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Original Article

Changes in the cytokine profile in patients with a combination of type 2 diabetes mellitus, hypertension, coronary heart disease and hepatic steatosis under conditions of obesity

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Abstract

The problem of the cytokine balance in patients with type 2 diabetes mellitus (T2DM), arterial hypertension (AH), coronary heart disease (CHD) and hepatic steatosis (LS) requires further study. This study aims to evaluate the content of cytokines in patients with named diseases depending on obesity. We examined 24 outpatients and divided them into 3 groups (overweight, class I obesity, and class II obesity). In addition to standard examinations, the levels of insulin, C-peptide, glycated hemoglobin, oral glucose tolerance test, non-esterified fatty acids, leptin, resistin, tumor necrosis factor α (TNFα), selectin, interleukin (IL)-6 and IL-2 were determined; triglyceride-glucose and leptin-resistin ratios, leptin-body mass and Castelli-I, -II indices were calculated. The cytokine profile in ObI was characterized by a significant increase in selection (181.5%; p=0.07) together with a decrease in IL-6 (59.3%, p>0.05) and TNFα (74.7%, p0.05), whereas in ObII - by an increase in leptin (219%, p<0.05) and IL-2 (122%, p>0.05), a slight decrease in increased selection (145%, p>0.05) compared to overweight group. We proposed formulas for cytokine profiles. In obese patients, cytokines correlated with transaminases, lipids, and T2DM, AH, and CHD duration. Cytokine profiles of patients with class I and II obesity are different, which can explain the obesity paradox; an exponential growth of leptin is observed in class II obesity.

Keywords: comorbidity of type 2 diabetes, hypertension, coronary heart disease, liver steatosis, leptin, resistin, selectin.

Introduction

Both adipose tissue and the liver produce a huge number of bioactive hormone-like cytokines, which play an important role in regulating various physiological and pathological processes. Adipose tissue produces leptin, adiponectin, visfatin, resistin, tumor necrosis factor-alpha (TNFα), interleukin (IL)-6, plasminogen activator inhibitor-1, angiotensinogen, insulin-like growth factor-1, apelin etc. [1]. Liver cytokines are just beginning to be studied; they include fetuins A and B, retinol-binding protein-4 (RBP4), selenoprotein P, fibroblast growth factor-21 (FGF-21), hepatocyte-derived fibrinogen-like protein-1, sex hormone-binding globulin (SHBG), angiopoietin-related growth factors-3, -4, -6, and -8; leukocyte cell-derived chemotaxis-2, SPARC-related modular calcium binding-1, growth differentiation factor-15 (GDF15; macrophage inhibitory cytokine-1), insulin-like growth factor-1, follistatin, activin E, lipocalin-13, Tsukushi factor, as well as TNFα and ILs [2, 3]. The interaction between cytokines of adipose tissue and liver may be provided by proinflammatory ILs and TNFα, which are common to both tissues, as well as selectins – transmembrane glycoproteins of cell adhesion, located on endothelial cells (E- and P-selectins), leukocytes (L-selectins), and platelets (P-selectins), which cause the migration of these cells to the inflammation foci in response to the proinflammatory cytokines increase [4]. However, the question of the role and levels of certain cytokines in clinical settings

needs to be studied [5], which determines the expediency and relevance of our study.

Our purpose was to evaluate the concentration of leptin, resistin, IL-2, IL-6, TNFα, and selectin in patients with a combination of type 2 diabetes mellitus (T2DM), arterial hypertension (AH), coronary heart disease (CHD), and hepatic steatosis depending on the presence and degree of obesity.

Material and methods

Study design and patients

We examined 24 outpatients with T2DM, AH, chronic forms of CHD with compensated heart failure of I-II functional class according to the New York Heart Association, and liver steatosis (males 11/45.8%, females 13/54.2%; mean age 55.83±0.89 years) according to the regulatory documents of the Ministry of Health of Ukraine in compliance with the principles of the Declaration of Helsinki. These patients were divided into clinically similar groups depending on body weight: patients of group 1 (G1) were overweight (mean body mass index [BMI] was 26.80±1.13 kg/m^2); patients of group 2 (G2) had class I obesity (mean BMI was 32.74±0.64 kg/m^2; $p<0.05$); and patients of group 3 (G3) had class II obesity (mean BMI was 37.57±0.64 kg/m^2; both $p<0.05$) (Table 1). In all patients, the duration of T2DM was up to 5 years. It is important, as certain differences in the cytokine content depending on diabetes duration have been described [6].

Laboratory data collection

Laboratory testing included standard analyses (liver tests, endogenous intoxication markers (creatinine, urea), lipid profile, and carbohydrate metabolism tests) and additional tests [levels of insulin, C-peptide, glycated hemoglobin (HBA1c) and oral glucose tolerance test, non-esterified fatty acids (NFA), adipokines (leptin and resistin), proinflammatory cytokines (TNFα, selectin, IL-6, IL-2)]. Apart from that, several complex indicators were calculated, namely, de Ritis index, triglyceride-glucose index (TGI), leptin to BMI ratio (L/BMI), leptin to resistin ratio (L/R), and two Castelli indices [I: total cholesterol/high-density lipoprotein cholesterol (HDL-C); II: low-density lipoprotein cholesterol (LDL-C)/HDL-C].

Statistical analysis

The results were processed statistically with the determination of the t-criterion, and the correlation analysis was carried out according to K. Pearson (r); in case of deviation of the parameter by ± (11–33) % from the normal value, the 1st degree of deviation $^{(\pm1)}$ was set, under the conditions of ± (34–66) % – the 2nd degree $^{(\pm2)}$, and in the case of a deviation of ± (≥67) % – 3rd degree $^{(\pm3)}$. P-values below 0.05 were considered significant.

Results

It was established that transaminase activity, indicators of endogenous intoxication (creatinine, urea), and blood lipids were increased in obese patients, but the differences did not reach significance levels.

Among the studied cytokines, the leptin content increased quite expectedly with an increase in body weight (G1: 16.46±5.45; G2: 18.67±2.70; G3: 36.00±0.62 ng/ml; p_{1-3}, $_{2-3}<0.01$). Under conditions of obesity, leptin level exceeded the value of overweight patients by 1.13 (class I obesity) and 2.19 (class II obesity) times, and selectin - by 1.81 (class I obesity) and 1.42 (class II obesity) times, respectively (Table 2, Figure 1).

Table 1: Baseline clinical characteristics of the subgroups of patients (except BMI, all $p>0.05$).

Parameter, units	G1, n=6	G2, n=7	G3, n=11
BMI, kg/m^2	26.80±1.13	32.74±0.64	37.57±0.64
T2DM duration, years	3.17±0.87	3.29±1.08	3.54±0.92
AH duration, years	7.67±4.11	12.57±4.35	9.27±3.04
CHD duration, years	1.47±0.74	1.93±0.70	1.82±0.77
Sex (males – 1, females – 2)	1.67±0.21	1.29±0.18	1.64±0.15
Age, years	56.83±1.25	56.14±2.04	55.09±1.36
Waist circumference/hip circumference	0.95±0.02	1.15±0.15	1.00±0.02

Table 2: Levels of the studied cytokines and markers that differed significantly or were involved in the correlation analysis in patients with type 2 diabetes, arterial hypertension, coronary heart disease, and liver steatosis under conditions of different body weight.

Parameter, units	G1, n=6	G2, n=7	G3, n=1
Creatinine, μmol/L	86.97±8.73	92.84±6.40	88.44±5.08
Leptin, ng/mL	16.46±5.45 [1]	18.67±2.70 [2]	36.00±0.62 [1,2]
Resistin, ng/mL	3.00±0.67	2.44±0.60	2.95±0.33
Selectin, ng/mL	142.38±35.00 [6]	258.43±49.11 [6]	205.95±44.92
IL-2, pg/mL	5.99±0.68	5.77±1.25	7.29±1.18
IL-6, pg/mL	3.86±1.99	2.29±1.05	3.45±0.82
TNFα, pg/mL	7.88±1.12	5.89±1.61	7.42±1.67
HBA1c, %	8.10±0.86 [8]	9.90±0.68	10.14±0.68 [8]
Insulin, mIU/mL	22.61±4.04	23.02±3.03	28.23±4.08
C-peptide, ng/mL	3.47±0.51	3.82±0.54	4.25±0.45
Fasting glucose, mmol/L	6.86±0.77 [3]	9.72±1.09 [3]	8.14±0.44
HDL-C, mmol/L	1.24±0.06	1.33±0.15	1.09±0.10
LDL-C, mmol/L	4.83±1.45	3.26±0.58	3.10±0.42
NFA, mmol/L	0.85±0.13	0.93±0.16	0.98±0.08
TGI, units	5.42±1.26 [7]	38.28±16.85 [7]	11.07±1.74
L/BMI, units	0.62±0.19	0.57±0.08 [5]	0.96±0.17 [5]
L/R, units	6.92±2.86	9.99±1.97	18.53±5.47
Castelli I	4.03±0.31 [4]	5.43±1.41	5.74±0.72 [4]

Note: [1-5] – $p<0.05$; [6-7] – $p=0.07$; [8] – $p=0.07$.

Therefore, in contrast to people who are overweight, the cytokine profile of patients with class I obesity was characterized by a significant increase in selection (181.5%; $p=0.07$) and a marked decrease in IL-6 (59.3%) and TNFα (74.7%). The formula for changes in the cytokine profile for patients with liver steatosis and class I obesity can be represented as follows: leptin $^{(+1)}$; resistin $^{(-1)}$; IL-2 $^{(0)}$; IL-6 $^{(-2)}$; TNFα $^{(-1)}$; selectin $^{(+3)}$. Instead, class II obesity was accompanied by a significant increase in leptin (219%, both $p<0.05$), some decrease in the already increased selection (145%) and an increase in IL-2 (122%), so the formula of cytokine status in case of class II obesity looks like this: leptin $^{(+3)}$; resistin $^{(0)}$; IL-2 $^{(+1)}$; IL-6 $^{(-1)}$; TNFα $^{(0)}$; selectin $^{(+2)}$.

Patients with comorbid class I obesity differed from patients with overweight not only by an increase in the selection level ($p=0.07$) but also by a higher fasting glucose level (9.72±1.09 *vs.* 6.86±0.77 mmol/L; $p<0.05$) and TGI (38.28±16.85 *vs.* 5.42±1.26; $p=0.07$), so, we may assume that the deterioration of carbohydrate control begins at the initial stage of obesity. Patients with comorbid class II obesity differed from individuals with overweight by a sharp increase in leptin (36.00±0.62 *vs.* 16.46±5.45 ng/mL; $p<0.01$), a less pronounced increase in selectin (205.95±44.92 *vs.* 142.38±35.00 ng/mL, $p>0.05$), as well as deterioration of carbohydrate metabolism control according to HbA1c increase (10.14±0.68% *vs.* 8.10±0, 86; $p=0.08$) and lipid metabolism worsening according to TGI increase (11.07±1.74 *vs.* 5.42±1.26; $p<0.05$) and the Castelli I index increase (5.74±0.72 *vs.* 4.03±0.31; $p<0.05$). In contrast to patients with class I obesity, individuals with more pronounced obesity were characterized by significantly higher leptin content (36.00±0.62 *vs.* 18.67±2.70 ng/mL; $p<0.01$) and L/BMI ratio (0.96±0.17 *vs.* 0.57±0.08; $p<0.05$), while according to the standard parameters of carbohydrate and lipid metabolism, the differences did not reach the level of significance.

According to the correlation analysis, in patients with steatosis and overweight, the content of resistin correlated with the CHD duration ($r=0.89$) and creatinine – an indicator of endogenous intoxication ($r=0.90$),

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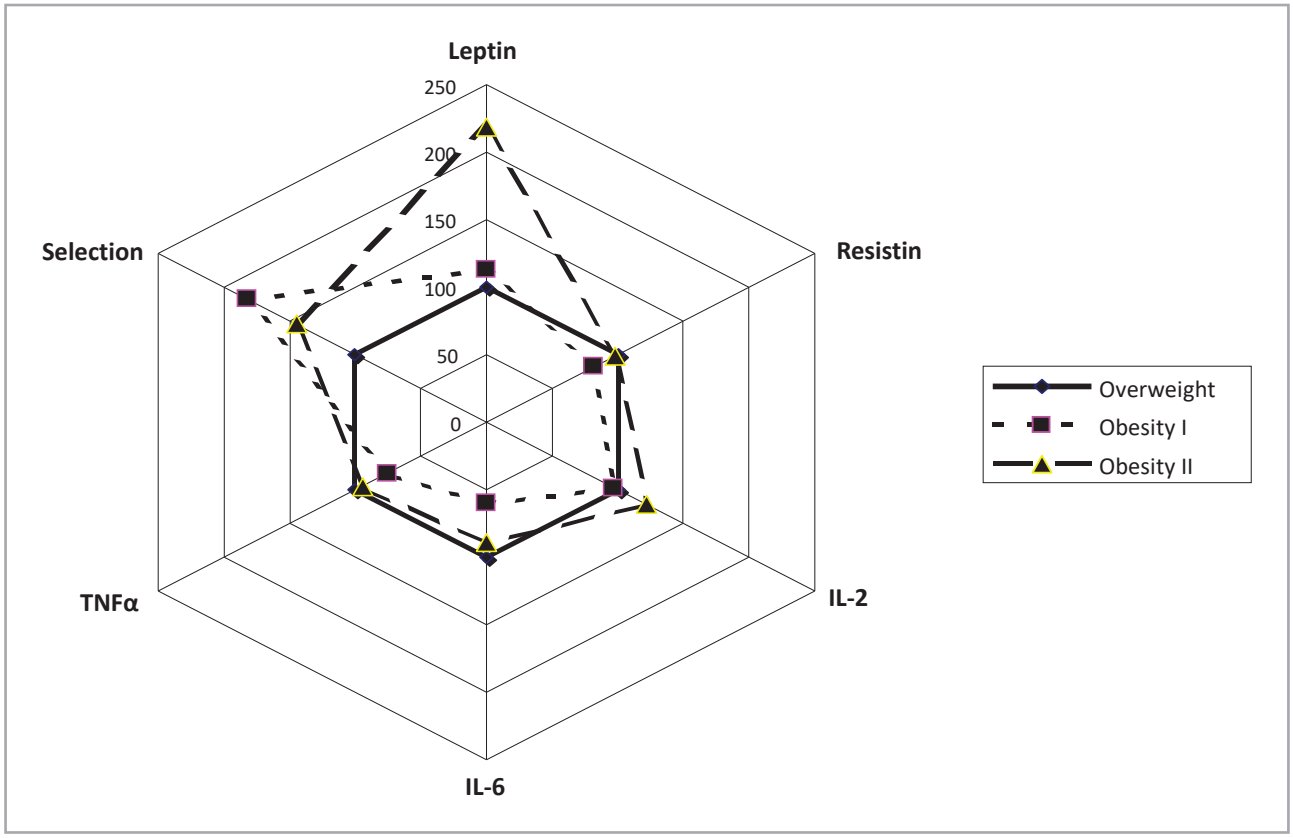

Figure 1: Relative values of leptin, resistin, IL-2, IL=6, TNFα, and selection in patients with overweight (100%) and obesity of class I and II.

which, in turn, correlated with the C-peptide level (r=0.85); in addition, L/R and L/BMI ratios correlated with each other (r=0.95), all p<0.05. That is, in patients with liver steatosis and overweight, resistin increase, which was associated with the activation of endogenous intoxication, inflammation, and insulin resistance, is unfavorable.

In patients with steatosis and class I obesity, numerous parameters of lipid metabolism and liver transaminases were included in the system of multidirectional correlations. TNFα significantly correlated with the AST (r=0.88), ALT (r=0.90), insulin (r=0.79), C-peptide (r=0.80), HDL-C (r=0.95), and LDL-C (r=0.82), which, in turn, were parallel to the IL-2 level (r=0.81); all p<0.05. The L/R ratio significantly correlated with the NFA level (r=0.81), selectin – with the IL-6 level (r=0.93), and leptin correlated with sex and was higher in females (r=0.90); all p<0.05. Correlation analysis in patients with steatosis and class II obesity revealed significant associations of leptin with creatinine (r=0.69) and LDL-C (r=0.73), of IL-6 – with age (r=0.67) and the waist circumference to hip circumference ratio (WC/HC; r=-0.63), of resistin – with IL-2 (r=0.81) and the AH duration (r=0.63), which, in turn, also correlated with IL-2 (r=0.66). The L/R ratio was proportional not only to L/BMI, as in overweight patients (r=0.83), but also to the T2DM duration (r=0.85), all p<0.05.

Discussion

Differences in the cytokine profile under the conditions of obesity of class I and II draw our attention: Class I was accompanied by a significant increase in selectin and a decrease in proinflammatory cytokines, and class II – was by a significant increase in leptin and a slight increase in proinflammatory IL-2. According to the literature data, an increase in selection is considered a marker of inflammation, endothelial dysfunction, and platelet activation [7], while its clinical value under the conditions of a combination of cardiovascular diseases and diabetes remains controversial [8], especially considering the presence of different forms of selection (E-, P -, L-). A polymorphism of the E-selectin gene (+A561C), which is associated with T2DM, AH, CHD, angina pectoris, and stroke [9], has been described. It may be assumed that the discovered increase of selectin triggers intercellular adhesion, which, in turn, stimulates the synthesis of proinflammatory cytokines. Interestingly, the leptin level does not increase

linearly in parallel to the increase in body weight and BMI but exponentially when it loses its physiological functions and acquires pathological ones [10]. Probably, a slight increase in leptin under conditions of overweight and class I obesity at least partially explains the described paradox of obesity – better survival of patients with overweight and class I obesity [11]. In addition, hyperleptinemia changes the gliovascular interface of the hypothalamus and potentiates hypertension [12]. Hyperleptinemia is also a key factor in carbohydrate intolerance [13], leptin resistance [14], oxidative stress [15], thyroid dysfunction [16], and future cardiovascular events [1, 17].

Conclusions

In patients with T2DM, AH, and CHD, the cytokine profile in case of class I obesity was characterized by a significant increase in selection (181.5%; p=0.07) and a decrease in IL-6 (59.3%, p>0.05) and TNFα (74.7%, p>0.05), whereas in case of class II obesity – by a significant increase in leptin (219%, p<0.05), a slight decrease in increased selection (145%, p>0.05) and an increase in IL-2 (122%, p>0.05) compared to individuals with normal weight and overweight. Cytokine profile formulas can be represented as follows: class I obesity: leptin $^{(+1)}$; resistin $^{(-1)}$; IL-2 $^{(0)}$; IL-6 $^{(-2)}$; TNFα $^{(-1)}$; selectin $^{(+3)}$; class II obesity: leptin $^{(+3)}$; resistin $^{(0)}$; IL-2 $^{(+1)}$; IL-6 $^{(-1)}$; TNFα $^{(0)}$; selectin $^{(+2)}$. According to the correlation analysis, in obese patients, the studied cytokines were associated with transaminases, parameters of lipid metabolism, and duration of all comorbid diseases (T2DM, hypertension, CHD).

Prospects for further research include the study of cytokine levels in patients with class III and IV obesity.

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Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the Danylo Halytsky Lviv National Medical University (approval ID: 347).

Consent to participate

Written informed consent was obtained from all participants in this study.

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