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THE RELATIONSHIP BETWEEN INSULIN RESISTANCE, ADIPOKINES, LIPIDS AND LOW-GRADE INFLAMMATION IN PATIENTS BY CARDIOVASCULAR DISEASE TREATED WITH STATINS

Maksymets T.A *https://orcid.org/0000-0003-2659-1083*
Sklyarova H.E. *https://orcid.org/0000-0003-3667-6304*

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

maksymets.t@gmail.com

Background. Hypolipidemic and hypotensive therapy enables to improve prognosis and decrease the risk of cardiovascular pathologies, however, an intensive regimen of prescribing statins for patients with arterial hypertension (AH) combined with obesity can promote development of hyperinsulinemia and insulin resistance (IR), which are predictors of type 2 diabetes mellitus development.

Aim: To investigate the relationship between insulin resistance, adipokines, lipids and low-grade inflammation in patients with cardiovascular disease treated by statins.

Materials and methods: 81 patients with overweight and/or obesity and AH or combination of AH and CAD were involved. Patients' age ranged from 35 to 79 years, among them there were 51 males and 30 females. ACE inhibitors and ARBs, diuretics were used for treatment of AH; atorvastatin was prescribed orally in the dose 20 mg or 40 mg per day permanently as hypolipidemic therapy.

Lipid spectrum, biochemical indices and glycated hemoglobin were determined according to common techniques on an analyzer "BioSystems" (Spain) using original kits of reagents. Concentration of insulin and hsCRP were determined on immunochemoluminescent analyzer "Immulite 2000" (Siemens, Germany). Insulin resistance index was calculated by the formula:

HOMA-IR=fasting insulin (mcIU/ml)*fasting glucose(mmol/L)/22.5

In HOMA-IR value > 2.77, patients were considered insulin resistant.

Leptin was measured using an ELISA kit DRG (USA). Adiponectin was quantified using the ELISA kit Mediagnost (Germany).

Results. It has been revealed in our investigation that body mass index, the level of systolic blood pressure, triglycerides, highly sensitive C-reactive protein and leptin were reliably higher in patients with IR, whereas adiponectin level was lower. These associations are confirmed by the results of studying correlations between IR index, adipokines, anthropometric and biochemical parameters. Thus, thorough evaluation of carbohydrate and adipokine profile in patients with cardiovascular pathology combined with obesity, who are subjected to hypolipidemic therapy, is an important factor in preventing type 2 diabetes mellitus.

Conclusion. Assessment of the risk of statin-induced diabetes mellitus is important for determining intensity of statin therapy, namely, presence of insulin resistance, levels of hsCRP and adipokines should be considered at the beginning and during treatment.

Keywords: insulin resistance, arterial hypertension, atorvastatin, leptin, adiponectin.

Background. Arterial hypertension (AH), dyslipidemia and insulin resistance (IR) are the main risk factors of cardiovascular pathology, which are combined within metabolic syndrome in overweight and obese patients. Angiotensin-converting enzyme inhibitors (ACE-inhibitors) and angiotensin receptor blockers (ARBs) are used for the treatment of hypertension in this category of patients. The choice of such medicines is caused by the fact that despite similar reduction of AH with various drugs, only ACE-inhibitors and ARBs improve sensitivity of tissues to insulin.

For correction of dyslipidemia, inhibitors of GMG-CoA-reductase (statins) are prescribed, which can provide sufficient decrease in concentration of low-density lipoproteins (LDL). The drugs of choice in this case are atorvastatin and rosuvastatin. Although hypolipidemic therapy enables to decrease a general risk of cardiovascular diseases, more data appear which prove that these medicines can increase the risk of development of type 2 diabetes mellitus in some categories of patients. Thus, assessment of patients' metabolic status is an important aspect, which includes medical history, anthropometric data, lipid, carbohydrate and adipokine profile (namely, leptin and adiponectin levels and their correlation), liver function indices and low-grade inflammation (highly sensitive C-reactive protein (hsCRP)).

The influence of abdominal obesity should also be considered as it plays an important role in development of AH, coronary artery disease (CAD) and IR due to regulation impairment in the system of adipokines, among which correlation between leptin and adiponectin is of utmost importance. Elevation of leptin concentration in blood serum is associated with AH and obesity, besides, leptin concentration correlates with IR and anthropometric parameters. Unlike leptin, low level of adiponectin in blood serum negatively correlates with IR, hyperinsulinemia, AH and hyperlipidemia. Hyperinsulinemia, irrespective of glycemic profile, can promote increase in noradrenaline level and increase sodium reabsorption with further increase in the volume of extracellular fluid and AH occurrence. Besides, it should be considered that hypertension in overweight and obese patients is associated with low-grade inflammation in perivascular adipose tissue [1].

To prevent death due to cardiovascular diseases and other causes, primary and secondary hospitalizations, it is necessary to achieve and maintain target levels of LDL at such level as long as possible, which can be accomplished by using statins [2].

Atorvastatin possesses a number of pleiotropic effects, including – anti-inflammatory and antithrombotic ones, can improve endothelium function, stabilize a lid of an atherosclerotic plaque, inhibit the processes of fibrosis and hypertrophy of the myocardium, accelerate its perfusion and angiogenesis, as well as has nephroprotective influence [3].

It should be noted that development of type 2 diabetes mellitus depends on many factors. On a patient's side, these are age, gender, body weight, physical activity, nutrition, family history of type 2 diabetes mellitus, smoking, initial condition of glucose metabolism, concomitant diseases (polycystic ovaries, hyperprolactinemia) and intake of some drugs (diuretics, β blockers). Concerning statin therapy, it is a chemical structure of a statin (pitava- and pravastatin do not have a negative influence on IR), duration of use and dose [4].

Taking into account conclusions of numerous comprehensive investigations and their subanalyses, which demonstrate that treatment with statins can provoke development of diabetes mellitus, the aim of our research was assessment the relationship between insulin resistance, adipokines, lipids and low-grade inflammation in patients with cardiovascular disease treated by statins.

Aim: To investigate the relationship between insulin resistance, adipokines, lipids and low-grade inflammation in patients with cardiovascular disease treated by statins.

MATERIALS AND METHODS

Clinical trial was conducted with accorandce to the Declaration of Helsinki, The Convention for the Protection of Human Rights and Biomedicine, Legislation of Ukraine and agreed by Ethical

Committee Danylo Halytsky Lviv National Medical University, protocol №3, 25.03.2019. All patients signed an informed consent before the study. 81 patients with overweight and/or obesity and AH or combination of AH and CAD were involved. None of them had diabetes mellitus, severe heart, kidney or liver failure that could influence the condition of insulin resistance. Patients' age ranged from 35 to 79 years, among them there were 51 males and 30 females. The patients received treatment according to recommendations of European Society of Cardiology (ESC, 2019). ACE inhibitors and ARBs, diuretics were used for treatment of AH; atorvastatin was prescribed orally in the dose 20 mg or 40 mg per day permanently as hypolipidemic therapy. Atorvastatin dose was administered depending on individual initial level of LDL, calculating percentage of reduction necessary for achieving target level.

Anthropometric measurements were performed to calculate body mass index (BMI), waist circumference (WC) was determined for establishment of the degree and nature of obesity according to the WHO criteria (1997) and International Diabetes Federation (IDF, 2006, 2015).

Lipid spectrum, biochemical indices (ALT, AST, uric acid, glucose) and glycated hemoglobin (HbA1c) were determined according to common techniques on an analyzer "BioSystems" (Spain) using original kits of reagents. Concentration of insulin and hsCRP were determined on immunochemoluminescent analyzer "Immulite 2000" (Siemens, Germany) using appropriate reagents "Immulite 2000 Insulin" (USA) and "Immulite 2000 hsCRP" (USA).

Insulin resistance index was calculated by the formula:

HOMA-IR=fasting insulin (mcIU/ml)*fasting glucose(mmol/L)/22.5

In HOMA-IR value > 2.77, patients were considered insulin resistant.

Leptin was measured using an ELISA kit DRG (USA). Adiponectin was quantified using the ELISA kit Mediagnost (Germany). Both ELISA procedures were performed using TECAN device.

Statistical analysis was performed by means of the library SciPy, programming language Python. The results of values are presented as mean arithmetic with statistical deviation. Normality of distribution was determined by Shapiro-Wilk test. Values with normal distribution are presented as confidence interval (95%), and the values, distribution of which significantly differed from normal, - as interval 25% and 75% percentiles. Comparison of groups was conducted by means of Mann-Whitney U test. To establish connections between indices, Spearman and Pearson rank correlations were applied.

RESULTS AND DISCUSSION

All patients were distributed into two groups depending on the presence of insulin resistance: 1st group (n=45) HOMA-IR<2.77 and 2nd group (n=36) HOMA-IR>2.77. Such distribution is caused by the necessity of considering insulin resistant status when choosing the regimen of hypolipidemic therapy to prevent the development of type 2 diabetes mellitus.

Anthropometric and hemodynamic parameters of the examined individuals are given in table 1.

Comparing groups, it was established that BMI, WC and systolic blood pressure were reliably higher in patients with IR ($p<0.01$).

ALT, AST indices, level of uric acid and glycated hemoglobin did not differ reliably between groups. Among parameters of lipid spectrum, a reliable difference was detected only for triglycerides (TG) ($p<0.01$).

Reliably higher concentration of hsCRP ($p<0.01$), glycated hemoglobin ($p=0.02$), glucose, insulin and leptin (all $p<0.01$) was detected in patients with IR (for all $p<0.01$), while adiponectin level was reliably lower in this group of patients ($p<0.01$). Respectively, correlation between leptin and adiponectin in the presence of insulin resistance shifted with leptin prevalence.

Results of correlations demonstrated distinct difference between the factors, which promote IR development and protective factors. A positive correlation was observed between the levels of leptin and BMI, WC, TG, Glucose, Insulin, HOMA-IR and hsCRP. Respectively, a positive correlation was observed between HOMA-IR and BMI, WC, ALT, Uric acid, TG, HBA1, Glucose and

Table 1

Antropometric characteristics, blood pressure, heart rate

Baseline Characteristic	1st group, HOMA-IR<2.77 n=45	2nd group, HOMA-IR>2.77 n=36	P
Age, years	59.0 (50.0;69.0)	55.9 (52.9;58.8)	0.04
BMI, kg/m^2	29.77 (28.35;31.19)	33.50 (31.67;35.33)	<0.01
WC, cm	102.29 (99.55;105.03)	110.00 (104.00;121.25)	<0.01
Systolic blood pressure, mmHg	137,62 (133,59;141,65)	144,86 (140,86;148,86)	0.02
Diastolic blood pressure, mmHg	79,24 (76,41;82,07)	81,47 (78,04;84,90)	>0.05
Heart rate beats per minute	75,22 (72,38;78,06)	74,61 (70,82;78,40)	>0.05

Table 2

The value of biohemical parameters, adipokines and hsCRP

Baseline Characteristic	1st group, HOMA-IR<2.77 n=45	2nd group, HOMA-IR>2.77 n=36	P
ALT, U/l	19.36 (16.31;22.41)	19.30 (13.35;37.63)	0.10
AST, U/l	24.40 (22.01;26.79)	24.35 (19.30;29.08)	0.38
Uric Acid, mmol/l	303.35 (264.30;398.65)	360.97 (331.57;390.37)	0.09
HDL, mmol/l	1.19 (1.10;1.28)	1.20 (1.12;1,28)	0.27
LDL, mmol/l	2.73 (2.19;3.63)	2.83 (2,43;3.23)	0.45
Cholesterol, mmol/l	4.65 (3.91;5.53)	5.06 (4.67;5.45)	0.16
Triglycerides, mmol/l	1.28 (1.01;1.79)	1.98 (1.53;2.51)	<0.01
hsCRP, mg/l	1.76 (0.82;2.85)	3.05 (1.67;5.39)	<0.01
HbA1, %	4.99 (4.79;5.19)	5.22 (4.78;6.11)	0.02
Glucose, mmol/l	5.60 (5.38;5.82)	6.19 (5.76;6.92)	<0.01
Insulin, μIU/ml	6.66 (5.96;7.36)	16.55 (12.95;23.15)	<0.01
Leptin, ηg/ml	12,20 (5.60;16,20)	24.90 (14.05;47.10)	<0.01
Adiponektin, μg/ml	13.23 (11.45;15.01)	8.41 (5.89;12.75)	<0.01
Leptin /Adiponektin	0.93 (0.47;1.79)	2.82 (1.75;5.34)	<0.01

Leptin levels. At the same time, adiponectin levels negatively correlated with ALT, Uric acid, TG, HBA1, Glucose, Insulin, HOMA-IR and hsCRP.

Comprehensive epidemiological investigations recorded that the higher arterial pressure and LDL level were, the higher was the risk of death due to cardiovascular diseases (stroke, infarction and heart failure) [2].

According to modern knowledge about pathogenesis of CAD, AH and type 2 diabetes

Table 3

Evaluation of correlations between serum adipokines and insulin resistance in the subjects with cardiovascular disease (n = 81)

	Leptin	Adiponektin	HOMA-IR
BMI	0,375	NS	0,307
WC	0,347	NS	0,378
ALT	NS	-0,486	0,263
Uric Acid	NS	-0,397	0,287
Triglycerides	0,220	-0,227	0,482
hsCRP	0,376	-0,231	0,252
HbA1c	NS	-0,320	0,290
Glucose	0,237	-0,456	-
Insulin	0,443	-0,332	-
HOMA-IR	0,442	-0,374	-

NS – non-significant

mellitus, abdominal obesity, hyperinsulinemia and IR are independent risk factors and predictors of development of such diseases. Hyperinsulinemia of compensatory nature, which appears to overcome IR, results in activation of sympathoadrenal and renin-angiotensin systems, which, in its turn, causes increase in the levels of catecholamines, renin and angiotensin II. Increased level of insulin inhibits activity of lipoprotein lipase, which causes hypertriglyceridemia and decrease in concentration of high-density lipoproteins (HDL). This mechanism explains associations of increased level of triglycerides with impairments of carbohydrate metabolism [4].

To confirm the role of IR and hyperinsulinemia in pathogenesis of essential hypertension, numerous investigations were conducted, especially in the recent decade. Most of them established a significant correlation between insulin resistance/hyperinsulinemia and AH [5].

Considering a key role of atherosclerosis in pathogenesis of cardiovascular pathology, it should be mentioned that administration of statins in patients with confirmed cardiovascular disease is a compulsory measure of secondary prophylaxis. LDL level is a target index in this case, and medicines of choice are atorvastatin and rozuvastatin, which are most frequently prescribed in clinical practice [2]. Levels of total cholesterol, LDL and HDL did not differ reliably between groups, since patients in both groups were taking atorvastatin.

However, a reliable difference was observed between levels of triglycerides, elevation of their concentration is associated with IR (TG-HOMA-IR r =0.48, $p<0.05$), as atorvastatin does not have influence on triglycerides.

Besides influence on LDL, inhibitors of GMG-CoA-reductase demonstrate a wide spectrum of pleiotropic effects, including anti-inflammatory, antioxidant, antithrombotic ones, can stabilize atherosclerotic plaques and improve endothelium function. These properties can significantly reduce the incidence of complications and mortality due to cardiovascular diseases; reduce the need in hospitalization, increase duration and quality of life [6].

For patients with existing type 2 diabetes mellitus, benefit of statins is undoubted, since decrease in incidence of cardiovascular events and general mortality was demonstrated in many investigations, besides, positive effect of their intake in patients with type 2 diabetes mellitus exceeded the one in patients without type 2 diabetes mellitus. In patients with confirmed type 2 diabetes mellitus, aggressive statin therapy is recommended, which should be prescribed irrespective of the initial LDL level [7].

However, treatment with statins can have possible side effects. These effects usually depend on many factors, such as type, dose and duration of statin administration, patient's peculiarities (age, kidney function, condition of carbohydrate metabolism), and interaction with other medicines, prescribed for an underlying or concomitant pathology. One of the described side effects is negative influence on glucose metabolism and development of new cases of type 2 diabetes mellitus on the background of hypolipidemic therapy. Although benefit of statin therapy for cardiological patients is undoubted, an issue of their diabetogenic influence remains urgent and disputable [8, 9, 10, 11].

Our investigation was hospital-based and included patients with cardiovascular pathology combined with obesity: 45 without IR and 36 – with IR, aged 58.17 (55.95;60.39) years.

Besides assessment of factors of cardiovascular risk associated with IR, analysis of indices of patients' metabolic status was conducted depending on HOMA-IR value (Table 2). The results of investigation showed that the patients with HOMA-IR < 2.77 had reliably lower values of BMI and WC, levels of uric acid, triglycerides, hsCRP, Glucose, insulin, leptin and increased amount of adiponectin.

Analyzing demographic, anthropometric, biochemical and immunological indices of patients with cardiovascular pathology, who were administered atorvastatin and antihypertensive drugs, attention was paid to the most important correlations, associated with IR. A reliable correlation was observed between HOMA-IR and triglycerides (r = 0.48), leptin (r = 0.44), WC (r = 0.38) and BMI (r = 0.31). Less evident correlations were recorded between HOMA-IR and HbA1 (r = 0.29), uric acid (r = 0.29), ALT (r = 0.26) and hsCRP (r = 0.25). The role of leptin is important in the development of this pathology, indices of which positively correlated with HOMA-IR (r = 0.44), hsCRP (r = 0.38), BMI (r = 0.37) and WC (r = 0.35). Thus, only adiponectin indices negatively correlated with HOMA-IR, hsCRP, TG, Glu and insulin (Table 3).

Despite the fact that statin-induced diabetes mellitus is considered prognostically favorable [12], it is important not to allow/induce its development. On the background of statin intake and normalization of LDL in patients with obesity, negative influence of other factors associated with obesity and IR is recorded. Such factors are hyperleptinemia, hypoadiponectinemia, hyperuricemia and they should be considered during treatment of patients. In normal response of tissues to insulin, intensive statin therapy can be administered, whereas treatment scheme should be corrected in IR (correction of dose, combination of statins with ezetimibe, replacement with other type of statin).

CONCLUSION

Assessment of the risk of statin-induced diabetes mellitus is important for determining intensity of statin therapy, namely, presence of insulin resistance, levels of hsCRP and adipokines should be considered at the beginning and during treatment.

Conflict of interest. The authors of this manuscript claim that there is no conflict of interest during the research and writing of the manuscript.

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ВЗАЄМОЗВ'ЯЗОК МІЖ ІНСУЛІНОРЕЗИСТЕНТНІСТЮ, АДИПОКІНАМИ, ЛІПІДАМИ ТА НИЗЬКО-РІВНЕВИМ ЗАПАЛЕННЯМ У ПАЦІЄНТІВ З СЕРЦЕВО-СУДИННИМИ ЗАХВОРЮВАННЯМИ, ЯКІ ЛІКУВАЛИСЯ СТАТИНАМИ

Максимець Т.А., Склярова О.Є.

Львівський національний медичний університет імені Данила Галицького, Львів, Україна

maksymets.t@gmail.com

Актуальність. Гіполіпідемічна та гіпотензивна терапія дозволяє покращити прогноз та знизити ризик розвитку серцево-судинної патології, однак інтенсивний режим призначення статинів хворим на артеріальну гіпертензію (АГ) у поєднанні з ожирінням може сприяти розвитку гіперінсулінемії та інсулінорезистентності (ІР), що є предикторами виникнення цукрового діабету 2 типу.

Ціль: дослідити зв'язок між інсулінорезистентністю, адипокінами, ліпідами та низькорівневим запаленням у пацієнтів із серцево-судинними захворюваннями, які лікуються статинами.

Матеріали і методи. Залучено 81 пацієнта з надлишковою масою тіла та/або ожирінням та АГ або поєднанням АГ та ІХС. Вік хворих від 35 до 79 років, серед них 51 чоловік і 30 жінок. Для лікування АГ використовували інгібітори АПФ та БРА, діуретики; аторвастатин призначали перорально в дозі 20 мг або 40 мг на добу постійно. Ліпідний спектр, біохімічні показники та глікований гемоглобін визначали за загальноприйнятими методиками на аналізаторі "BioSystems" (Іспанія) з використанням оригінальних наборів реактивів. Концентрацію інсуліну та високочутливого С-реактивного протеїну (вчСРП) визначали на імунохемолюмінесцентному аналізаторі «Immulite 2000» (Siemens, Німеччина). Індекс інсулінорезистентності розраховували за формулою:

HOMA-IR =інсулін натще (мкМО/мл)*глюкоза натще (ммоль/л)/22,5

При значенні HOMA-IR > 2,77 пацієнти вважалися інсулінорезистентними.

Лептин вимірювали за допомогою набору ELISA DRG (США). Кількісне визначення адипонектину проводили за допомогою набору ELISA Mediagnost (Німеччина).

Результати. У нашому дослідженні було виявлено, що індекс маси тіла, рівень систолічного артеріального тиску, тригліцеридів, вчСРП та лептину були достовірно вищими у пацієнтів з ІР, тоді як рівень адипонектину був нижчим. Це підтверджується результатами вивчення кореляційних зв'язків між HOMA-IR, адипокінами, антропометричними та біохімічними параметрами. Таким чином, ретельна оцінка вуглеводного та адипокінового профілю у хворих із серцево-судинною патологією в поєднанні з ожирінням, яким проводиться гіполіпідемічна терапія, є важливим чинником профілактики цукрового діабету 2 типу.

Висновок. Оцінка ризику виникнення статин-індукованого цукрового діабету є важливою для визначення інтенсивності гіполіпідемічної терапії, а саме наявність інсулінорезистентності, рівень вчСРП та адипокінів слід враховувати на початку та під час лікування.

Ключові слова: інсулінорезистентність, артеріальна гіпертензія, аторвастатин, лептин, адипонектин.