A.S. Golderova, A.N. Romanova, S.D. Efremova, E.A. Alekseeva,
Z.N. Krivoshapkina, V.E. Tarasova, F.A. Zakharova

Interrelation of a Leptin Level with Factors of Cardiovascular Risk at Stable and Unstable Stenocardia

ABSTRACT

The research detected a leptin level at patients with ischemic heart disease subject to the course of stenocardia (stable or instable). We established that destabilization of the IBS course is characterized by elevation of leptin level and increase of a number of its correlation relationship with factors of cardiovascular risk in comparison with patients with stable stenocardia.

Keywords: coronary heart disease, leptin, cytokines

INTRODUCTION

Leptin is a protein hormone structurally similar to the first class of cytotokine, secreted by fatty cells and a controlled by genome, causing obesity [13]. A leptin level of serum correlates with the general content of fat in an organism [8], reflects a total power reserve of fatty tissue and can change in energy imbalance [3, 10]. So, leptin raises with increasing fatty tissue mass, and its production in hypodermic and fatty cellulose is higher, than in visceral fatty depots [7]. Leptin count in blood directly depends on body weight [2]. It is known that the hyperleptinemia is associated with obesity [9]. Leptin promotes development of atherosclerosis and atherothrombosis at apolipoprotein - E-scarce mice [12]. It is known that leptin is an independent predictor of myocardial infarction at patients with arterial hypertension [6]. Leptin activates immune cells and stimulates the cellular immune reaction, influences production of pro-inflammatory cyitokine by direct impact on a vascular wall [1, 11]. Change of leptin levels, free fatty acids, adipokin are one of the possible reasons of development of insulin resistance in patients with myocardial infarction [5]. Research of features of interrelation of leptin level with factors of cardiovascular risk depending on a form of the course of coronary heart disease (CHD) is of great significance.

MATERIALS AND METHODS

In this research 61 men with the diagnosis CHD aged from 42 till 70 years (middle age 54,9 ±7,17 years) were included. Of them 32 men had stable stenocardia (middle age of 54,7±1,1 years), and 29 men with a sharp coronary syndrome without rising ST segment on the electrocardiogram (firstly diagnosed stenocardia, unstable stenocardia) (middle age of 55,3±1,5

years). Anthropometrical parameters were measured at the patients: growth, body weight, circle of a waist, hips, thorax. The body mass index (BMI) was calculated as follows: BMI = body mass (kg) / growth $(m)^2$.

Laboratory researches included determination of ASAT (aspartataminotransferase), ALAT (alaninaminotransferase), alkaline phosphatase, GGT (gamma glutamil transferase), LDG (laktat degidrogenase), a kreatinkinase, glucose, the general protein, albumine, urea, uric acid, a kreatinkinaza the general, the general cholesterol, triglycerides, HDL in blood serum. All biochemical indicators were determined on the automatic biochemical Labio analyzer with application of the commercial reactants "Biocon" (Germany). The LDL and VLDL and a rate of atherogenicity were determined by standard methods. Concentrations of C-reactive protein (CRP) and cytokines in blood serum were defined by method of the immunofermental analysis:IL-4, IL-6, IL-8, IL-10, IFN-γ (interferon- gamma) and TNF-α (tumor necrosis factor-alpha) (Vektor-Best, Novosibirsk), as well as leptin level (DRG diagnostic", USA).

Immunophenotyping of lymphocytes was conducted by a method of flow cytometry (FACSCalibur, Becton Dickinson) with use of monoclonal antibodies with a threefold tag: CD3FITC + CD4RPE + SD45RPE-Cy5; CD3FITC + CD19-RPE + SD45RPE-Cy5; CD3FITC + CD8RPE + SD45RPE-Cy5; CD16FITC + CD19RPE + CD3RPE-Cy5; with one tag of CD25-RPE; Sd11b-RPE; CD71-FITC; (Dako); CD54-RPE, CD95-RPE (BectonDickinson), CD62L-FITC (Sorbent, St. Petersburg).

All patients gave the informed consent for being involved in the research. The protocol of research was approved by the local ethical committee in compliance of the Helsinki declaration (2000). The statistical analysis of the obtained data was carried out by means of the package SSPS version 17.0. Equality of selective averages was checked by Student's criterion (at normal distribution) and to nonparametric U-criterion of Mann-Whitney for independent selections (at abnormal distribution). Data of the comparative analysis are presented in the table as follows: median (25th and 75th percentile). For definition of correlation ratio between the studied quantitative data an analysis with calculation of Spirmen's correlation ratio was carried out. Distinctions at $p \leq 0,05$ were considered statistically significant.

RESULTS AND DISCUSSION. Results of the comparative analysis showed that both groups of patients were age comparable, however significant distinctions in growth of a body were noted (r=0,03), waist circles (r=0,005), hips (r=0,000) and a thorax (r=0,027) were noted, i.e. with anthropometrical distinctions: at persons with unstable stenocardia these indicators were much higher (173,03 ± 1,35 cm; 104,51 ± 1,85 cm; 106,46 ± 1,34 cm; 108,42 ± 1,62 cm), than at

patients with stable stenocardia (169,25 ± 1,03 cm; 93,47 ± 2,94 cm; 93,26 ± 2,88 cm; 101,15 ± 2,72 cm), respectively. However on BMI both groups had no significant distinctions: at unstable stenocardia 30,13 ± 0,84 kg/m^2, and at persons with stable stenocardia - 29,97 ± 0,84 kg/m^2. The research of leptin level showed that of all examined patients (n=61) only 22 men (36%) had leptin level within norm, the rest 39 patients (64%) had the higher level. The leptin level shouldn't to exceed 13,8 ng/ml at men aged 20 years and more.

Fig. 1. Distribution of patients with CHD on the leptin level

The comparative analysis of distribution on the leptin level depending on the CHD form established that at unstable stenocardia a number of patients with the leptin level exceeding the norm was significantly higher, than at stable stenocardia ($\chi 2 = 5,66$; p=0,032) (fig. 1).

The analysis of laboratory indicators of blood showed that at unstable stenocardia at patients the quantity of leukocytes (p=0,007), concentration of VLDL atherogenous fraction (r=0,027) and aterogen ratio significantly increases (p=0,031), and concentration of anti-atherogenous HDL (p=0,000) was significantly reduced, than at stable stenocardia (табл 1). Along with it, at patients with unstable stenocardia increase of level of pro-inflammatory cytokine of IL-8 (p=0,008), TNF-α (p=0,009) and concentration of CRP (p=0,013), and also decrease in a level of anti-inflammatory cytokine of IL-4 is noted (p=0,047), these parameters testifying to activation of inflammatory processes at destabilization of a course of coronary heart disease. Average value of the leptin concentration in blood serum of patients with unstable stenocardia (32,45 ± 4,71 ng/ml) was significantly higher (p=0,049) than at stable stenocardia (20,58 ± 3,66 ng/ml).

The correlation analysis of the leptin level with major factors of cardiovascular in the general selection of patients (n=61) established direct correlations of the leptin level with BMI (r=0,510; p=0,000), with circles of hips (r=0,509; p=0,000), thorax (r=0,496; p=0,001) and waists (r=0,461; p=0,001). Indirect distinction in correlation ratio is confirmed by data of the authors [4] testifying that the leptin level in serum increases at increase in mass of fatty tissue and its production in hypodermic fatty cellulose is higher than in visceral fatty depots.

Table 1

Biochemical and immunological parameters of blood at patients with CHD
median (25th percentile; 75th percentile)

Blood indicators	stable stenocardia n=32	unstable stenocardia n=29	p…
Leukocyte, x 10^9 / l	5,70 (4,80; 6,30)	6,55 (5,77; 9,57)	0,007
HDL, mmol/l	1,30 (1,00; 2,00)	0,86 (0,77; 1,16)	0,000
VLDL, mmol/l	0,59 (0,44; 0,79)	0,82 (0,62; 1,13)	0,027
Aterogenost coefficient	3,13 (2,73; 4,29)	4,15 (3,32; 6,05)	0,031
CRP, mg/ml	4,41 (2,64; 9,11)	8,55 (4,52;17,05)	0,013
IL-8, pg/ml	5,00 (2,80;16,49)	18,26 (10,12; 31,05)	0,009
TNF-α, pg/ml	3,11 (2,20; 5,50)	8,68 (4,43; 13,83)	0,000
IL-4, pg/ml	3,00 (1,50; 4,00)	1,99 (1,70; 2,83)	0,047
Leptine, ng/ml	20,58 ± 3,66	32,45 ± 4,71	0,049

For the purpose to assess interrelations of the leptin level at stable and unstable stenocardia the correlation analysis is carried out. At patients with stable stenocardia the level of leptin had direct correlations with BMI (r=0,424; p=0,016), quantity of leukocytes (r=0,479; p=0,009), concentration of atherogenous triglycerides (r=0,410; p=0,022), and also the return correlation with the relative quantity of lymphocytes (r = - 0,377; p=0,044), i.e. only 4 relations were established.

The analysis of interrelations at unstable stenocardia revealed the greatest number of interrelations: direct correlations with BMI (r=0,456; p=0,017), with a thorax circle (r=0,455; p=0,019) and waists (r=0,410; p=0,034). From indicators of peripheral blood the level of a leptin directly correlated with the relative quantity of monocytes (r=0,770; p=0,000), concentration of erythrocytes (r=0,492; p=0,020) and with value of speed of subsidence of erythrocytes (r=0,456; p=0,033). For biochemical indicators direct correlations with aterogenic ratio (r=0,455; p=0,019), concentration of enzymes ASAT (r=0,482; p=0,019), GGT (r=0,611; r=0,001), general kreatinkinaza (r=0,428; p=0,033) and return correlation with the level of anti-atherogenous HDL (r = - 0,482; r=0,015). The analysis of interrelations of the leptin level with immunological parameters established positive correlations with pro-inflammatory cytokine of IL-6 (r=0,681; p=0,000), IL-8 (r=0,551; p=0,002), IFN-γ (r=0,492; p=0,011) and also regulatory cytokine of IL-10 (r=0,628; p=0,001). The leptin level at patients with unstable stenocardia had also close direct correlation with the relative maintenance of T-lymphocytes (CD3+) (r=0,592; p=0,004) and the

return correlations with the relative quantity of NK cells (CD16+) (r= - 0,438; p=0,041), the level of an expression of molecules of adhesion of CD62L+ (L-selektina) (r= - 0,442; p=0,039), CD11b + (αм-chain of an integrin) (r = - 0,512; p=0,015). Nature of communications specifies that the leptin level grows with growth of cardiovascular risk. In figure 2 various cases of interrelations of the leptin level with the relative content of monocytes and a pro-inflammatory cytokine of IL-6 are presented, at a stable current of CHD interface - minimum, and at destabilization – maximum.

Fig. 2. Various nature of interrelations of the leptin level with inflammation markers (monocytes and IL-6) at patients with stable stenocardia (on the left) and unstable stenocardia (on the right)

The data of the correlation analysis obtained by us testify to various nature of the established interrelations at patients depending on the CHD form. The number of correlations at unstable stenocardia (19 communications) was more than 4,75 times, than at stable stenocardia (4 interrelations). It should be noted that at patients with unstable stenocardia the strongest positive correlations of the leptin level with inflammatory markers are: monocytes (r=0,770; p=0,000), IL-6 (r=0,681; p=0,000) and regulatory cytokine of IL-10 (r=0,628; p=0,001), and also relative content of T-lymphocytes (CD3+) (r=0,592; p=0,004).

Thus, at unstable stenocardia there is growth of the concentration of leptin and amount of its interrelations not only with traditional factors of cardiovascular risk (BMI, a waist circle, a dislipidemiya, etc.), but also with markers of inflammatory process, activation of immune system that gives the chance to consider increase of the leptin level as one of predictive criteria of destabilization of the current of CHD increases.

CONCLUSION

1. The interrelation of leptin with BMI is much more expressed in comparison with indicators of the circle of a waist, hips and a thorax.
2. At patients with unstable stenocardia the leptin level is significantly increased in comparison with patients with stable stenocardia.
3. Destabilization of a current of CHD is characterized by sharp increase in number of correlations interrelations of the leptin level (in 4,75 times) with factors of cardiovascular risk and markers of inflammatory process.

REFERENCES

1. Adipokiny i ikh sootnoshenie u bol'nykh s metabolicheskim sindromom [Adipokines and their ratio at patients with a metabolic syndrome] / O.I. Mitchenko, V.YU. Romanov, K.O. YAnovskaya (i dr.) // Ukr. kardiol. zhurn. [Ukr. cardiol. journal] – 2011. – №6. – P. 71–78.
2. Andreeva V.O., Shabanova L.Yu. Taktika vedeniya devochek-podrostkov s disfunktsiej yaichnikov i izbytochnoj massoj tela [A way of surveillance of teenage girls with dysfunction of ovaries and excess body weight] / V.O. Andreeva, L.Yu. Shabanova // Reproduktivnoe zdorov'e detej i podrostkov [Reproductive health of children and teenagers]. – 2011. – № 1. – P. 26 –35.
3. Inyushkina E.N. Leptin – anareksigennyj regulyatornyj polipeptid s respiratornoj aktivnost'yu [Leptin is anorexia regulatory polypeptide with respiratory activity] / E.N. Inyushkina // Vestnik Samarskogo gosudarstvennogo universiteta [Bulletin of Samarsky state university]. – 2006. – № 2(42). – P. 168–174.
4. Leptin: fiziologicheskie i patologicheskie aspekty dejstviya [Leptin: physiological and pathological aspects] / M.A., Kovarenko, L.A. Ruyatkina, M.S. Petrishheva i [dr.]. // Vestnik NGU. Seriya: Biologiya, klinicheskaya meditsina. 2003. T.1. Issue.1. P. 59-74.
5. Uroven' leptina, adiponektina i svobodnykh zhirnykh kislot u patsientov s razlichnoj massoj tela na fone infarkta miokarda s pod"emom ST [A level of leptin, adiponektin and free fatty acids at patients with various body weight at myocardial infarction with raising of ST] / L.V. Kvitkova, D.A. Borodkina, O.V. Gruzdeva [i dr.]. – Klinicheskaya ehndokrinologiya. – 2013. –№3. – P. 8–12.
6. Association of plasma leptin levels and complexity of the culprit lesion in patients with unstable angina / L. Dubey, H. Zeng, S. Hashmi [et al.] // Int. J. Cardiol. – 2008. – Vol. 126. – № 2. – P.183–189.
7. Flier J.S. Leptin expression and action: new experimental paradigms / J.S. Flier // Proc. Natl. Acad. Sci. USA. – 2002. – Vol. 94. – P. 4242–4245.
8. Increased expression of C-reactive protein and tissue factor in acute coronary syndrome lesions: correlation with serum C-reactive protein, angioscopic findings, and modification by statins / R.P. Andriea, G. Bauriedelad, P. Braunb [et al.] // Atherosclerosis. – 2009. – Vol. 202. – P. 135–143.
9. Leptin concentrations in relation to overall adiposity, fat distribution, and blood pressure in a rural Chinese population. / F.B. Hu, C. Chen, B. Wang [et al.] // Int J Obes Relat Metab Disord. – 2001. – №25. – P. 121–125.

10. Leptin secretion from adipose tissue in women: Relationshipto plasma levels and gene expression / F. Lonnqvist, L. Nordfors, M. Jansson [et. al.] // J. Clin. Invest. – 2002. – Vol. 99. – P. 2398–2404.
11. Nair P. The Effects of leptin on airway smooth muscle responses // Am. J. Respir. Cell Mol. Biol. – 2008. – Vol. 39. – P. 475-481.
12. Recombinant leptin promotes atherosclerosis and thrombosis in apolipoprotein E-deficient mice / P.F. Bodary, S. Gu, Y. Shen [et al.] // Arterioscler. Thromb. Vasc. Biol. – 2005. – Vol. 25. – P. 119–122.
13. Tritos N. Leptin: Its role in obesity and beyond / N. Tritos, C.S. Mantzoros // Diabetologia. – 1997. – Vol. 40. – P. 1371–1379.

AUTHORS:

1. Golderova Aytalina Semenovna – Doc. of Med. Sc., chief research associate, head of the Office of adjustment mechanism research (OAMR) FSBSI "The Yakut scientific center of complex medical problems", hoto68@mail.ru
2. Romanova Anna Nikolaevna - Doctor of Med. Sc., chief research associate FSBSI "The Yakut scientific center of complex medical problems"
3. Efremova Svetlana Dmitriyevna, junior research associate of laboratory of immunopathology of OAMR FSBSI "The Yakut scientific center of complex medical problems"
4. Alekseeva Elizabeth Aleksandrovna – junior researcher of laboratory of an immunopathology of OIMA FGBNU "The Yakut scientific center of complex medical problems"
5. Krivoshapkina Zoya Nikolaevna – senior research associate of laboratory of biochemistry of OIMA FGBNU "The Yakut scientific center of complex medical problems"
6. Tarasova Vera Evstafyevna – cardiologist of ORIT, State Budgetary Institution Yakut City Clinical Hospital
7. Zakharova Fedora Apollonovna – Doc. of Med. Sc., professor of department of normal and pathological physiology