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Content of sP-selectin and Cytokines in Blood of Patients with Type 2 Diabetes Mellitus and Arterial Hypertension Depending on Diabetes Compensation Condition

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Abstract

The goal of our research has been to study sP-selectin and cytokine content changes, namely IL-2, IL-6 and TNF-α in blood plasma of patients with type 2 diabetes mellitus with varying compensation for the disease and arterial hypertension, as well as to investigate a possible interrelation between sP-selectin and cytokines. To achieve the goal 137 patients with type 2 diabetes mellitus with AH of the I-II stages and without AH (72 women and 65 men) have been examined. The levels of sP-selectin, IL-2, IL-6, TNF-α in blood serum were determined by means of immunoenzymatic assay method. As a result, a reliable increase in sP-selectin level in blood serum has been detected in patients with type 2 diabetes mellitus with deterioration of diabetes compensation along with AH; increase in IL-6 level in groups with good and satisfactory compensation for diabetes and TNF -α in groups with bad compensation with the presence of AH. A reverse correlation between the content of sP-selectin and TNF-α in groups of those examined with non-compensated type 2 DM, associated with AH and without AH, stronger in a group of patients with type 2 DM and AH. The level of sP-selectin in blood increases with the deterioration of type 2 DM compensation along with AH. Most probable reverse correlations between sP-selectin and TNF-α levels in groups of patients with insufficient compensation for diabetes may indicate a mutually potential role of these factors in the development and progression of type 2 DM decompensation and AH.

Keywords: sP-selectin, interleukin -2, interleukin -6, tumor- necrotic factor -α, diabetes mellitus type 2, arterial hypertension

1. Introduction

Diabetes mellitus (DM) and arterial hypertension (AH) are among the most common diseases in the world. Over 80% of patients with type 2 diabetes mellitus suffer from arterial hypertension. Especially dangerous is a combination of these diseases, as long as in this case cardiovascular risk and death rate in this category of patients significantly increases. P-selectins are adhesive molecules, which are exposed with erythrocytes and platelets in the process of activation, they provide their interaction with leukocytes. P-selectin of erythrocytes is kept in Weibel-Palade bodies. Its production is stimulated by inflammatory response cytokines, hyperglycemia and vasospasm. P-selectin plays an important role in the process of endothelial cell interactions with blood cells, coagulation, blood clot formation, including atheromatous plaques and is considered to be the marker of hyperactivity of blood clots. A soluble sP-selectin is detected in the blood Violation of P-selectin expression may be one of the factors in development of cardiovascular events (Aref S. *et al*., 2005; Glowinska B. *et al*., 2005; Jiao J.A. *et al*., 2010). Up to now discussing and less researched is the problem of sP-selectin in the pathogenesis of endothelial dysfunction development in patients with diabetes and its taking part in acute cardiovascular conditions. R. Füth and other authors has revealed direct connection between diastolic dysfunction of myocardium and the level of sP-selectin despite existence of diabetes and ischemic heart disease. However, such scientists as V. Bláha, T Dogru and K. Gokulakrishnan in their research have discovered reliable increase of sP-selectin in patients with type 2 diabetes, which closely correlated with weight index, markers HOMA R and hyperglycemia. (Bláha V *et al*., 2006; Dogru T *et al*., 2006 Gokulakrishnan K *et al*., 2006). In case of AH and type 2 DM combination, a complex of not only hemodynamic changes, impairment of blood coagulation properties, specific to HD, is observed, but also disorders of carbohydrate and lipid metabolisms and signs of systemic inflammation, peculiar to type 2 DM are notable (Yang HI. *et al.,* 2016). Data analysis in contemporary literature proves that chronic systemic inflammation is an important component of type 2 DM development, atherogenesis, remodeling of vascular wall,

development of AH, IHD. Cytokines are the endogenous mediators, which regulate the intensity and duration of an immune inflammatory response. Normally, they realize interrelation between non-specific resistance of the body and specific defense. A numerous group of cytokines, especially interleukin (IL) and tumor necrosis factor α (TNF-α), participate in regulation of the basic body functions both in normal functioning and in a pathological condition. They provide close correlation between different groups of cells (Mattu H. S. *et al.,* 2013). It is supposed that type 2 DM is a result of acute-phase inflammatory response activations, in the course of which, release of cytokines occurs (Corbin KL. *et al.,* 2016; Ibarra Urizar A. *et al.,* 2016). However, at present the biological and pathophysiological effects of P-selectin in the human body, moreover, the influence of P-selectin on diabetes compensation condition and the course of AT, its interrelations with other adipocytokines have been investigated partially and this problem remains the theme of scientific discussions. Thus, the goal ofour investigation was to study the changes of sP-selectin and cytokine contents, namely IL-2, IL-6 and TNF-a, in blood plasma of patients with type 2 DM with varying compensation for the disease and arterial hypertension, as well as to investigate a possible interrelation between sP-selectin and cytokines.

2. Materials and Methods

The investigation involved 137 patients with type 2 DM with AH of the I-II stages and without AH (72 women and 65 men), who sought consultation at the endocrinology department of Danylo Halytskyi Lviv National Medical University. Anthropological examinations, which included measurement of the patients' weight and height, were conducted for all patients. Presence of obesity and its degree was diagnosed based on classification criteria of the World Health Organization (1997) with determination of body mass index (BMI): according to the formula: BMI = body weight (kg) / height (m^2). The diagnosis of type 2 DM was made according to the WHO experts' recommendations. Compensation for type 2 DM was verified according to the order of the Ministry of Health of Ukraine No 1118 dated 21.12.2012. The diagnosis of AH was made based on case history data, clinical and instrumental examination after exclusion of symptomatic hypertension according to WHO recommendations.

After standard procedure of diagnostics, the patients' daily blood pressure was monitored with electronic automatic device for measurement of blood pressure VAT41-2. Analysis of data was carried out by means of «ARIADA» software (with automatic calculation of standard indices).

To compare sP-selectin levels in groups with good, satisfactory and unsatisfactory diabetes compensation and presence or absence of AH, all examined patients were divided into groups according to the condition of type 2 DM compensation and presence of AH:

1st (n=18; age=54,21±1,40 years; BMI =32,31±1,22 kg/m^2) – with good compensation for type 2 DM (HbA1c≤7%) and normal blood pressure;

2nd (n=14; age=57,25±1,94 years; BMI=30,00±1,32 kg/m^2) – with good compensation for type 2 DM (HbA1c≤7%) and AH (SBP =125,3±1,07 mmHg; DBP =86,74±4,54 mmHg);

3rd (n=22; age=52,23±1,92 years; BMI=30,81±1,58 kg/m^2) – with satisfactory compensation for type 2 DM (HbA1c 7.1-8%) and normal blood pressure (SBP =123,5±2,68 mmHg; DBP =72,49±2,62 mmHg);

4th (n=12; age=53,40±2,84 years; BMI=34,33±2,50 kg/m^2) - with satisfactory compensation for type 2 DM (HbA1c 7.1-8%) and AH (SBP =145,7±4,55 mmHg; DBP =89,17±2,99 mmHg)

5th (n=46; age=53,66±0,71 years; BMI=30,61±0,65 kg/m^2) – with unsatisfactory compensation for type 2 DM (HbA1c ≥8%) and normal blood pressure (SBP =121,4±0,87 mmHg.; ДАТ=73,38±0,70 mmHg.);

6-th (n=25; age=53,67±0,78 years; BMI=32,91±0,90 kg/m^2) - with unsatisfactory compensation for Type 2 DM (HbA1c ≥8%) and AH (SBP =143,9±1,25 mmHg; DBP =86,00±1,13 mmHg);

Blood for analysis was taken after 12 hours of fasting. Level of sP-selectin in the blood was determined by the method of immunoenzymatic assay with the kit, manufactured by "Bender MedSystems GmbH" (Austria); level of insulin in the blood was determined by the method of immunoenzymatic assay ("ELISA") with the kit of reagents, manufactured by "DRG" (Germany); level of HbA1c was determined by means of high-pressure cation exchange chromatography method with automatic analyzer "Biorad D-10"; TNF-α, IL-2 and IL-6 were detected with the kits of reagents manufactured by "Diaclone" (France) in the clinical laboratory of Lviv regional endocrinological dispensary.

Statistical processing of the results was performed by means of parametric methods of Student t-test and correlation analysis with determination of Pearson's code, and in case of normal distribution inconsistency–by means of Kolmogorov–Smirnov test and correlation analysis with determination of Spearman's correlation coefficient. While comparing the groups, ANOVA test was applied. The results are presented in the form of the mean value and standard deviation and correlation coefficient r. The reliable value was considered to be the one at $P<0.05$.

In clinical investigation, safety measures for a patient's health, stipulated for such cases, protection of his/her rights, human dignity, moral and ethical standards were followed according to the principles of Helsinki Declaration (Human Rights Declaration), European Convention on Human Rights and Biomedicine, and corresponding laws of Ukraine.

3. Results

Differences in sP-selectin, cytokines and insulin content depending on the presence of AH and condition of diabetes compensation in examined groups of patients are shown in Figure 1 and 2.

The level of sP-selectin in blood is significantly higher in groups of patients with type 2 DM and AH, in comparison with the groups of corresponding diabetes compensation without AH, which is demonstrated in Figure 1. Besides, it was found that in case of unsatisfactory type 2 DM compensation, independent of AH presence, sP-selectin level also increases significantly. In analysis of cytokine contents, depending on condition of DM compensation, an increase in TNF-α level in blood serum was detected in the group with poor type 2 DM compensation and AH in comparison with group of patients with diabetes mellitus without AH and IL-6 in blood serum in groups with good and satisfactory type 2 DM compensation and AH in comparison with a groups of the corresponding compensation without AH.

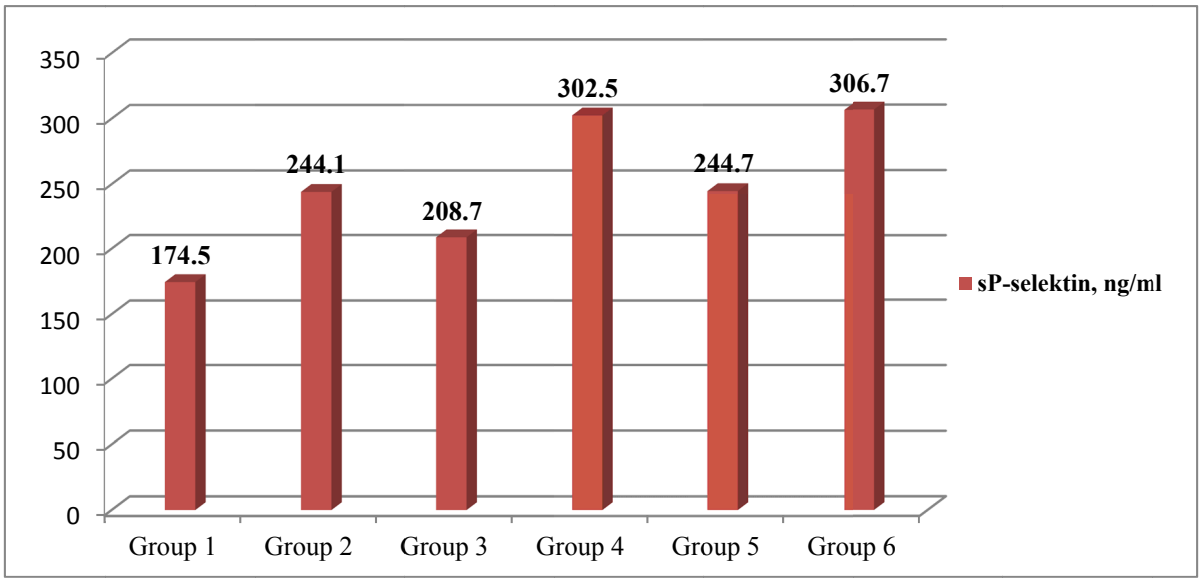

Figure 1. Content of sP-selectin in blood serum in group of patients depending on diabetes mellitus compensation and presence of AH

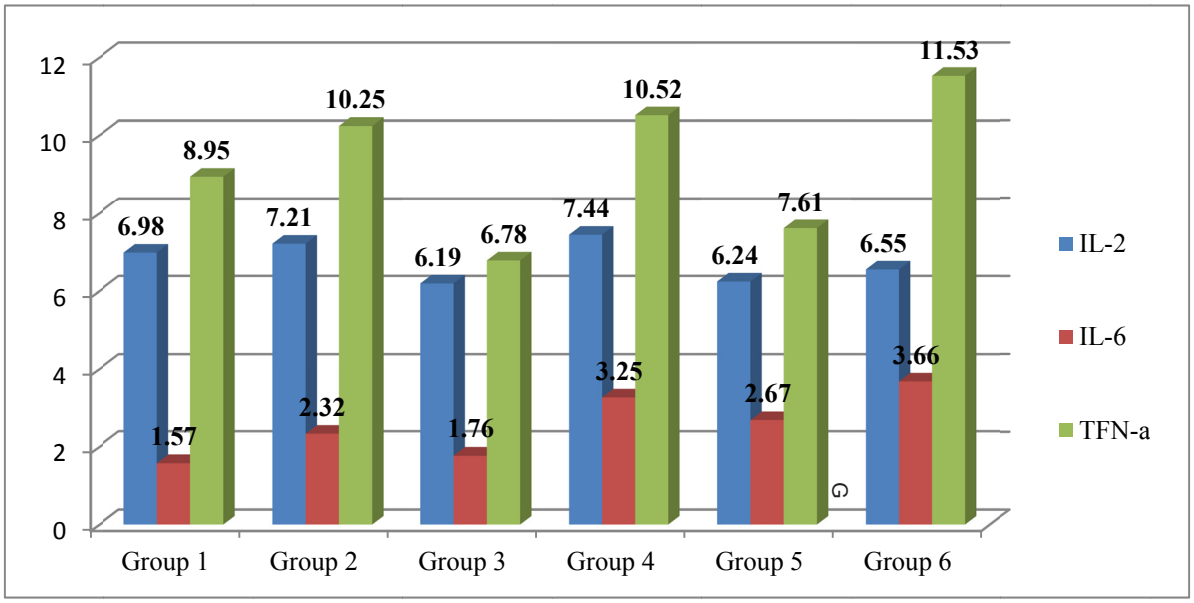

Figure 2. The content of cytokines in the serum of patients in groups depending on the compensation of diabetes and presence of hypertension

Results, obtained in correlation analysis, are presented in Table 3.

Table 3. Correlations between sP-selectin and mediators of non-specific inflammation in groups of patients with type 2 DM and AH as well as without AH with varying compensation for the disease.

Pair of variables	Group 1; r	Group 2; r	Group 3; r	Group4; r	Group 5; r	Group6; r
selectin &IL-2	-0.258	-0.322	**-0.058** P=0.037	0.800	-0.063	**-0.364** P=0.0128
selectin&IL-6	-0.267	-0.108	0.126	0.100	0.036	0.014
selectin&TNFα	-0.467	-0.571	-0.036	0.500	**-0.310** P=0.010	**-0.394** P=0.0067

Note: Characters in bold are statistically reliable coefficients of linear correlation ($p<0.05$).

4. Discussion

Some scientists consider that the level of type 2 DM compensation does not have any compensation is associated with P-selectin level (Penman A. *et al.,* 2015). Thus, Yang S. together with co-researchers proved that high concentration of blood glucose significantly increases P-selectin level. It confirms the results we obtained concerning increase in sP-selectin level in case of compensation deterioration for type 2 DM (Yang S. *et al.,* 2015). Segal HC and co-authors analyzed 14 biomarkers in blood, related to inflammation, thrombosis, atherogenesis, damage to heart cells and neurons at the onset of transient ischemic attack or ischemic stroke in population investigation of Oxford Vascular Study. They revealed reliable relations between the following biomarkers of chronic inflammation and the above-mentioned processes: IL-6, C-reactive protein and P-selectin. It also enables us to confirm the revealed changes in sP-selectine and IL-6 levels in patients' blood in the examined groups (Segal H.C. *et al.,* 2014). The role of AH in sP-selectin content increase in patients with type 2 diabetes mellitus, revealed in this investigation, should also be mentioned. Previously we detected a significant increase in sP-selectin level in blood of patients with diabetes mellitus and AH in increased duration of type 2 DM (Urbanovych A.M., 2014). The reverse correlation between sP-selectin and TNF-α in groups with poor compensation for type 2 DM with and without AH, and even stronger in group with type 2 DM and AH, should also be taken into consideration. TNF-α is a pleiotropic cytokine with wide range of immunological and non-immunological responses (it has cytotoxic and pro-inflammatory effects). The recent investigations prove that TNF-α is involved in pathophysiological chains and clinical signs of AH, cardiac failure, and myocardial infarction. *In vivo* TNF-α induces synthesis of pro-inflammatory cytokines, in particular IL-6, promotes formation of hyperglycemia, causes lacticacidemia, obesity, cachexia; *in vitro* stimulates production of IL-6, adhesion molecules and procoagulative blood activity. There are evidences that haemodynamic pressure overload is a significant stimulus for hyperproduction of TNF-α by cardiomyocytes and non-cardiomyocytes (Bautista L.E. *et al.,* 2005; Chae C.U. *et al.,* 2001; Tabet J.Y. *et al.,* 2002; Kovalyova O. *et al.,* 2002). IL-6 is a multifunctional cytokine which influences different organs and systems. IL-6 inhibits the production of TNF-α and completes the development of an inflammatory process (Danielson KK. *et al.,* 2016). Respectively, the highest levels of these cytokines were detected in the groups of patients with poor type 2 DM

compensation and AH. IL-2, in its turn, activates cytotoxic T-cells, monocytes, microphages, which increase the synthesis and secretion of IL-6, TNF-α. The latter exhibits a similar activity as IL-2 concerning inflammatory and immune reactions (Nelaeva AA. *et al.,* 2013).

AH is associated with endothelial dysfunction and oxidative stress. Endothelial dysfunction is considered to be an important component of insulin resistance in type 2 diabetes mellitus and AH. It results in activated state, characterized by increased agglutination and platelet aggregation, hypersecretion of P-selectin by platelet membranes. It was also observed in our patients in case of compensation deterioration for type 2 DM with AH. It has been proven that P-selectin level is increased in type 2 diabetes mellitus with marked angiopathies (Blankenberg S. 2003; Penman A. *et al*., 2015; Kolahdouz P. *et al*., 2015). Respectively, the increased level of sP-selectin may be regarded as an early marker of vascular complications of type 2 DM (Woollard KJ. *et al*., 2014). D. Bednarska-Chabowska with co-authors, investigating the level of selectin in patients with types 1 and 2 diabetes mellitus, revealed that in case of type 2 DM , associated with AH, presence of AH causes increase in P-selectin level, which coincides with our results. Moreover, these authors made a conclusion that the degree of endothelial tissue damage in diabetes mellitus depends on concomitant hypertension. P-selectin concentration increase is proportional to the degree of existing damage to vascular wall (Bednarska-Chabowska D. *et al.,* 2002).

5. Conclusions

The level of sP-selectin in blood increases with deterioration of type 2 diabetes mellitus compensation and AH.

Highly reliable correlations between the levels of sP-selectin and TNF-α in groups of patients with poor compensation for diabetes mellitus may confirm a mutually potential role of these factors in the development and progression of type 2 DM decompensation and AH.

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