Antibody avidity in patients with different stages of type 2 diabetes mellitus in Azerbaijan Republic

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Aim: To evaluate some biochemical and immune parameters such as IgA, IgM, IgG, circulating immune complexes (CIC), phagocytic index, T-lymphocytes, as well as avidity of antibodies in type 2 diabetic patients. **Materials and Methods:** All patients (n = 57), depending on the level of glycemia and disease duration, were divided into three groups: in the stage of compensation, subcompensation and decompensation. As a biochemical marker, level of glycated hemoglobin (HbA1C) was also determined. Immunologic parameters such as IgA, IgM, IgG, CIC, antibody avidity were measured in patients and in control group including 20 healthy subjects. **Results:** Immunoglobulins and circulating immune complex values increased and the phagocytic number decreased in all three stages of the disease. Also, value of high-avid antibodies in diabetes mellitus (DM) patients decreased in 74, 52 and 27%, respectively, in the three groups, whereas in healthy subjects it is 95%. Conversely, increased amount of low-avid antibodies was observed in 26, 48, 73% of the patients, respectively (5% in control). **Conclusions:** Metabolic changes in patients with type 2 DM negatively affect the avidity of antibodies. A significant decrease of high-avid G class antibodies with abnormal conformation and low protective function occurs, which points to the disorders in protective function of B-system immunity.

KEY WORDS: Antibody avidity, circulating immune complex, diabetes

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Introduction

Diabetes mellitus (DM) remains as one of the relevant aspects of clinical medicine and public health, in view of the increasing number of patients in all countries of the world, as well as in Azerbaijan. According to the World Health Organization (WHO) report, there are 175 million diabetic patients in the world. Expert estimation of disease prevalence allows an estimation that by 2010, the number of diabetic patients will be more than 230 millions, and by 2025, this number will run up to 300 million and 80–90% of these patients will be type 2 diabetic patients.[1-5]

Recently, it has been considered that hyperactivity of B-system immunity is generally observed in type 2 diabetic patients. It manifests as a high value of B-lymphocytes, increased number of plasma cells, high level of antibodies (IgM, IgG, IgE) and rise in quantity of circulating immune complexes.[6] Some authors suggest that circulating immune complexes (CIC) concentration in blood has correlated relation with complement system, as well as with severity of the metabolic disease course.[7]

Materials and Methods

Clinical trials were carried out in the blood samples of 57 type 2 diabetic patients (21 men, 36 women). Type 2 DM was diagnosed based on the criteria of American Diabetes Association.[2] Most of the patients were under treatment in the self-supporting Endocrinological Dispensary, Baku. Antibody avidity as well as immunologic parameters in blood serum of patients was studied at the Institute of Bioorganic Chemistry named after M. M. Shemyakin and U. A. Ovchinnikov in Moscow. Based on the disease duration and glycemia, patients were divided into three groups: under 1 year [stage of compensation (n = 27)], from 6–10 years [stage of subcompensation (n = 12)], over 10 years [stage of decompensation (n = 18)].

Total protein was measured using biuret test. Albumin was determined with bromocresol. Commercially available kits (Human - Wiesbaden city, Germany

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Lachema - Brno city, Czech) were used for the determination of calcium. Creatinine and urea levels were measured by kits (Lachema, Czech).[8]

Value of T-lymphocytes was determined by the Jondal method and phagocytic activity of neutrophils was determined through their ability to absorb yeast cells.[9] The main classes of immunoglobulins (IgG, IgA, IgM) were measured by the method of Manchini.[10]

CIC concentration [CIC-enriched fraction, precipitated with 3.5% polyethylene glycol (PEG)] was determined by the method of Grinevich.[9] To 0.3 mL blood serum was added 0.6 mL of 0.1 M borate buffer (4.275 g borax, 3.410 g boric acid diluted in 1 L distilled water). This mixture was divided into two parts (0.3 mL each). To one of them, 2.7 mL borate buffer (control) was again added, and to the second part 2.7 mL PEG was added. The final concentration of PEG was 3.5%. After incubation at 4°C for 18–20 hours, the mixture was centrifuged for 15 minutes at a speed of 3000 rpm and CIC precipitation was separated. Optical density was determined by a spectrophotometer at a wavelength of 450 nm.

Plasma glucose was measured by glucose-oxidase method.[8] Glycated hemoglobin ($HbA1_C$) level was measured using tiobarbituric acid colorimetric method.[11]

To estimate B-system protective function, analysis of the conformational position of IgG molecule and antibody avidity was carried out. For this, IgG_1 was reduced from the blood serum of patients following diethylaminoethyl cellulose (DEAE) -sefarosa chromatography. The purity of preparations obtained was verified by polyacrylamide gel electrophoresis and Uchterloni radial immunodiffusion method using commercial IgG human anti-immunoglobulin antiserum, as well as by Edman N-region sequencing method of Fab and Fc. Fc fragment was obtained by immunoglobulin hydrolysis with trypsin. Hydrolysis was carried out in 0.05 M NH_4HCO_3 buffer, pH 7.8, 37°C for 24 hours, at a proportion of ferment:substrate = 1:20. The fragments obtained were separated using DEAE-sefarosa ion-exchange chromatography.[12]

Avidity, high- and low-avid antibody ratio was determined by test systems for express diagnostics of clinical and preclinical forms of immunologic deficiency.[13]

Data were presented as means ± SEM. Mann-Whitney U-test was used to compare the results between the groups.

Results

We studied some biochemical parameters as well as antibody avidity in different stages of type 2 DM. In long and permanent derangements of carbohydrate metabolism, i.e., in decompensation stage of DM and in the absence of adequate treatment, the level of Hb_1A_C increases. At the same time, like hemoglobin other body proteins undergo unenzymatic glycosylation. These can cause receptor dysfunction, thickening of membrane, metabolic disturbance, which are typical of the progression of DM.

The levels of $HbA1_C$ and other biochemical parameters are given in Tables 1 and 2.

As shown in Table 2, there are no significant differences in the biochemical parameters in the stages of compensation and subcompensation. Creatinine and urea levels are significantly higher in the stage of decompensation, which is a result of diabetic nephropathy.

It should be mentioned that disease duration has a negative affect on cell immunity, especially on its functional activity. Levels of IgA, IgG, CIC and T-lymphocytes in patients with disease duration of 1 year were significantly lower than in patients with disease duration of 6, 10 years and more. However, in patients with disease duration of 6–10 years and over 10 years, the level of IgG was noticeable, but feebly marked. These results are given in Table 3.

As evident from Table 3 with long duration of disease, levels of IgA, IgG and CIC increased respectively. Conversely, parameters of T-system immunity were reduced.

As it appears from Table 3, the amount of T-lymphocytes in peripheral blood of patients with disease duration less than 1 year is approximately close to the level of this parameter in the blood of practically healthy people.

Table 1: Glycemia and HbA1C levels of the groups studied (mean ± SE)

Diabetic patients	Number of patients	Mean daily glycemia (mmol/L)	Hb_1A_C%
In the stage of compensation	27	5.74 ± 0.14	6.5 ± 0.55
Subcompensation	12	8.1 ± 018**	7.7 ± 3.31
Decompensation	18	8.74 ± 0.58	10.5 ± 0.82*
Control	20	3.8 ± 0.22	5.4 ± 0.91

*$P < 0.01$; **$P < 0.001$

Table 2: General biochemical parameters from type 2 diabetic patients (mean ± SE)

Patient groups	Number of patients	Total protein (g/L)	Albumin (g/L)	Creatinine (mmol/L)	Urea (mmol/L)
Compensation	27	79 ± 0.12**	47 ± 0.2**	92.8 ± 2.38**	5.87 ± 0.31**
Subcompensation	12	76 ± 0.2*	40 ± 0.7**	101.7 ± 0.31**	9.8 ± 0.01**
Decompensation	18	52 ± 0.4**	32 ± 0.6**	345 ± 18.91**	12.85 ± 0.67**
Control	20	78.45 ± 1.31	44.6 ± 1.17	78.9 ± 0.2	6.4 ± 0.2

*P < 0.01; **P < 0.001

Table 3: Immunologic parameters and antibody avidity in type 2 DM patients

Parameters	Control	Stages of disease		
		Compensation(n = 27)	Subcompensation (n = 12)	Decompensation (n = 18)
T-lymphocytes, %	58.5 ± 2.2	56.1 ± 0.92	42.1 ± 2.7	36.8 ± 4.9**
IgG, mg/mL	14.5 ± 0.5	16.92 ± 1.7	20.89 ± 1.1	21.79 ± 1.3
IgM, mg/mL	2.18 ± 0.22	2.61 ± 1.7	2.91 ± 0.7	2.87 ± 0.81
IgA, mg/mL	1.75 ± 0.1	2.2 ± 0.3	3.1 ± 0.2	3.7 ± 0.1
CIC, mg/mL	0.51 ± 0.19	0.73 ± 0.24**	1.43 ± 0.28**	1.39 ± 0.19**
Phagocytic index, %	92 ± 2.2	71 ± 2.1**	69 ± 1.8*	51 ± 1.7**
High-avid antibodies,%	95 ± 2	74 ± 1.8**	52 ± 2.7	27 ± 1.5
Low-avid antibodies, %	5 ± 1.2	26 ± 1.4	48 ± 4.1	73 ± 1.7

*P < 0.01; **P < 0.001

With the longer duration of disease, immune status disorders obtain the deeper character.

Discussion

As an immunodeficiency state, DM obtains more recognition from the scientists.[14,15] Nevertheless, contradictory information is available in literature about changes in cell and humoral immunity.[16-18] Early revelation of disease in these patients may prevent some late complications. In spite of numerous studies carried out, the relationship between immune changes and metabolic disorders in diabetic patients is still not clear.[19,20]

One of the indicators presenting immune status state and autoimmune processes is the level of circulating immune complexes (CIC) in blood. As is well known, long time circulation of CIC in body, even at an insignificant increased level, can cause its deposition in tissues, high platelet aggregation and adhesion, which result in microcirculatory injury, vessel congestion, tissue damage, necrosis and other consequences. Hence, it is not unreasonable to assume that CIC plays a large role in the pathogenesis of diabetic late complications (micro- and macroangiopathy, neuropathy). In such a way it has been determined that duration of DM and diabetic macroangiopathy are characterized by derangement of cellular differentiation of T-lymphocytes, depression of their proliferative activity and lowered level of T-suppressors, hyperimmunoglobulinemia of all classes and presence of CIC in the blood serum. It can be assumed that immune system changes lead to complications of DM and aggravate metabolic disorders of mentioned pathology.[21,22]

The findings represent high concentration of CIC in patients with late stages of DM (compared with control), which agrees with the literature data. This gives grounds to assume that CIC, as a result of autoimmune reactions, plays a vital role in the pathogenesis of late complications of DM.[23,24]

It is known that antibodies of G_{1-3} subclasses play a key role in the mechanisms of immunologic recognition of alien antigens, which further initiates an immune response involving cascade of molecular and cell-mediated mechanisms of natural protection and this leads to selective destruction and elimination of alien antigens and pathogens.[25-27] However, not always there is linear dependence between the amount of G class specific antibodies and their protective activity.[28] Protective function of serum is determined not only by affinity and idiotypic spectrum of antibodies, but also to a greater extent by the ratio of high- and low-avid specific antibodies in it. Only high-avid antibodies of G class form polyvalent bond with antigen epitopes and lead to realization of effector function of antibodies, i.e., involving molecular and cell mechanisms of body

natural protection in immune response.[13,29]

Secretion of low-avid antibodies of G class with anomalous conformation and low functional activity as mentioned before, leads to several consequences, and among them, the most essential is the formation of small, soluble immune complexes circulating for a long period in blood, which are incapable of activating classical way of complement system and to involve cascade of Fc gamma and CR-depended cell-mediated defense mechanisms[30-32] Such small, soluble CIC have cytophilic activity, i.e., ability to bind with tissues. Owing to these reasons it can be considered that allergens and atopens circulating for a long time in blood lead to hyperactivity of B- and T-system immunity and suppression of antibacterial activity of macrophage cells.[33] Increased level of low-avid IgG explains the persistence of allergens and atopens in body.[34]

In this way pathogenesis of DM can be viewed as a chain of interrelated mechanisms, in which the trigger role is played by a deficiency of protective function of B-system immunity concerned with secretion of G class antibodies with low avidity and protective activity.[15,17,27]

Therefore, further study of immunologic mechanisms of diabetic complications can be used for early diagnosis of these complications and for the development of new effective treatment modes.

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