symptoms in the form of depression. Injury to the corpus callosum develops intellectual impairment,

damage to the thalamic and basal ganglia which lead to cognitive and mental impairments, including changes in memory, speech, and mood.

In the control group, which includes patients with non-epilepsy - cognitive disorders were found in 15 (75%) patients, from which 12 (60%) of patients have arterial hypertension and circulatory encephalopathy, 30 (30%) endocrine pathologies, and arterial hypertension (6%). Patient with brain injury - 2 (10%).

From this number, mild grade dementia was detected in 55% of patients with non-epilepsy. They have memory impairment - (11%), patients in this group more or less cope with the difficulties of daily life, speech disorder was observed in 13%, erased personality traits, including critical thinking, communication skills impairment - in 19% of patients. 35% of patients with non-epilepsy also have moderate dementia. They have memory impairment (56%). Speech disorder (31%), personal qualities are erased, patients are not able to follow the hygienic norms - (65%)

Severe dementia occurs in 10% of patients with non-epilepsy, they have reduced intellectual abilities (80%), the person becomes unrestrained and careless and engages in socially unacceptable behaviors, is attracted to alcohol (31%). Urinary incontinence is also expressed (42%).

According to our studies, the development of dementia is exacerbated by a changing living environment. Clinical signs depend on the patient's premorbid personality traits. Individuals with good social skills continue to function adequately despite their reduced intelligence. Elderly socially isolated individuals with poor hearing abilities are less likely to compensate for impaired intellectual ability. The first symptoms of cognitive impairment are forgetfulness, although it is often difficult to detect in its early stages.

There is quite a high rate of dementia in both types of patients with epilepsy and non-epilepsy. Patients with epilepsy find it difficult to learn new material.

Learning problems (32%) are common in patients with epilepsy when the disease has started before the age of 18.

In conclusion, with continuous adequate antiepileptic treatment and medical supervision, complete cessation of epileptic seizures is achieved in 70-75% of patients with adequate psychotherapy and an adequate psycho-social environment, although treatment of cognitive impairment should be considered in accordance with our research, symptoms of dementia is highly prevalent during epilepsy.

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DEMENTIA IN PATIENTS WITH EPILEPSY

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SUMMARY

Introduction. In today's world, the number of dementia patients is dramatically increasing. It is due to several factors. The number of patients with epilepsy is also high. According to a study conducted

in 2012, 8.8 out of every 1000 people in Georgia have epilepsy. There should be up to 35,000 individuals with active epilepsy in the country.

The aim of the topic is to determine the prevalence of dementia in patients with epilepsy who have not previously been diagnosed with dementia.

Materials and methods. The study was conducted in Batumi, Georgia at a high-tech clinic in the medical center. During 2018-2020, the study included 82 people with epilepsy (confirmed) aged from 20 to 80 years. To diagnose dementia, we studied the patient's medical history, physical examination, and assessed their mental and cognitive functions. We conducted a Mini-Mental State Examination. Surveyed family members because often the patient found it difficult to accurately describe the symptoms. We paid special attention to the starting point of the disease, duration, and progress of the worsening of the symptoms. The examined patients underwent magnetic resonance imaging of the brain.

Results and conclusion. According to our study variable degree of cognitive impairment was found in epileptic patients. In patients with severe epilepsy and dementia, the intellectual capacity is reduced (55%), Urinary incontinence is expressed in (25%), In conclusion, with continuous adequate antiepileptic treatment and medical supervision, complete cessation of epileptic seizures is achieved in 70-75% of patients with adequate psychotherapy and an adequate psycho-social environment, although treatment of cognitive impairment should be considered in accordance with our research, symptoms of dementia is highly prevalent during epilepsy. Most of the respondents follow the established way of life.

Keywords: Dementia, epilepsy, Georgia

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COVID INFECTION AND GUILLAN-BARRE SYNDROME

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თემის აქტუალობა. *ორ წელზე მეტია COVID-19 საქართველოში, ისე, როგორც მთელ მსოფლიოში, მძვინვარებს.*

თემის მიზანია, *შემოგთავაზოთ საინტერესო კლინიკური შემთხვევა, რომელიც კოვიდ ინფექციასა და გიიენ-ბარის სინდრომს უკავშირდება. დავსახოთ პრევენციის გზები.*

გამოყენებული მასალა და მეთოდები. *ჩვენს მიერ შესწავლილი იყო კლინიკაში კოვიდ ინფექციის დიაგნოზით პაციენტები 2020 წლის ოქტომბრიდან 2021 წლის პირველ თებერვლამდე. სულ კლინიკას მომართა 544 პაციენტმა, სტაციონარული მკურნალობა ჩაიტარა 477 პაციენტმა. აქედან კაცი 242- იყო, ქალი-240. მათგან მხოლოდ ერთს. ხოლო პოსტკოვიდურ პერიოდში ორი კვირიდან სამ თვემდე სტაციონარს მომართა 270 პაციენტმა, მათგან მხოლოდ 3 მათგანს დაუფიქსირდა გილენ-ბარეს დაავადება.*

სტატიაში გახილულია ორი შემთხვევა ჩვენს კლინიკაში მოხვედრილი სამი პაციენტიდა, რომელთაც გიენ-ბარეს სინდრომი დაუდასტურდათ.

ჩვენი აზრით, დიაგნოზის დასასმელად საკმარისია პაციენტის სიმპტომების გათვალისწინება და მისი ფიზიკური შემოწმება. ამ დაავადების ტიპიურ სურათს წარმოადგენს კუთების (პროგრესირებადი) სისუსტის სწრაფი გამოვლენა, რასაც თან სდევს რომლებიც სხეულის ორივე მხარეს უჩვეულო შეგრძნებები,მყეს-ძვალთა არეფლექსია. ამრიგად, პირველი დიაგნოსტიკური ნაბიჯები ანამნეზის შეკრება და პაციენტის გასინჯვა იყო, შემდეგ ჩატარდა ლუმბალური პუნქცია. თავისა და ზურგის ტვინის გამოსაკვლევად ასევე ჩატარდა ელექტრომიოგრაფია – ჩონჩხის კუნთების ელექტრულ პოტენციალთა რეგისტრაცია ნერვებისა და მათ მიერ ინერვირებული კუნთების მოქმედებას გამოსაკვლევად.სადაც ნერვის გამტარობა ნელა და ბევრად ჩამორჩებოდა ნორმას. ასევე ჩატარდა ხერხემლის მაგნიტურ-ბირთვულ რეზონანსული კვლევა, სადაც ზურგის ტვინის ფესვები ზომებში მომატებული.

ამრიგად, SARS-CoV-ის ინფექციის დროს ზიანდება პერიფერიული ნერვული სისტემაც. საჭიროა ყურადღება მივაქციოთ იმ კოვიდპაციენტებს, რომლებშიც დაავადების პირველი ნიშანი სწორედ ნევროლოგიური გამოვლინება შეიძლება იყოს. დაავადების ადრეულ ეტაპზე დიაგნოსტიკა მისი სწორად მართვისთვის და ვირუსის გავრცელების თავიდან ასაცილებლად იქნება მნიშვნელოვანი. დაავადების პრევენცია გულისხმობს სამედიცინო დაწესებულებაში დროულ მიმართვას, რადგან დაავადების პროგნოზი პირდაპირ არის დამოკიდებული დროულად ჩატარებულ გამოკვლევებსა და მკურნალობაზე.

INTRODUCTION.

For three years COVID-19 has been raging in Georgia as well as all over the world. Many clinics including ours have become a battle place against covid infection [1,2]. According to scientists lengthening of symptoms is very common for many virus and bacterial infections [5,6,7]. MERS-CoV can cause severe scattered encephalomyelitis. There were cases of encephalitis after brainstem infection and Guillan-Barre syndrome. Severe scattered encephalomyelitis can be formed in case of HCoV-OC43 infection. Immunosuppressed people are particularly at high risk. The virus was found in the brain tissue of immunosuppressed people having encephalomyelitis caused by HCoV-OC43. The same doubt exists towards SARS-CoV2 [1,2,3,4].

The Guillan-Barre syndrome is not uncommon in the post infection period. The frequency of Guillan-Barre syndrome after 30 days from any kind of flu equals 16,6 cases from 100 000 people (in vaccinated people the frequency is 0,76 from 100 000 people). In Great Britain 21,5 Guillan-Barre cases were described for every 10 million vaccinated people during 6 weeks after the vaccination [8,9].

MATERIAL AND METHODS.

We have studied patients with covid infection in our clinic from October 2020 to February 2021. In total 544 patients were admitted to the clinic, 477 were treated on a stationary basis. Among them 242 were men and 240 – women, the median age was 47 years. In the post-covid period 270 patients were re-admitted to clinic within two weeks to three months. Among them only 3 had Guillan-Barre disease (GBS). All the patients had a Brighton criteria level of diagnostic certainty 1 or 2.

The majority of the patients were admitted to the intensive care unit for severe respiratory distress, and peripheral nervous system involvement became evident at weaning off sedation and gaining conscience. In accordance with the Brighton criteria, the interval between coronavirus diagnosis and the beginning of weakness was between 24 h and 35 days.

The aim of our manuscript is to introduce interesting clinical cases which are related to covid infection associated Guillan-Barre syndrome and to find ways of prevention.

RESULTS.

Patient - 61year-old male, had covid-19 one month prior to the onset of symptoms. Disease started with severe tiredness, weakness of lower limbs and progressed during the day. Symptoms started in both limbs with symmetrical muscle weakness, which started from the proximal parts of lower limbs and spread to upper limbs in several hours. The weakness was followed by paresthesia in fingers. Paresthesia (a feeling of an ant crawling) and numbness of legs “socks” and hands – as “gloves” was accompanied by pain in the back area. There were autonomic changes, including increased blood pressure, sinus tachycardia, sweat, face reddening, reflexes were decreased and later disappeared. We diagnosed the person by clinical symptoms, nerve conduction studies, analysis of cerebrospinal fluid, MRI of the spine was done as well.

A female patient, 55 years old, on admission to the clinic, was complaining about balance difficulty and inability to walk or climb stairs, difficulties in face and eye movement, including swallowing, chewing and speaking, severe pain in limbs and back, which worsened at night, complications in urination and defecation, accelerated heartbeat, breath difficulties. When she was admitted to the clinic she had symmetrical weakness – areflexia, atonia, the feeling of pin pricking in fingers and toes, ankles and wrists, leg weakness which later spread to the upper part of the body.

On the eighth day a patient had dry cough and 38 ± 2 ° C. We have seen vitreous blurring of both lungs with X-ray images of chest cavity. The analysis of nose smear confirmed positive analysis of SARS-CoV-2. After three days the strength in lower limbs was 4 points, but in the upper limbs it was 3, clinical laboratory analysis showed lymphocytopenia. On the fifth day there weren't F waves during nerve conduction studies.

To diagnose this patient, we took into consideration the patient's symptoms and her physical check-up. The typical image of this disease is quick ongoing weakness, which is followed by unusual feelings in the limbs and areflexia. Therefore, the first diagnostic steps were to collect anamnesis and check the patient, then we did lumbar puncture. We did electromyography- electrical potential registration of skeleton muscles to examine the action of nerves and innervated muscles, where nerve conductivity was slow and significantly lagged to the norm. We also conducted MRI research on the spine, where the roots of the spinal cord were increased in size. So, during SARS-CoV infection, the peripheral nervous system damages too.

Third patient, a male, is 37 years old and became symptomatic 10 days after vaccination. The disease began with a tingling sensation in the fingers and toes, a weakness started in the feet and covered the upper extremities as well, with difficulty climbing stairs, swallowing, and speaking was affected too. Symptoms within a few days were accompanied by difficulty urinating, tachycardia, low blood pressure.

Areflexia was noted during the neurological examination. The patient underwent lumbar puncture and electroneurography, where acute motor-axonal neuropathy was seen. The patient underwent several plasmapheresis sessions after diagnosis as well as immunoglobulin was administered intravenously for five days.

About two weeks after the onset of the first symptom, the patient's condition was improved and he was able to walk independently in six months.

The patients were treated with low molecular weight heparin at high doses for primary prevention of SARS-CoV-2 induced thrombosis. In the one patient who performed CSF analysis, reverse transcription polymerase chain reaction assay on CSF for SARS-CoV-2 was negative. Nerve conduction studies demonstrated acute inflammatory demyelinating polyneuropathy in 3 cases, according to Hadden criteria.

Interestingly only three cases of GBS were admitted to our hospital. We believe it worthwhile to communicate our experience and to raise awareness to healthcare professionals dealing with COVID-19 patients regarding the frequent peripheral nervous system involvement of SARS-CoV-2 infection.

Two patients underwent a blink reflex test, which showed a demyelinating pattern in either the facial and/or the trigeminal nerves, suggesting frequent cranial nerve involvement. Neuromuscular weakness is a common occurrence in the intensive care unit; nevertheless, it is usually due to critical illness myopathy and neuropathy, the differential diagnosis of which is based on electrophysiological tests. Both of these diseases usually present as symmetric flaccid limb weakness; however, they must promptly be distinguished considering the proven effectiveness of intravenous immunoglobulin or plasma exchange in GBS. Amongst our cohort, 3 patients were treated with intravenous immunoglobulin and two received plasma exchange.

DISCUSSION.

As COVID 19 is an easily contagious disease, which caused the worst pandemic in the 21st century, it is important to pay attention to patients whose first signs may be neurological symptoms. Diagnosis at the early stage of the disease will be important later on to manage correctly symptoms. Disease prevention means applying to the hospital on time, because disease course directly depends on relevant examination and timely treatment.

Intensivists should bear in mind that difficulties in spontaneous breathing and failure of weaning from mechanical ventilation should be a red flag of GBS. Neurologists should be aware of the major significance of proper diagnosis, as GBS related to Covid infection can be treated with excellent results. Both must have in mind that treatments, particularly efficacious when administered in the early phase of the disease.

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COVID INFECTION AND GUILLAN-BARRE SYNDROME

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SUMMARY

Introduction. For three years COVID-19 has been raging in Georgia as well as all over the world. Many clinics including ours have become a battle place against covid infection.

The aim of this work is to introduce interesting clinical cases which are related to covid infection associated Guillain-Barre syndrome and to find ways of prevention.

Materials and methods. We have studied patients with covid infection in our clinic from October 2020 to February 2021. In total 544 patients were admitted to the clinic, 477 were treated on a stationary basis. Among them 242 were men and 240 – women, the median age was 47 years. In the post-covid period 270 patients were re-admitted to clinic within two weeks to three months. Among them only 3 had Guillan-Barre disease (GBS). All the patients had a Brighton criteria level of diagnostic certainty 1 or 2.

Results and conclusion. As COVID 19 is an easily contagious disease which caused the worst pandemic in the 21st century, it is important to pay attention to patients whose first signs may be neurological symptoms. Diagnosis at the early stage of the disease will be important to manage correctly and prevent its spread. Disease prevention means applying to the hospital on time, because disease course directly depends on relevant examination and treatment.

Keywords: COVID-19, Guillan-Barre syndrome

A B S T R A C T S

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ANALYSIS OF SPECIFIC LABORATORY MARKERS IN PATIENTS WITH COVID-19 SYMPTOMS

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COVID 19 pandemic is the greatest challenge not only for the 21st century medicine, but all mankind. COVID infection management process is the subject to continuous updating. Time proved that post COVID complications appeared to be even more pressing healthcare problem. The prolonged and unidentified course of post COVID respiratory complications is particularly prominent.

Among the frequent symptoms of respiratory nature developed after COVID infection is a dry, annoying cough with varying degrees of respiratory failure. The mentioned complaint persists leading to social discomfort of the person. Consequently, monitoring of post COVID respiratory complications puts on the agenda the need in investigation of respiratory system functional status, especially, in patients with post COVID respiratory complications by spirometric computed tomography. Studies in this direction are scarce, and the urgency of the issue – acute, respectively.

The results of the study presented will be of great importance to ensure active planning for the prevention of post COVID complications and develop disease management, especially in recovered COVID patients with post COVID respiratory complications. In addition, the results of the study are important not only from scientific but also in clinical perspective, as the results can be used to improve managing post COVID complications.

Therefore, due to the urgency of the issue, the aim of the present study is to investigate the functional status of the lungs and bronchi in recovered patients with post COVID respiratory symptoms - annoying dry cough and acute respiratory failure. The initial study involved 76 patients (18-70 years of age; 46 women; 30 men), with a variety of complaints: dry, annoying cough, shortness of breath, tiredness, dyspnea, pain in joints and chest, memory problems, concentration or sleep disorders, muscle pain and/or headache, increased heart rate, loss of smell or taste, depression or anxiety, insomnia, numbness in hands and /or lower extremities, ringing in the ears, pain, fever, worsening of symptoms after physical or mental activity, skin rash.

The diagnostic program includes the study of covid infection inflammatory markers, such as: D-Dimer, Coagulogram, C-reactive protein, Procalcitonin, total IgE. In patients with respiratory complications, computed spirometry is performed using Spirolab 3.

According to the analysis of obtained results in patients with various post COVID symptoms were revealed: changes in D-dimer levels – in 15 cases (19%); of coagulogram markers, fibrinogen concentration was elevated in 65 (85%) patients. Continuous monitoring of coagulogram results revealed that despite intensive treatment, an elevated level of fibrinogen persisted for a long time. Elevated C-reactive protein (CRP) was observed in 21 (27%) patients with post COVID syndrome, whereas procalcitonin level was increased in only 6 (8%) cases. Investigation of total serum IgE will enable to prove allergic genesis of COVID complications.

The study found that total IgE was elevated in 25 (32%) of cases. The extent to which an elevated common marker correlates with COVID infection is a subject for further study. In addition, significant changes in spirometry results were also noted in 25 (32%) patients with prominent respiratory symptoms, namely dry, annoying cough, and shortness of breath. Spirometry showed mild or moderate obstruction in only 18 (23%) cases and restrictive changes in single cases. The above reaffirms the need for spirometric study in patients with post COVID respiratory complications.

The above study proved the need for laboratory monitoring of inflammatory markers in most post COVID cases, especially in patients with post COVID symptoms. It is also important to monitor and analyze the immunological marker -IL 6, further study of which is of great interest.

Keywords: covid-19, symptoms, laboratory markers

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INFLUENCE OF COVID-19 INFECTION ON IMPAIRED WOUND HEALING IN POSTOPERATIVE PERIOD

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Global challenge of COVID-19 infection motivated researchers worldwide to an unprecedented effort towards understanding the key mechanisms of this disease. Study results reported cases of impaired wound healing processes during the postoperative period in patients with COVID-19 infection. Postulated theories such as pericyte alteration due to angiotensin converting enzyme 2 receptor association and inflammatory mediators causing endotheliopathy are thought to be the likely cause of the thrombo-inflammatory effects of SARS-CoV-2. However, the definitive cause is yet to be identified. Our Aim was to study the cause of impaired wound healing due to SARS-Cov-2 infection in postoperative period. We obtained study results (published in more than 40 articles, from databases - PubMed and Google Scholar) of impaired wound healing due to SARS-Cov-2 infection in postoperative period.

Study results demonstrated that postsurgical wound healing relies on adequate hemostasis, proliferation and remodeling. The proclivity of SARS-CoV-2 to cause microvascular insult and endotheliopathy creates challenges and complications in postsurgical care. Surgical site complications in patients with postoperative SARS-CoV-2 infection have been documented in many case studies, commonly including severe sepsis, wound dehiscence, osteomyelitis and loss of skin grafts in transplant surgeries. SARS-CoV-2 infects patients by binding to the angiotensin-converting enzyme 2 (ACE2), this enzyme is expressed in the alveolar cells, cardiac myocytes and vascular endothelium. Pericytes of the circulatory system have one of the highest expressions of ACE2 receptors. The key roles of pericytes are maintaining endothelial integrity. Apoptosis and detachment of these cells due to the effects of the SARS-CoV-2 infection may be the reason for the micro-vasculopathy. In addition, elevated levels of Angiotensin II leads to formation of free radicals resulting in oxidative stress, mitochondrial dysfunction and thrombosis.

Further research on pericytes and ACE2 association may aid in the understanding of micro-vasculopathy. Surgical procedures which are only deemed obligatory must be performed with great measure to prevent SARS-CoV-2 infection in the postoperative periods. Routine screening for SARS-CoV-2 and examination of surgical sites would promote early detection and fewer complications.

Keywords: Covid-19 infection, wound healing, micro vasculopathy, postoperative period.

A. PORGADOR

CAR- AND IC-BASED CANCER IMMUNOTHERAPY, AND DIAGNOSTICS FOR PRECISION CANCER IMMUNOTHERAPY

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Harnessing the immune system to eradicate cancer proves efficient results in recent years. Rapidly emerging immunotherapy approaches are CAR-based and IC-based immunotherapy. CAR-based adoptive cell therapy was proven efficient but is facing the danger of an overt immune response leading to potential hazard of severe side effects, autoimmunity or even death. Improved control of the expression of the

engineered CAR is needed to reduce the risks of on-target-off-tumor life-threatening side-effect, for therapy of solid tumors. CARTIV is a novel platform that employ synthetic promoters to induce CAR gene expression only in the tumor microenvironment, based on combination of promoter-responsive elements that their combined stimuli represent the tumor microenvironment.

MAb-based blocking of the immune checkpoints involving the CTLA4-B7 and PD1-PDL1inhibitory axes enhance T-cell–based adaptive immune responses in patients with cancer. We identified innate immune checkpoint involving the NK-expressed NKp44 receptor (isoform 1) and PCNA located from the cancer cell nucleus to the cancer cell membrane. Mab 14-25-9, recognizing the membrane-associated from of PCNA can be employed for blocking the NKp44-PCNA IC and thus enhancing the function of natural killer cells in cancer immunotherapy.

As discussed, IC blocking (ICB) therapy have made a remarkable contribution to prolonging the survival of some cancer patients. However, the major medical problem is that only 5–40% of patients respond to single-agent of ICB, and nonresponding patients are susceptible to severe aberrant affects. IcAR is a platform is to predicate response to ICB-based immunotherapies, to spare non-responders from the side-effects of ineffective treatments, and to propose combinations of immunotherapies. IcAR is based on the ability of an engineered live-cell-based artificial reporters' system to quantify the availability and suppression activity of immunomodulatory proteins in fresh and FFPE fixed tumors.

Keywords: CAR-based and IC-based immunotherapy, cancer, immunotherapy

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PHARMACOLOGICAL MANAGEMENT OF COPD IN PATIENTS INFECTED WITH COVID-19 (LITERATURE REVIEW)

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Patients with Covid-19 are prescribed antiviral therapy along with the symptomatic treatment, whereas patients with asthma and COPD are prescribed symptomatic treatment like corticosteroids (oral and inhaled). Irrational pharmacotherapy can could lead to exacerbations and even death. One of the strongest predictors of poor outcome is the degree of hypoxia during the acute course of the SARS-Cov-2 infection. That's one of the reasons, why pre-existing respiratory illness such as asthma or COPD can increase the need of ventilator support in the patients with COVID-19.

The presence of COPD increases the mortality rate by 1.5-fold in patients with SARS-CoV-2. COPD patients with COVID-19 would require standard-of-care management with both corticosteroids and antimicrobial drugs.

Since Covid-19 is known to worsen the symptoms in COPD patients, drug classes like long-acting muscarinic antagonists, long-acting β2-agonists and inhaled corticosteroids are being used. Glucocorticoids are often used as an empirical treatment for severe COVID-19. Unlike specific cytokine inhibitors, glucocorticoids are considered to be nonspecifically effective against cytokine storm by inhibiting multiple inflammatory processes.

All of them act as bronchodilators, thereby inhibiting the exacerbation of COPD and bronchial asthma induced by infection with viruses, including Sars-COV-2. The route of administration of these drugs can be through nebulizers, pressurized metered-dose inhalers (pMDI) with spacer and dry powder inhalers. However, the effects of these drugs on Sars-COV-2 replication and infection-induced inflammation in the human airway are unknown.

Furthermore, the antiviral therapy targeting the Covid-19 virus acts by inhibiting the RNA-dependent RNA polymerase, defecting the viral replication process. Cytokine-target therapy, recommended by the NIH (National Institutes of Health) and the American Association of Infectious Diseases: Baricitinib + dexamethasone (in severe patients); Combination of interlkin-6 blocker - tocilizumab + dexamethasone.

Conclusion: The COPD patients, regardless of their infection with Covid-19 are recommended to follow regular therapy of medications. They are to be monitored closely as delays in diagnosis and treatment could adversely affect the patient's prognosis. Studies are yet to be concluded on whether Covid-19 in COPD patients needs different pharmacological management.

Keywords: COVID-19, COPD, asthma, pharmacological management.

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CORONAVIRUS INFECTION – NEW RISK FACTOR OF MEDICAL PERSONNEL MORBIDITY

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A new factor, the SARS CoV-2 coronavirus, has joined to the traditional biological risk factors for healthcare workers in pandemic conditions and causing a disease of varying severity, which is explained by the constant contact of healthcare workers with both COVID-19 patients and laboratory biological material and medical waste. Today, the number of confirmed cases of COVID-19 among healthcare workers is increasing worldwide. Medical workers, providing care to patients with a new coronavirus infection, are themselves at risk for COVID-19, and some of them get this infection while performing their professional duties. The article presents a review of foreign literature on the problem of infection and incidence of COVID-19 in medical workers in different countries, including Georgia, as well as the possibility of classifying this disease as an occupational one, which is confirmed by data from some European countries. Based on the analyzed data, the main ways of infection of medical staff, variants of the course of the disease and their severity were determined.

Features of clinical manifestations, ways of spreading a new coronavirus infection, relatively high mortality of doctors working in high-risk areas emphasize the relevance of the COVID-19 problem and its prevention in order to preserve the health of medical workers.

Keywords: Covid-19, medical personnel, risk factor, morbidity

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PEDIATRIC MULTISYSTEM INFLAMMATORY SYNDROME (PIMS) IN CHILDREN WITH COVID-19 INFECTION (REVIEW OF THE LITERATURE)

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Background: From the beginning of the coronavirus disease 2019 (COVID-19) pandemic, it became evident that children infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remain mostly asymptomatic or mildly symptomatic. Pediatric Inflammatory Multisystem Syndrome (PIMS) is a new condition that happens weeks after someone has been infected with COVID-19 and is known to cause inflammation throughout the body. The temporal association between the pandemic peaks and outbreaks of PIMS seems to be in favor of a post-infectious, immune-mediated mechanism.

Aim: The aim of this study was to analyze scientific data on PIMS as its manifestation in children is associated with a 10 times greater risk for hospitalization than other complications of Covid-19. The clinical features, pathogenesis and the treatment of PIMS and its further relationship with Covid-19 are explored.

Methods: The scientific databases - Google Scholar and PubMed were used to review articles published since 2021. The keywords for the search included Pediatric Inflammatory Multisystem

Syndrome, PIMS, Kawasaki Disease, Acute Rheumatic Fever, SARS-CoV-2 and Hyperinflammatory shock.

Discussion: A number of COVID-19 otherwise healthy children were found to experience a rare multisystem hyperinflammatory syndrome, most of whom had a clinical history of exposure to COVID-19 and/or SARS-COV2 clinical diagnosis. Clinical presentations included fever, cardiac and gastro-intestinal symptoms, mucocutaneous manifestations and hematological or other organ dysfunctions. PIMS has been hypothesized to be the rheumatic fever of the 21st century based on the similarities between the pathogenesis, symptoms and course of both diseases. This unexplained syndrome also showed some clinical similarities with other inflammatory illnesses, such as Kawasaki disease and Toxic Shock Syndrome. However, it was subsequently defined as a separate condition, referred to as pediatric inflammatory multisystem syndrome temporally associated with COVID-19 (PIMS) in the UK and multisystem inflammatory syndrome in children (MIS-C) internationally. Unlike Kawasaki disease, which tends to affect children below the age of five years, PIMS generally affects children of school age. A high proportion of patients are male and usually do not have any major underlying health conditions. There exists a relationship between the cases of COVID-19 and PIMS, alongside the delayed onset of PIMS symptoms in patients who are SARS-CoV-2 negative but show evidence of prior exposure. This supports the theory that PIMS is a post-infectious complication. The spectrum of severity of PIMS ranged from standard hospitalization to pediatric intensive care unit management. No specific diagnostic tests for PIMS exist and are largely based on symptomatic manifestation, blood tests and other medical investigations.

Treatment is symptomatic and patients are managed by a multidisciplinary team comprising pediatric cardiologists, immunologists, rheumatologists and critical care specialists. There is a lack of data regarding treatment; however, current therapies include anti-inflammatory agents (such as intravenous immunoglobulins and corticosteroids) and remedies targeting specific inflammatory cytokines.

Conclusion: PIMS is a new and rare systemic inflammatory disease that mainly affects children including those infected with COVID-19. More data is becoming available, however there is also an evident gap in literature regarding risk factors and long-term impacts for patients with PIMS. Research addressing some of these issues will improve not only the timely diagnosis of children with PIMS but also the treatment approach.

Keywords: Pediatric Inflammatory Multisystem Syndrome, PIMS, Kawasaki Disease, acute rheumatic fever, SARS-CoV-2, Hyperinflammatory shock

A. P. KAPLAN, K. JOSEPH, B. GHEBREHIWET

COVID 19 AND THE BRADYKININ-FORMING CASCADE

SARS-Coronovirus-2 is the cause of a worldwide pandemic, which to date, has killed 6 million people. The main cause of fatalities is a severe pneumonia leading quickly and profoundly to an acute respiratory distress syndrome with severe hypoxia. There is evidence of release of a multitude of inflammatory cytokines, activation of complement, particularly via the alternative complement pathway, and a dramatic activation of both pathways for bradykinin formation; namely, the extrinsic or tissue kallikrein pathway, and the intrinsic or factor XII-dependent pathway of plasma. The tissue kallikrein pathway consists of 15 homologous gene products, 3 of which can produce bradykinin (KLK1, 2, and 12). KLK 1 is he most prominent and is found in lung, pancreas, kidney, and salivary glands. When released it cleaves low molecular weight kininogen (LK) to release lysyl-bradykinin. A plasma aminopeptidase removes the N-terminal lysine to produce bradykinin. Bradykinin induces fluid leakage and, in the lung, a pulmonary edema-like state, by interaction with B-2 receptors along small venules. The plasma pathway involves sequential activation of factor XII, conversion of prekallikrein to kallikrein, and cleavage of high molecular weight kininogen (HK) to release bradykinin. It is of interest that circulating free RNA is an activator of factor XII-dependent bradykinin formation, and SARS-Coronavirus-2 is an RNA based virus.

The products of these two bradykinins forming cascades are lys-bradykinin and bradykinin. One step in their degradation is to remove C-terminal arginine which produces des-arg^{10} lys bradykinin or des-arg^{9} bradykinin, respectively, and these are not yet inactive. They both interact with the B-1 "bradykinin" receptor and can perpetuate vascular permeability until there is further degradation by angiotensin converting enzyme (ACE). This step also seems to be relevant for COVID 19 pathogenesis.

The virus binds to lung alveolar type II epithelia cells by attaching to angiotensin-converting enzyme-2 (ACE-2) expressed at the cell surface via its viral spike protein. A transmembrane serum protease (TM PRSS2) activates the viral spike protein and enables cell entry. Studies of gene expression in bronchioalveolar lavage specimens of patients with COVID 19, when compared with normal control specimens, reveal upregulation of multiple components that lead to bradykinin formation and downregulation of factors that control the process.

All kallikreins (tissue, plasma) and kininogens (LK and HK) are upregulated, the B-2 receptor is increased over 200-fold, and the B-1 receptor over a thousand-fold. C1 INH, the critical plasma control protein is downregulated which would render the plasma cascade labile and overreactive. ACE is decreased 8-fold so that bradykinin and des-arg$^{10/9}$ bradykinin would not be inactivated normally. Although viral binding to ACE-2 limits its enzymatic activity, ACE-2 has relative specificity for inactivating the des-arg degradation products i.e. those reactive with the B-1 receptor. Altogether a "bradykinin storm" result. Initiation of the plasma cascade can be induced by viral RNA however 4 separate viral proteins tested as recombinant entities all bound factor XII and HK and activated the plasma bradykinin-forming cascade including the spike protein, a nucleocapsid protein, membrane protein M, and an envelope protein.

Finally, antagonism of the B-2 receptor has been shown to reduce SARS-CoV-2 replication in bronchial epithelium which may be a feedback control mechanism, thus therapy with Icatibant could be helpful not only to antagonize permeability effects of bradykinin, but also to retard viral replication. Lanadelumab, a monoclonal antibody inhibits plasma kallikrein which lasts two weeks per injection and could also be beneficial. While immunization has focused on the spike protein needed for cell entry, the three additional proteins noted above that activate complement as well as the bradykinin-forming cascade could be additional targets that could increase the effectiveness of immunization by broadening the anti-viral response.

Keywords: Covid-19, bradykinin, cytokines

A. P. KAPLAN

THE BRADYKININ-FORMING CASCADE IS ACTIVATED IN ANAPHYLAXIS AND IS MAST CELL DEPENDENT

Anaphylaxis includes a multitude of possible symptoms including urticaria, peripheral angioedema, asthma, laryngeal edema, and hypotension among those that are typical. The most common and best studied causes of anaphylaxis are allergy to foods, drugs, and insect stings. Two of the elements that are a cause of anaphylactic fatalities are profound hypotension and laryngeal edema. It is of interest that laryngeal edema is associated with all of the causes of angioedema that are mediated by bradykinin e.g. C1 inhibitor deficiency, angioedema due to ACE inhibitors or angioedema due to factor XII mutation. Further, should bradykinin levels be elevated in the arterial circulation rather than being confined to the venous side of the circulation, it is a cause of hypotension that is proportional to the bradykinin level. When severe allergic reactions proceed, among the released constituents is mast cell heparin, a form of heparin that is over-sulfated relative to the heparin used for anticoagulation, and is an activator of factor XII, thereby initiating bradykinin formation. A second factor XII activator that can also be secreted is polyphosphate polymers. In rodent systems, mast cell heparin induces hypotension and increases vascular permeability each of which is abrogated if the heparin is de-sulfated i.e. is no longer capable of activating factor XII. In a mouse model of allergen-induced anaphylaxis, there is release of mast cell heparin, thereby

anticoagulating the blood, with cleaved HK and elevated bradykinin levels. Mice were protected from hypotension if the B-2 receptor is deleted or of there is a homozygous null mutation of factor XII. Studies of human anaphylaxis in 10 patients (Sola-Cunill A, et. al. JACI 2015;135:1031-1043) demonstrated high molecular weight kininogen (HK) cleavage that was proportional to the severity of the reaction with diminished levels of native factor XII and prekallikrein, all consistent with activation of the bradykinin-forming cascade.

Keywords: The Bradykinin-Forming Cascade, Anaphylaxis, Mast Cell

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CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF SARS-COV-2-INDUCED HYPERCOAGULABLE STATE (A RETROSPECTIVE STUDY)

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Introduction. SARS-CoV-2 has shown a great propensity to produce hypercoagulable state and increase the risk of thrombosis/embolism in infected individuals. We aimed to study the prevalence of this complication, the mortality rate, clinical and epidemiological characteristics in patients with COVID-19.

Methods. All cases of the patients that were diagnosed with COVID-19 and hospitalized in the First University Clinic of TSMU during the year 2021 have been reviewed retrospectively. The patients that developed thrombosis/embolism while being in hospital were chosen to assess the demographic characteristics, hospitalization periods, underlying comorbidities, and the mortality rate.

Results. Out of 4868 cases 71(1.458%) were complicated with thrombosis or embolism. 50.7 % of the patients (n=36) were male, 49.29% (n=35) female. Age range was 26 to 92, mean age 64.9 (SD 14.128). The duration of hospitalization period varied between 1-39 days (SD 120). Pulmonary embolism developed in 88.73 % of the patients (n=63), lower extremity thrombophlebitis in 11.26 % (n=8), stroke in 7 % (n=5) (4 in the precerebral arteries, 1 in the cerebral arteries), acute myocardial infarction in 2.8 % of the patients (n=2), intracardiac thrombus was detected in 2.8 % (n=2), 2.8 % (n=2) had lower extremity arterial thrombosis, 1.4 % (n=1) portal vein thrombosis, 1.4% (n=1) –unspecified venous thrombosis/embolism. Majority of the patients had underlying comorbidities: 71.8 % (n=51) - primary (essential) hypertension, 46,5%(n=33) - MV/TV/AV insufficiencies, most of them had combined lesions of more than one valve. 30.9% (n=22) had type II Diabetes Mellitus, 22.5 % (n=16) - atrial fibrillation/flutter, 21.1 % (n= 15) - obesity, 19.7% (n=14) - heart failure, 15.5% (n=11) - pre-existing defects of coagulation cascade. 11.3% of the patients (n=8) had a history of chronic ischemic heart disease or myocardial infarction, 7 % (n=5) - chronic bronchitis, 5.6% (n=4) -conduction disorders of the heart, 5.6% (n=4) - previous coronary intervention, 2.8 % (n=2) - stroke, 2.8 % - emphysema, 2.8% - interstitial lung disease/pulmonary fibrosis, 2.8 % - chronic kidney disease, 2.8 % - liver insufficiency/chronic viral hepatitis, 2.8 % - underlying malignancy, 1.4 % (n=1) - primary pulmonary arterial hypertension. 1 patient was pregnant, 3 patients had concomitant bacterial pneumonia. Mortality rate was 45% (n=32).

Conclusion. Thrombosis often with subsequent embolism (especially in pulmonary artery) is one of the most concerning complications of COVID-19, characterized by high mortality rate. Patients with comorbidities and underlying hypercoagulable state are at higher risk of developing this complication.

Keywords: SARS-CoV-2, Thrombosis, mortality rate, comorbidities.

O. KONIASHVILI [1,2], L. GABUNIA [3], M. ROBAKIDZE [1], G. KHACHIASHVILI [1], A. MAGHALASHVILI [1]

THE DEVASTATING EFFECT OF POLYPRAGMAZY IN HOSPITALIZED PATIENTS WITH COVID-19 – A RETROSPECTIVE STUDY

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Introduction. Polypragmazy is defined as the use of five or more medications on the daily basis. It is quite common in older individuals and the population, suffering certain chronic conditions. Exposure to Polypragmazy is inevitable in certain conditions, considering the severity of the illness, however, the probability of pharmacokinetic and pharmacodynamics interactions is worrisome. Hereby we aimed to investigate the correlation between Polypragmazy and clinical outcome in hospitalized patients with SARS-CoV-2 infection.

Methods. We gathered information on 300 patients retrospectively and assessed the correlation between Polypragmazyand the outcome of the disease. All of the selected individuals were hospitalized due to SARS-CoV-2 infection. The 10th revision of the International Classification of Diseases (ICD-10) was used in the medical services and hospitalization databases.

Results. Of the selected 300 patients, 197 had experienced prescription 5 or more drugs (Polypragmazy). Antipsychotic medicines were linked to severe COVID-19 morbidity and an elevated risk of mortality in individuals infected with the virus. Antibiotics were provided to 150 patients (76,14%), anticoagulants to 54 (27,41%), aspirin to 42 (21,3%), clopidogrel to 10 (5,07%), hydroxychloroquine to 17 (7,6%), corticosteroids to 156 (179,1%), and supplements to 123 (62,4%). Nearly one in every seven people (n=28) were exposed to potentially harmful drug-drug interactions, with increased bleeding risk and QTc prolongation being the most prevalent effects. Hydroxychloroquine in combination with either ritonavir/lopinavir can prolong QT interval and cause ventricular arrhythmias (Torsade de Pointes) other than upper mentioned medications, tramadol, rofecoxib, diltiazem, piperacillin/tazobactam, isoniazid, clarithromycin, and furosemide can cause drug-drug interaction with potentially harmful effects. Although Hydroxychloroquine has few drug interactions with antithrombotics (with exception of apixaban, dabigatran, rivaroxaban), other extensively mused medications (LPV/r) can increase blood levels of apixaban, rivaroxaban and ticagrelor (high risk).

Conclusion. Polypragmazy and certain medication classes have been linked to a higher risk of negative clinical outcomes in COVID-19 patients when taken in combination. Future study is needed to determine which portion of the relationships is the result of iatrogenic dangers particular to the drugs used, and which part is the result of the severity of the disorders that have conditioned the use of numerous medications. Future study is needed to determine which portion of the relationships is the result of iatrogenic dangers particular to the drugs used, and which part is the result of the severity of the disorders that have conditioned the use of numerous medications.

Keywords: Polypragmazy, COVID-19 patients, Torsade de Pointes, iatrogenic diseases.

M. JOSHI [1], P. S. PERERA [1], N. H. KORRAPATI [1], N. GAMKRELIDZE [2]

PATHOGENESIS OF COVID-19 INDUCED PAROTITIS (REVIEW OF THE LITERATURE)

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Background: By the time COVID-19 was declared a pandemic by the WHO, medical personnel were aware of the main clinical manifestations affecting the respiratory tracts. However, minor manifestations such as sialadenitis, mainly of the parotid glands (referred to as parotitis), later emerged as more cases were reported and thorough research was conducted.

Methods: Google Scholar, PubMed and Research4life were used for searching the articles. Appropriate filtering was applied in all the databases for the material that included a publication date within 2 years, keywords such as 'parotitis', 'sialadenitis', and 'COVID-19' and in the English language. The resulting articles were screened and those pertinent to the topic were analyzed. The references used in them were also examined to aid us with any further relevant material.

Discussion: With the progression of the COVID-19 pandemic and millions infected so far, there is a rise observed in atypical manifestations of the infection, one being parotitis. Salivary glands are sites of immune privilege and act as repository for SARS-COV-2. Salivary gland ductal epithelia and serous acinar cells show large expression of ACE2 receptors acting as an entry point for SARS-COV-2. Viral transmission into the salivary glands most commonly occurs mainly via a hematogenous spread.

Persistence of infection in salivary glands leads to inhibition of antibody production and increased risk of reinfection. Residual damage of the oral cavity persists in the vast majority of patients after the infection has seemingly cleared, suggesting that the oral cavity is a preferential target for SARS-COV-2 infection.

Salivary gland ectasia has been observed upon histological analysis and intraparotid lymphadenitis, temporomandibular joint abnormalities, facial pain, and masticatory muscle weakness are also seen to occur along with parotitis.

Conclusion: Due to the elevated emergence of parotitis in covid patients and its possible severe complications, its inclusion as a clinical characteristic of COVID-19 rather than a minor manifestation has now become increasingly important. More investigation should be conducted for understanding mechanisms of COVID-19 parotitis and proper treatment should be targeted to ensure complete patient recovery.

Keywords: Parotitis, COVID-19, atypical manifestation, sialadenitis, parotid glands.

N. KIKVADZE [1], G. GORGADZE [1], SH. KHETSURIANI [2]

INCREASED RISK OF COVID-19 COMPLICATIONS DURING PREGNANCY AND POSSIBLE MECHANISMS OF VERTICAL TRANSMISSION

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The emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused unprecedented crisis over the world since beginning of 2020. Pregnant women are among very sensitive population that require particular attention during emergencies and infectious diseases. Previous studies revealed that pregnant women with different viral respiratory disease were at high risk of developing obstetric complications and adverse perinatal outcomes related the altered body habitus, physiology and immune-suppressive state. Additionally, the Centres of Disease Control and Prevention (CDC) reported that pregnant women with COVID-19 were more likely to be hospitalized compared to non-pregnant at

an equivalent age (31.5% versus 5.8%) and nearly quarter of them develop pneumonia and have an increased risk of mortality up to 35%. The reduction in total lung capacity and inability to clear secretions can make pregnant women more susceptible to severe respiratory infections. Besides, the modulations of the maternal immune system during pregnancy, such a programmed cytokine switch from Th1 to Th2 profile is a major predisposing factor for COVID-19 infection. The pathophysiological events behind the increased risk of obstetric complications are basically the cytokine storm and the activation of circulating cells as macrophages, T lymphocytes and endothelial cells.

Research outcomes indicate extremely controversial arguments about possible mechanisms of vertical transmission of this infection. Placenta is an important, but not completely effective barrier for the vertical transmission of infections. Some clinical reports of confirmed neonatal infection have led to concerns of a potential mechanisms (direct infection of syncytiotrophoblast with subsequent transmission through the cytotrophoblast, also infection via trafficked maternal cells has been suggested, as evidenced by an observed expression of ACE2 protein in infiltrating maternal cells in human placentas with chorioamnionitis) for vertical transmission of COVID-19 infection. Alternatively, studies indicate that syncytiotrophoblasts are often infected with virus, but the fetus is not always infected. Study results demonstrated possible transmission via placenta through other alternative receptors (DPP4 and CD147) and proteases also. Neonatal infection can occur either through an ascending infection through the vagina and cervical canal/during vaginal birth via direct infant contact with maternal virus.

In conclusion, high-quality data revealed COVId-19 infection as key factor for complications during pregnancy. Future investigations are essential for understanding the possibility of SARS-CoV-2 virus vertical transmission.

Keywords: Covid-19, pregnancy, immunological response, vertical transmission.

N. H. KORRAPATI, N. K. AHMED, M. H. PERERA, L. GABUNIA

DERMATOLOGICAL MANIFESTATIONS RELATED TO COVID-19 (CROSS-SECTIONAL STUDY)

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Introduction: Numerous cases recording cutaneous manifestations related to the SARS CoV infection have thrown light on the importance of considering these manifestations as prominent Covid-19 symptoms.

Aim: to study dermatological symptoms related to covid-19 infection.

Methods: An observational study was carried out in the form of a detailed survey targeting participants with a history or ongoing Covid-19 infection in the past 8 months, inquiring on possible skin manifestations experienced.

Results: A total of 100 participants participated in this study, aged between 15 to 60 years with the mean being 25.83. 58.4% (n-59) were female participants and 41.6% (n-41) were male participants. While the majority, 70% (n-70) belonged to South Asian, Asian, and middle eastern descent, 30% (n-30) were Caucasian.

Results revealed that several participants had pre-existing skin conditions such as eczema 9.9% (n-10), psoriasis 2% (n-2), acne 21.8% (n-22), rosacea 2% (n-2), urticaria 2% (n-2) and 2% (n-2) had diabetes. 54 participants had covid between the months of December 2021 to March 2022, consistent with the rise in omicron cases. Fever, dry cough, fatigue, and headache were the most common symptoms the participants had. 70% (n-70) of the participants report using NSAIDs mostly for treating their symptoms.

Results showed, only 20%(n-20) had cutaneous manifestations out of which 5% (n-5) had Maculopapular rash, 4% (n-4) had Xerosis, 2% (n-2) had Pruritis, 1% (n-1) had Vesiculopapular rash, 3% (n-3) had Chilblain/pernio manifestations ,1% (n-1) had Erythema multiforme, 2% (n-2) had Livedo reticularis, 1% (n-1) had Purpuric vasculitis and 2% (n-2) had Urticarial rash. These manifestations, along with general symptoms, lasted for more than 3 days for 76.2% (n-16). Vaccination history showed 93.1%

(n-94) of the participants were fully vaccinated with a booster shot whereas, 65% (n-65) were vaccinated before their infection.

Conclusion: Maculopapular rash, Xerosis, Chilblain/pernio were the more common cutaneous manifestations observed in those infected with covid-19, which are thought to develop because of the high viral exanthem. These manifestations were more commonly seen among women 75% (n-15) of European and Caucasian descent 50% (n-10) possibly due to the increase in expression of hACE2 receptors in skin tissues having fewer melanocytes.

Cutaneous manifestations although not common can be seen more commonly in female patients aged above 30 of European and Caucasian descent when infected with covid-19 and should be considered as noteworthy symptoms.

Keywords: covid-19, sars-cov-2, dermatological symptoms, maculopapular rash.

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MANAGEMENT OF DIALYSIS PATIENTS INFECTED WITH COVID-19 (LITERATURE REVIEW)

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The global impact and emergence of Covid-19 disease (officially known as SARS-COV-2) is responsible for several symptomatic anomalies with regards to Human Health most of which are attributed to the Respiratory System, however greater insight is gained as time passes of a ripple effect endowed by Covid-19 upon other organ and organ systems more specifically conditions prompting the need for Dialysis.

Little over 2 years into the pandemic bestowed upon us by Covid-19 lies a greater understanding of how a multi-organ symptomatic effect is witnessed as an infection of Covid-19 is endured.

Essentially, several factors enabled by Covid-19 cause renal distress resulting in chronic kidney disease, some of which include targeting of kidney cells in particular due to cell receptor compatibility, lack of oxygen due to respiratory insufficiency, cytokine storms causing inflammation and blood clots that coagulate in the renal pathways amongst others actively being researched to understand the several ways Covid-19 affects Kidneys.

Patients with preexisting needs for Dialysis or those of whom must succumb to such measures, may it be during or along recovery phase from Covid-19, are accommodated with additional challenges to the physiological responses to not just Covid-19 alone but the trials of Dialysis itself and the strain the body endures as the treatment ensues. This implies the Edema, Shortness of breath due to fluid buildup and sanitary complications encountered in treating Patients requiring Dialysis in contrast to the symptoms observed by Covid-19 disease are correlative under several circumstances. Differentiating and accurately addressing said symptoms to a declining response to the Covid-19 infection or a side-effect of the Dialysis procedure is one of many obstacles encountered by Physicians.

Furthermore Covid-19 safety protocols and guidelines make Dialysis more demanding due to the aspect of traveling to areas of higher susceptibility or exposure to Covid-19 (Hospitals, Out-patient clinics, other patients), Expenses to cost of treatment due to greater sanitary control and monitoring, Shortage of Equipment due to greater inflow of patients suffering from renal complications due to Covid-19 leaving them short-staffed and ill equipped.

Conclusion: Providing a greater prospective through compiled literature to understand the renal complications induced by Covid-19, the procedural and diagnostic complications faced by physicians alongside hospital administrative departments. Solutions garnered over the course of adapting to Covid-19 to help control the influx of patients and alternative methods such as Peritoneal Dialysis over hemodialysis.

Keywords: SARS-COV-2, Cytokine storms, Dialysis, Kidney Damage.

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POSTCOVID RADIOLOGICAL CHANGES IN CHILDREN

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Although coronavirus infection is relatively mild in children, coronavirus can cause polyorgan damage later in the period, which we call postcoid complications. The postcoid period includes 4 weeks or more after disease transmission. Literary data on changes in the respiratory system as a result of prolonged covitis infection are scarce. According to radiologists in England, different types of symptoms lasted for 42.6% of patients 60 days or more after the onset of the disease. Prolonged course was observed in 12.9% of children aged 2-11 years and 14.5% in children aged 12-16 years.

Our aim was to detect radiographic changes in respiratory tract damage in both the severe and mild course of the disease in the postcoid period. We think sharing our data by combining radiological and clinical data will not be without interest.

We monitored 175 patients who came to us between September 2021 - September 2022, all of whom had a Covid infection and a negative PIS test during the adjustment period. Patients were conditionally divided into 3 groups.

1. A history of severe or moderate Covid pneumonia was reported and was considered cured. Patients in this group approached us with various types of non-respiratory complaints: general weakness, fatigue, drowsiness, etc. Half of the patients were included in this group, -80 patients, - 46%.
2. Patients who transmitted covid infection, and were left with respiratory changes; Cough, shortness of breath during exercise, respiratory failure- amounted to 28% 49 patients.
3. Patients who, according to the anamnestic data, had a temperature reaction during the visit to the clinic, but a negative PCAR test. In this group, 26% - 46 patients were found.

All patients underwent radiographic examination. Clinical status assessment CT scan did not require any of the patients. Patients ranging in age from 8 months to 17 years, patients with acute respiratory changes underwent radiographic examination upon admission to the clinic and on days 6-7 of the illness. Considering the clinical condition.

Patients who developed a severe form of Covid pneumonia and showed respiratory changes, the annoying cough was in most cases radiographically- Enhanced vascular image in the medial fields by thickening of the bronchial walls, the so-called donut sign. The low location of the diaphragm arches, consequently a decrease in the cardio thoracic index, which was the cause of abnormal changes in the cardiovascular system.

The postcoid period includes a period of more than 4 weeks. The main reason for the adjustment was coughing. Hyperpneumatization has been reported radiologically in all cases; Impoverished vascular image, unstructured root,. Lowering diaphragm arches, reduced cardio thoracic index of 0.4% or less.

In the case of acute clinical course, radiological changes developed in stages, Decreased pneumatization and infiltration of weak intensity appeared in the central and lower fields, unstructured vascular picture. Repeated radiological examination was performed on the 5th-5th day of the disease due to clinical symptoms. Infiltrative changes became intense, spreading to the periphery and basal segments.

The process was bilateral and asymmetric, with predominantly unilateral injury. Although complete healing was not possible, radiographic changes persisted for 4 weeks and longer in the disease.

The process was bilateral and asymmetric, with predominantly unilateral injury. These X-ray changes were not observed in all patients. Some patients did not show any abnormal changes on the radiograph despite the severe clinical course. Different radiographic picture is found in children aged one year, in case of prolonged and / or temperature reaction, low-transparency radiographic picture included root area and central fields in the form of fine-grained infiltrates, e.g., Graund glass syndrome. X-ray

changes were reversed more slowly than in adults. Unfortunately, due to the small amount of time we have no results in the data period after 2 months,

Conclusion: In the case of postcovid syndrome, radiological changes do not always indicate a severe course of the disease. In the case of clinical recovery, radiological changes are manifested in the 4th week of the disease and for a longer period. X-ray examination should be performed based on clinical symptoms.

Radiological changes are manifested in the root area and central fields, in the form of small transparencies infiltrates of low transparency, in contrast to other types of viruses changes continue in the postcovid period. Severe course of the disease does not always mean detection of postcovid syndrome and vice versa. In the case of a mild course of the disease it is possible to develop different degrees of polyorgan damage.

Keywords: Covid-19, postcovid, radiological changes, children

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CORONAVIRUS AND STROKE

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Scientists have identified a sharp increase in the incidence of stroke among younger patients compared with the same age group before the pandemic. According to studies by American scientists, the incidence of vascular complications in the form of a stroke in patients with COVID-19 is eight times higher than in patients with influenza (1.6% and 0.2%, respectively). Strokes occur in perfectly healthy people. Among 800 patients of the Batumi Medical Center, there were -102 (12.75%) patients diagnosed with stroke (ischemic and hemorrhagic), Coronavirus was detected in them only after they were admitted to the hospital with a stroke. After suffering from covid for 2 weeks to 3 months 570 patients aged 18-90 years old came to the clinic with neurological complaints, among them with a diagnosis of stroke - 115 (20.17%), among them vertebrobasilar arterial syndrome, the transient cerebral attack was recorded - in 148 (25.96%) patients.

We have studied 2 cases of ischemic stroke in male patients aged 18 and 22, athletes. In anamnesis, they denied chronic diseases, drinking and smoking, and a family history of stroke. In one of them, an 18-year-old boy, on the 40th day after Covid, a decrease in strength in the arm and leg began against the background of high blood pressure, after which a left-sided hemi syndrome developed. Radiological examination revealed an acute ischemic disorder in the right hemisphere. According to clinical and laboratory data, the D value of the dimer was high. (D dimer increased by 20 times). And no pathology was detected in the study of the vessels of the brain and heart.

A second case is a 22-year-old man. an athlete who does not smoke does not take alcohol and denies any chronic disease in anamnesis. A month after the covid, numbness and disorientation began. A radiological examination of the brain revealed an acute ischemic disorder in the frontal lobe, no disorders of the cardiovascular system were detected. According to clinical and laboratory studies, inflammatory markers - procalcitonin and C reactive protein - were sharply increased in the blood. And so, during the course of COVID-19, endothelial functions are disrupted - increased blood clotting is observed and blood clots form and strokes develop.

Studies have shown that it is increased blood clotting that causes Post-Covid Syndrome. Judging by the tests, blood clotting markers in those who have had COVID-19 remain at a high level for a long time, although there are no signs of inflammation. People between the ages of 18 and 30 are hospitalized with brain damage a few months after recovering from an infection. In the future, we recommend testing for COVID in all young stroke patients, especially those with no pre-existing known conditions. Stroke is considered the most severe consequence of the coronavirus.

Keywords: covid-19, stroke, post-covid syndrome

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CONGENITAL TUBERCULOSIS ASSOCIATED WITH MATERNAL MILIARY TUBERCULOSIS

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Goal of the research: In the countries of high tuberculosis prevalence morbidity of women of reproductive age is quite high, with increasing risk of development of this disease in the period of pregnancy and hence, with the risk of congenital tuberculosis in the newborns.

In the infants and especially in the newborns tuberculosis infection is characterized with short incubation period and rapid progression. Therefore, the children of this age are subject of high risk and consequently, tuberculosis diagnosis and timely treatment are of critical significance.

Symptoms of the disease are non-specific that makes early diagnosis complicated and therefore, it requires high suspect to be considered. The symptoms include: respiratory deficiency, fever, hepatomegaly, low birth weight, lethargy or excitability.

Clinical form of congenital tuberculosis corresponds to the way of infecting of the fetus: 1. at a time of trans-placental infection to primary tuberculosis complex in the liver, and enlarged lymph nodes can compress the common bile duct, causing obstructive jaundice. 2. In case of infection via aspiration the disease develops as a type of bacterial pneumonia. 3. In case of oral way of infection – the abdominal tuberculosis, mesenterial lymphatic nodes or tuberculosis enteritis is developed.

This report summarizes the investigation of the case in Georgia. Two-week-old girl was delivered to our clinic. She was vaccinated by BCG vaccine. She had respiratory distress, hyperthermia, hepatomegaly, jaundice. 2 weeks before delivery, the mother was diagnosed by miliary tuberculosis. According to the result of conducted tests (chest x-ray examination, Mantoux skin test, examination of bronchial lavage for tuberculosis mycobacteria), the patient was diagnosed by miliary tuberculosis, right lung primary tuberculosis complex, left paratracheal lymph node tuberculosis. She received treatment with 4 tuberculosis medications during 4 months in the clinic and further outpatient treatment lasted for additional 6 months. As a result of conducted treatment, the patient was fully recovered.

Keywords: Congenital tuberculosis, miliary tuberculosis

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ALLERGIC PATHOLOGIES OF THE RESPIRATORY SYSTEM ASSOCIATED WITH TOBACCO DUST EXPOSURE

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In the enterprises of the tobacco industry, new progressive technological methods for processing tobacco and high-performance aggregates, automated flow lines are being widely introduced, and the main manufacturing processes are being mechanized. All this leads to the improvement of the safe and hygienic working conditions of the people employed in various manufacturing phases of tobacco processing.

Accordingly, the interest in the sensitizing effect of tobacco dust on the respiratory system is also increasing, as in previous years, in the cases under such conditions, when a high concentration of chemicals (including tobacco dust) was detected, main attention was paid to their toxic effects, which, as we know, are revealed much earlier than allergic effects.

According to the existing data, tobacco dust causes hypertrophic and atrophic rhinitis and pharyngitis, as well as chronic bronchitis. Data on allergic respiratory lesions in persons exposed to tobacco dust are fragmentary and contradictory. The workers of tobacco-fermentation plants, who are under long-term continuous exposure to tobacco dust, develop catarrhal lesions of the upper respiratory tract (rhinitis, pharyngitis).

In the clinical analysis of cases of chronic bronchitis among the workers of this occupational group, it is possible to distinguish 4 main forms of the disease (revealed in some individuals in various ways): 1. Chronic dust bronchitis; 2. allergic bronchitis; 3. Chronic bronchitis "smoker"; 4. chronic infectious bronchitis.

Occupational allergic diseases of the respiratory organs (mainly bronchial asthma) in tobacco fermentation enterprises are also observed among experienced workers and are characterized by asthma attacks precipitated by tobacco dust.

We found that among tobacco manufacturing workers with a long working experience there is a high number of cases of bronchial asthma, asthmatic bronchitis, allergic dermatitis, urticaria, and eczema.

The possibility of a differential clinical assessment of the pathology of the respiratory system in workers engaged in tobacco cultivation and workers in tobacco manufacturing is the basis for successful therapy and the development of a rational complex prophylaxis for the prevention of these diseases.

Keywords: tobacco, dust, respiratory system, workers.

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THE IMPACT OF THE COVID PANDEMIC ON LIFESTYLE OF PEDIATRIC POPULATION

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Background: The ongoing Covid-pandemic has put our life routine in the face of many challenges, which undoubtedly reflect in the daily lives of schoolchildren in more than one way.

Aim: The aim of our research was to collect and analyze data to find out the changes and problems with the learning process, daily routine, emotional status, lifestyle, and duration of using computer technologies in children. Also, to find out if all these changes correlate with a specific age category.

Methods: We conducted a Google Forms 4-part survey, covering different aspects of children's lives including: Lifestyle, Productivity, and Psycho-emotional State. Qualitative, plausible answers and open-ended questions were used. Also, open columns were provided for additional comments. The questionnaire was completed by parents and older family members of adolescents. The data was processed with SAS (Statistical Analysis System).

Results: From 430 participants, 50% report an increase in time of using electronic devices from 2 to 6 hours. 67,7% confirm a significant reduction in physical activity with no apparent weight gain. 9.3% complain of impaired vision. 60% report a decrease in motivation to study and 36,7% state a considerable decline in productivity.64,4% manifest signs of aggression, while 32,3% seem to be depressed to their parents. The degree of motivation, involvement and concentration in the lesson process by more than 50% is assessed with an average score. Surprisingly, 26.3% rate the quality of learning as the highest. 61.9% of family members state that it takes a lot of effort to involve an adolescent in the learning process. Only 8.6% of the respondents mentioned the severity of the relationship problems with the teenager's peers.

Discussion: As it turned out, most parents point out a lot of problems. There is a correlation between the degree of sleep disturbance and the increase in excitability, which is logical and is caused by nervous system tension and lack of rest. There may also be an increase in a depressive background and self-loathing in the relationship. Thus, it is logical for parents to be dissatisfied with online learning.

Conclusion: Persistence of contemporary circumstances has a negative effect on the children population's mental health and development.

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Keywords: Covid-pandemic, mental health, lifestyle, schoolchildren.

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CASE REPORT: PEDIATRIC PATIENT WITH LICHENOID RASH – CLINICAL AND MORPHOLOGICAL CORRELATION

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Introduction: PLEVA – Pityriasis lichenoides et varioliformis acuta is a rare inflammatory skin condition with various clinical manifestations. The exact underlying cause is unknown. It may last for a few weeks to years, and may getting better and worse before going away on its own. Timely diagnosis helps us to avoid unnecessary examinations and none useful treatment. Therefore, it's very important to know clinical manifestation of PLEVA and choose the rational methods for diagnostic.

Case: A 9-year-old boy attended our clinic with a history of 2-month eruption on the skin with mild itching. Initial rash – a lot of reddish macular eruptions with a single small blister. He was treated with antihistamines, glucocorticosteroid creams with mild improvement, and then with system glucocorticosteroid with some exacerbation on the whole body excluding a head. It was done various clinical and laboratory examinations, without any results. Physical findings – the trunk and extremities were covered by the erythematous–to–reddish or purpuric round/ovoid shiny papules, 5-8mm in diameter. Some papules were presented as a vesicular and pseudo vesicular summit evolving to a central necrosis and a hemorrhagic crust, without manifestation of systemic signs. To prove our suspicion on PLEVA we referred the patient for skin biopsy with morphologic examination. Pathomorphology: The granular layer was kept. There was parakeratosis, most part of keratinocytes were necrotized. Superficial layer of epidermis contained neutrophils in form of microabscesses. Pronounced hydropic degeneration, tense lichenoid infiltration in the dermo-epidermal area, also revealed extravasates, small areas of pseudoepithelial hyperplasia, CD8 - positive. Skin rash was decreased in 2 weeks, without any medications, just skin care products for sensitive skin and in 2 months absolutely disappeared.

Conclusion: Resistant reddish macular-papular eruption with vesicles, is a condition for suspicion on PLEVA. That's why it is not necessary to look for the various causative factors or associated disorders besides of lymphoproliferative diseases. As it lasts for months and even years and mainly doesn't have the specific treatment, it's difficult to assure patient and/or their parents not to do a lot of none useful examinations; for proving of diagnosis is preferable to do morphological examination of skin and for treatment use just symptomatic therapy.

Keywords: PLEVA, lichenoid rush, pediatric patient, case report.

E. NIKOLEISHVILI, M. KATCHARAVA, M. DARAKHVELIDZE

ONLINE PHARMACY DURING THE COVID-19 PANDEMIC, REALITY AND PROSPECTS

School of Health Sciences; University of Georgia; Georgia

The opportunity to purchase medicines online has been around since the 1990s. It has become especially relevant today when the whole world is fighting COVID-19 disease.

The Public Health Centers made the decision to announce social distancing and quarantine for preventing the spread of COVID- 19. After, during few months the use of online pharmacy has been increased in the world including in Georgia as well.

Trends of use of Internet pharmacies are observed not only in developed countries such as the United States, Canada, the United Kingdom, etc. but also in developing countries. The obvious advantages of buying medicines on the internet are buying the medicine without leaving home, bringing it to the desired time and place, and keeping it confidential if necessary.

Online pharmacies have been in operation for more than two decades. Research in various countries has revealed a lack of awareness about the purchase of medicines by online pharmacies. However, the attitude of most of the respondents towards the online pharmacy turned out to be positive. The online pharmacies made available not only medicines but also cosmetics and care products, baby foods, medical equipment, etc.

Today, the use of online pharmacies is facing challenges everywhere, implying the misuse of its services and capabilities. Even in countries with well-developed pharmacovigilance systems, many cases of misuse of online pharmacies have been reported. The results of research show, it is quite difficult to control unlicensed, illegal online pharmacies, their functioning endangers the health of the patients and their financial condition. The biggest threat to modernity is the misuse and drug abuse of opioids, tranquilizers, sleeping pills, and others. Unlicensed (illegal) online pharmacies can be considered a special threat in this direction.

The advantages of working in online pharmacies are the ability to store and analyze a large amount of data across the country about the need and turnover of medicines; It is also possible to sort the data according to the age of the patient and the nosology of disease, the use of which will be very useful when planning public health programs.

Of course, along with the development of e-health and telemedicine, the prospect of online pharmacy is huge. Successful online pharmacy promotion requires the licensing of online pharmacies and employed pharmacists, the protection of drug safety, and raising public awareness.

In many cases, online pharmacy owners themselves are also pharmaceutical manufacturers, which helps to reduce the price of the drug and, consequently, increase the availability. Online sales of medicines in Georgia are provided by pharmacies PSP and Aversi.

The Department of Pharmacy at the University of Georgia is providing the research to assess the level of awareness of online pharmacy among university students and academic staff. The results of this research will be helpful to raise the level of awareness among the students and academic staff of the University regarding the advantages and disadvantages of the online pharmacy; Also, will assess the regulations that govern the sale and use of medications during COVID-19 and impact on public health.

Keywords: E-health, Online Pharmacy, Pharmacovigilance

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L. KVACHADZE, D. BOLKVADZE, M. LOLADZE, L. KAVTARADZE

SOME SEROLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF ANTISTAPHYLOCOCCAL POLYCLONAL IMMUNOGLOBULIN

G. Eliava Institute of Bacteriophages, Microbiology and Virology

The purpose of the work is studying serological and biochemical characteristics of antistaphylococcal polyclonal immunoglobulin. The first stage of the work was dedicated to studying of staphylococcal strains for their use in the form of immunogens. From 102 bacterial cultures from various clinics of Georgia, 30 strains were selected with stable characteristics (Staphyl.aureus – 24, Staphyl.epidermidis – 6). Culture and morphological features, enzymatic activity, susceptibility to novobiocin and staphylococcal bacteriophage. In addition, the ability to synthesize microcapsule, proteolytic and hemolytic characteristics were also studied.

The following immunogens were prepared for immunization of animals-producents (a goat of local breed) - heat-inactivated staphylococcal culture (5 bn/ml), anatoxin-an absorbed, PV leukocidin, bacterial hyaluronidase. The above-mentioned immunogens were administered subcutaneously, in the

shoulder area, in increasing dose. After fifth and sixth injection of antigens into the animals-producents, blood samples were taken from the jugular vein and hyperimmune serum was separated. Using the method of serum ethanol fractionation (Chon et al.), the antistaphylococcal polyclonal immunoglobulin was obtained. Protective antibodies were observed in all series (C1, C2, C3). Antibodies against alpha-toxin in the Lh reaction reached 150 IU/ml; titer of antibacterial antibodies in the reaction of passive hemagglutination (RPHA) was 1:3200-1:12800; titer of anti-leukocidin antibodies in the RPHA – 1:640-1:2560; and titter against hyaluronidase - 1:320-1:640. In normal serums, RPHA values of the mentioned antigens were in 1:10 -1:80 range. The samples of normal serum and immunoglobulin were examined in the immunoenzymatic assay (IEA). Value of normal (control) serum in relation to alpha-toxin was 0,081. Positive index in relation to alpha-toxin was equal to 10,08. Positive index of antibacterial antigens was 2,3287, PV leukocidin - 4,3968, hyaluronidase - 0,9214. The following was used in IEA: Anti-Goat IgG (H&L) in rabbit Affinity Purified, Polysciences, Inc.exp.10.2022.

Optical density of immunoglobulin samples (C1, C2) at the temperature of 37^{O}C for 30 days compared to the temperature regime +4 ^{O}C, at the wavelength of 400 nm, was not significantly increased and was equal to 0,035 units, and at the wavelength of 540 nm increased only by 0,028 units. Obtained data confirm a thermal stability of the studied preparation. The similar data was obtained in the gel electrophoresis of immunoglobulin samples in the polyacrylamide gel. Obtained profiles of the samples, seasoned in various temperature regimes, were identical, which confirms a thermal stability of the studied preparation. Series of immunoglobulin were studied in vitro in terms of sterility and in vivo on laboratory animals, in accordance with the requirements of the European Pharmacopoeia. The results showed that immunoglobulin is sterile, atoxic, apyrogenic, harmless (areactogenic). Therapeutic features of immunoglobulin were studied. Experiments on white mouses showed that the preparation with the activity of 10 IU/ml protects test animals from Dcl (definitely a lethal dose) of the culture and toxin with 90-92%. Control mice that received only similar dose of the culture and toxin, died with 100% during 24 hours. The work is ahead for enzymatic (pepsin) treatment of molecules of protection antibodies and obtaining of F(ab') 2-fragmnets of antibodies, studying their therapeutic and anaphylactogenic features.

Keywords: serological, biochemical characteristics, antistaphylococcal polyclonal immunoglobulin

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ავტორთა საყურადღებოდ!

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2. სტატიის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და უნდა შეიცავდეს ციტირებული ლიტერატურის სიას, ცხრილებს და გრაფიკებს. მიმოხილვითი და ზოგადთეორიული სტატიების მოცულობა უნდა შეთანხმდეს ჟურნალის რედაქციასთან.
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Page of Editors-in-chief

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გამოჩენილი ქართველი მეცნიერი და საზოგადო მოღვაწე. დიდი ანატომი. საქართველოში კლინიკური მორფოლოგიის ფუძემდებელი. თბილისის სახელმწიფო სამედიცინო ინსტიტუტის კურსდამთავრებული (1935). მედიცინის მეცნიერებათა კანდიდატი (1941). მედიცინის მეცნიერებათა დოქტორი (1949), პროფესორი (1953), საქართველოს მეცნიერებათა დამსახურებული მოღვაწე (1965), საქართველოს მეცნიერებათა აკადემიის აკადემიკოსი (1979). საქართველოს მეცნიერებათა აკადემიის ექსპერიმენტული მორფოლოგიის ინსტიტუტის დირექტორი (1959-2006), საპატიო დირექტორი (2006-2012). ჯილდოები: ღირსების ორდენი, ლენინის ორდენი, შრომის წითელი დროშის ორდენი, ხალხთა მეგობრობის ორდენი, საპატიო ნიშნის ორდენი. 300-მდე სამეცნიერო ნაშრომის, 9 მონოგრაფიის ავტორი.

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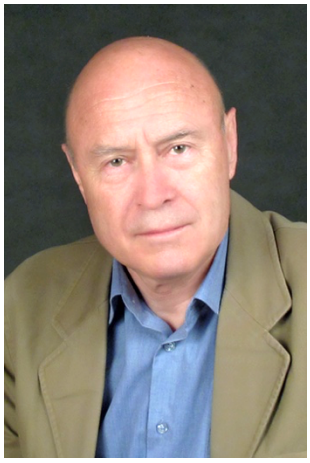

ბორის კორსანტია - მთავარი რედაქტორი 2013-2020 წლებში
გამოჩენილი ქართველი მეცნიერი, იმუნოლოგი. საქართველოში ვირუსოლოგიის ერთ-ერთი ფუძემდებელი. ვიტებსკის სახელმწიფო სამედიცინო ინსტიტუტის კურსდამთავრებული (1964). ლენინგრადის ექსპერიმენტული მედიცინის ინსტიტუტის ასპირანტი (1964-1967), მედიცინის მეცნიერებათა კანდიდატი (1967), ლენინგრადის სსრკ ჯანდაცვის სამინისტროს გრიპის ინსტიტუტის დოქტორანტი (1972-1975), მედიცინის მეცნიერებათა დოქტორი (1975), პროფესორი (1980), მედიცინის და ბიოლოგიურ მეცნიერებათა აკადემიის აკადემიკოსი. საქართველოს ექიმთა პოსტდიპლომური განათლების ასოციაციის დამფუძნებელი, ვიცე-პრეზიდენტი, კონფერენციების სამეცნიერო დირექტორი.
290 სამეცნიერო ნაშრომის და 5 მონოგრაფიის ავტორი.

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ექიმი დერმატოვენეროლოგი. თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის, კანისა და ვენერიულ სნეულებათა დეპარტამენტის ასოცირებული პროფესორი. თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის კურსდამთავრებული (2001).საქართველოს მეცნიერებათა აკადემიის ბიოტექნოლოგიის ინსტიტუტის ასპირანტი იმუნოლოგიასა და ალერგოლოგიაში (2001-2003), თსსუ დერმატო-ვენეროლოგიის რეზიდენტი (2002-2005).
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