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IMPACT OF HYPERGLYCEMIA ON ERYTHROCYTE'S MEMBRANE BAND 3 PROTEIN (BP3)

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T2DM remains one of the main causes of death and disability in the developed world. Approximately 9.3% of adults worldwide have diabetes. More than 50% of diabetics are undiagnosed and untreated leading to a risk of various complications: heart diseases, stroke, peripheral vascular disease, and microvascular problems [1]. T2DM is a chronic, progressive condition that causes hyperglycemia over a period of years as a result of a slow decline in β-cell function after chronic IR.

To prevent T2DM the pathogenic mechanisms of IR must be modified by slowing down, blocking, or reversing the dysfunction of the β-cells. Early diagnostic of carbohydrates metabolism disorders will make it possible to prevent the chronic complications of diabetes [2]. We investigated the erythrocytes membrane proteins in groups of patients with insulin resistance, prediabetes, and T2DM.

2. Material and methods

2.1 Patients. Blood was obtained upon signed informed consent from healthy volunteers, and also from patients with IR, prediabetes, and T2DM, who were admitted to the National Institute of Endocrinology (Georgia) from April 2022 to March 2022. All studies were carried out by the Helsinki Declaration. The study protocol was proven by the Ethical Committee for Human Studies of the Faculty of Medicine of Javakhishvili Tbilisi State University and the Multidisciplinary High School of the Society of Rheology (Tbilisi, Georgia).

Different patient groups were investigated:
1. Patients with IR (n=20, average age - 42±3.4 years);
2. Patients with prediabetes (n=20, average age - 48±2.4 years; no prior history of insulin resistance);
3. Patients with T2DM (n=20, average age 55±5.5 years) with an average age of the disease less than 1 year (without a previous record in the history of IR and prediabetes).
2.2 Erythrocytes Preparation. Blood samples were used after all clinical analyses were completed. Erythrocyte membrane isolation was performed by the Hast method [3].

Blood samples, collected in tubes containing anticoagulants were centrifuged at 3000g for 15 min. The obtained erythrocyte sediment was washed 3 times with a 1:4 volume of solution A, containing 130 μM KCl, and 20 μM Tris-HCl (pH-7.4). For hemolysis of the obtained erythrocyte sediment, the 1:10 volume of solution B, containing 5 μM Tris-HCl, and 1 mm EDTA, was added and the resulting mixture was left all night (for about 15 hours). The next day the suspension was centrifuged at 12,000 g for 20 min. The obtained precipitate was washed again with solution "B" 2-3 times before bleaching. The precipitate was washed again with a 1:10 volume of "A" solution.

The membrane protein content was quantified using the DC (detergent compatible) DC protein assay and was solubilized in Laemmli buffer [4]. Protein analytical electrophoresis was performed under dissociated conditions in a 12.5% gradient polyacrylamide gel with 1 mm thick and 6 ml volume with 0.1% sodium dodecyl sulfate SDS, by heating the samples for 10 min at 100°C and loading 20 μg of membrane proteins on an 8% gel for protein staining by colloidal 0.2% Coomassie Blue G-250 [12]. A set of standard proteins (kDa) as electrophoresis markers were used.

The data obtained by the B3p analytical electrophoresis method were analyzed with the texture analysis system (TAS plus, Leitz, Germany) which allows calculating quantitatively of the electrophoresis results.

2.3 Statistical analysis. An analysis of variance (ANOVA) (SPSS-12 for Windows) was used for the comparative analysis of the data.

3. Results

3.1. Bend 3 protein (B3p) expression level determination. Table 1 and Figure 2 show B3p (100 kDa) levels in erythrocytes from healthy volunteers and patients with IR, prediabetes, and T2DM. As seems from the data results, the level of B3p in the IR group statistically significantly did not differ from its level in erythrocytes from healthy volunteers, in patients with prediabetes, its content decreased by 24% in comparison to the level in the healthy volunteers and patients with diabetes iB3p level accounts 89% of healthy volunteers’ level.

Table 1. B3p levels in erythrocytes from healthy volunteers and patients with IR, prediabetes, and T2DM

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers (control)</th>
<th>IR</th>
<th>Prediabetes</th>
<th>T2DM</th>
</tr>
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<tbody>
<tr>
<td>B3p (kDa)</td>
<td>1,47±0.04</td>
<td>1.38±0.07</td>
<td>1.13±0.04</td>
<td>1.31±0.02</td>
</tr>
</tbody>
</table>

As follows from Figure 2, in patients with disorders of carbohydrate metabolism the expression of B3p (90-100 kDa) is reduced in comparison to the control level and appears the oligomerized form of B3p (oB3p) (180 kDa); the expression of the oB3p is maximal in patients with prediabetes.

3.3 Level of HbA1C in patients’ blood. Figure 3 shows alterations of HbA1C in patients with IR, prediabetes, and T2DM. As seems from this data in patients’ blood with IR and prediabetes HbA1C statistically significantly did not change in comparison to the control level; in patients with T2DM level...
of HnA1C statistically increased in comparison to the control level. Hb A1 is considered a standard test to monitor glycemic status.

![Graph](image)

**Figure 3.** HbA1C level in patients with IR, prediabetes, and T2DM

**Discussion.** B3p (90 - 100 kDa) is the most abundant integral protein of erythrocytes' membrane associated with several proteins of the cytoskeleton (spectrin, actin, band 4.2), playing a very important role in the regulation of the flexibility and rigidity of the erythrocyte's membrane [5].

As follows from the results of our studies, in patients with disorders of carbohydrate metabolism the expression of B3p (90-100 kDa) is reduced in comparison to the control level and appears the oligomerized form of B3p (oB3p, 180 kDa). The level of B3p in the erythrocytes from the IR patients group statistically significantly did not differ from its level in healthy volunteers, in patients with prediabetes, its content decreased by 24%, and in patients with T2DM - by 11% in comparison to the levels in healthy volunteers, while expression of the oligomerized form of B3p (oB3p) is maximal in patients with prediabetes.

There are two mechanisms of post-translational modifications of B3p, modulating its clusterization capability: glycosylation and tyrosine phosphorylation. Glycosylation appears to prevent oxidative cross-linking of B3p and therefore reduces its clustering ability. In contrast, tyrosine phosphorylation via phosphotyrosine kinases (PTKs) or phosphotyrosine phosphatase (PTP) [6], promotes oxidatively modified B3p clustering [20]. It has been proposed that B3p, as a redox sensor, is controlled by phosphorylation. Under oxidative stress conditions, rapid, intense Tyr-phosphorylation of B3p, sets off a series of events affecting the interaction of B3p’s with cytoskeletal proteins and triggering the membrane's resistance alterations, its destabilizing, and as a result, causing the erythrocytes hemolysis [7,8].

According to our earlier study results, in patients of all studied groups (IR, prediabetes, and T2DM) an increase in MDA content and a decrease in TAA level in blood serum were detected;

TAA was especially low in patients' blood with prediabetes, which indicates an exceptionally high intensity of oxidative stress in patients of this group [9]. Therefore, it seems that in the process of modification of erythrocyte membranes of patients with disorders of carbohydrate metabolism participate both, hyperglycemia and oxidative stress conditions associated with diabetes. Apparently, in patients with prediabetes prooxidant conditions (low TAA) initiate the tyrosine phosphorylation of B3p in erythrocytes membranes facilitating oxidatively modified B3p clusterization (oligomerization of B3p), whereas, in patients with T2DM in conditions of the especially high level of glycemia, glycosylation mechanisms prevail, which restrain oxidative cross-linking of B3p in erythrocytes (the oB3p is not detected).

It is possible to be suggested that: the high sensitivity of B3p to hyperglycemia and hyperglycemia-induced oxidative stress determines its responsibility for the alterations in erythrocytes' osmotic resistance and further hemolysis, as one of the first consequences of hyperglycemia.
References:

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ВЛИЯНИЕ ГИПЕРГЛИКЕМИИ НА БЕЛОК ПОЛОСЫ 3 (ВЗР) 
ЭРИТРОЦИТАРНЫХ МЕМБРАН

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РЕЗЮМЕ
Для того чтобы предотвратить развитие диабета типа 2 и его хронические осложнения, необходима ранняя диагностика нарушений метаболизма углеводов и их проявлений. Мы исследовали мембранные белки эритроцитов в группах пациентов с инсулинорезистентностью, предиабетом и диабетом типа 2.

Кровь была получена от здоровых добровольцев и пациентов с диабетом типа 2, предиабетом и инсулинорезистентностью. Содержание белка полосы 3 (ВЗР) в изолированных мембранах эритроцитов определяли с помощью метода аналитического электрофореза. ANOVA использовалась для сравнительного анализа данных.

У пациентов с нарушениями углеводного метаболизма экспрессия ВЗР (90–100 кДа) была снижена по сравнению с контрольным уровнем и проявлялась олигомеризованная форма ВЗР (180 кДа). Предполагаем, что в процессе модификации мембран эритроцитов пациентов с нарушениями углеводного метаболизма участвуют как гипергликемия, так и окислительные механизмы, обусловленные диабетом.
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SUMMARY

To prevent T2DM and its chronic complications early diagnostic of carbohydrates metabolism disorders is necessary. We investigated the erythrocytes membrane proteins in groups of patients with insulin resistance, prediabetes, and T2DM.

Blood was obtained from healthy volunteers and patients with T2DM, prediabetes, and IR. Band 3 protein (B3p) content in isolated erythrocyte membranes was determined with the analytical electrophoresis method. ANOVA was used for the comparative analysis of the data.

In patients with disorders of carbohydrate metabolism the expression of B3p (90-100kDa) was reduced in comparison to the control level and appeared as the oligomerized form of B3p (180kDa).

Therefore, it seems that in the process of modification of erythrocyte membranes of patients with disorders of carbohydrate metabolism participate both, hyperglycemia and oxidative stress conditions associated with diabetes.

Keywords: band 3 protein, erythrocytes, hyperglycemia