ORIGINAL ARTICLE
(CC BY-SA)
UDC: 616-008.9:616.43
https://doi.org/10.2298/VSP180626132S

The correlation between metabolic syndrome quantification scores and numerous laboratory parameters related to this syndrome

Korelacija između kvantifikacionih skorova metaboličkog sindroma i brojnih laboratorijskih parametara udruženih sa njim

Branko Srećković*, Igor Mrdović[†‡], Ivan Soldatović[§], Mirko Resan[‖¶], Nenad Janeski, Emina Čolak[††], Hristina Janeski[‡‡], Mirjana Šumarac-Dumanović[‡§§], Miloš Joković[‡‖‖], Nebojša Ivanović*[‡], Jasna Gačić*[‡], Vesna Dimitrijević-Srećković[‡§§]**

***Clinical Center "Bežanijska kosa", Belgrade, Serbia; Clinical Centre of Serbia, [†]Clinic for Emergency Internal Medicine, [††]Institute of Medical Biochemistry, [§§]Clinic for Endocrinology, Diabetes and Metabolic Diseases, [‖‖]Clinic for Neurosurgery, Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, [§]Institute for Medical Statistics and Informatics, Belgrade, Serbia; Miltary Medical Academy, [‖]Clinic for Ophthalmology, Belgrade, Serbia; University of Defence, [¶]Faculty of Medicine od the Military Medical Academy, Belgrade, Serbia; **Clinical Centre Zemun, Belgrade, Serbia; [‡‡]University Children's Hospital, Belgrade, Serbia**

Abstract

Background/Aim. Metabolic syndrome (MS) is characterized by basic cluster risk factors – waist circumference (WC), glucoregulation disorders, hypertension, hypertriglyceridemia, low HDL-cholesterol followed by associated factors such as insulin resistance (IR), C-reactive protein (CRP), uric acid, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, hyperhomocysteinemia (HHcy), nonalcoholic fatty liver disease (NAFLD) and microalbuminuira. The aim of this study was to analyze basic and associated factors of MS in patients with and without MS as well as correlation of siMS score, siMS risk score with basic and confounding factors of MS. **Methods**. The study included 148 overweight [body mass index (BMI) 25–30 kg/m^2 and obese patients (BMI > 30 kg/m^2)], age 30–75 years, classified into two groups: I – with MS (68 patients); II – without MS (80 patients). For quantification of MS, siMS score was used as a method, and siMS risk score was used as atherosclerotic complications risk indicator. **Results.** Patients with MS had statistically higher values of WC, hypertension, triglycerides ($p < 0.001$), glycemia ($p = 0.006$), as well as values of associated factors of MS [homeostatic model assessment (HOMA-IR)] ($p = 0.002$), CRP ($p = 0.01$), uric acid ($p < 0.001$), alanin transaminase (ALT) ($p = 0.007$) i gammaglutamyl transferase (GGT) ($p = 0.001$) and lower values of HDL-cholesterol ($p < 0.001$) compared to patients without MS. siMS score has shown correlation with associated factors of MS (log HOMA IR, logCRP, uric acid, ($p < 0.001$), fibrinogen ($p = 0.005$), liver enzymes logALT ($p = 0.001$) and log GGT ($p < 0.001$) and renal parametars (creatinine ($p = 0.013$) and serum protein ($p = 0.006$). siMS risk score correlated significantly with homocysteine, platelets, uric acid, blood urea nitrogen, albumins and proteins. **Conclusion.** In our study we found that patients with MS had higher values of associated factors of MS (HOMA-IR, CRP, uric acid, ALT, GGT), which was confirmed by correlation with siMS score. siMS score further indicated that IR, CRP, fibrinogen, uric acid and NAFLD are associated factors of MS. siMS risk score is another score that indicated that obesity and hyperprotein diet aggravates HHCy with age, increasing the risk for renal dysfunction and promoting atherosclerotic complications.

Key words:
biomarkers; homocysteine; metabolic syndrome; risk assessment; risk factors.

Apstrakt

Uvod/Cilj. Metabolički sindrom (MS) karakterišu osnovni faktori rizika [obim struka (OS), poremećaji glikoregulacije, hipertenzija, hipertrigliceridemija, nizak HDL-holesterol] kao i pridruženi faktori rizika – insulinska rezistencija (IR), C-reaktivni protein (CRP), mokraćna kiselina, inhibitor aktivacije plazminogena-1 (PAI-1), fibrinogen, hiperhomocisteinemija (HHci), nealkoholna masna bolest jetre (NAMBJ) i mikroalbuminurija. Cilj rada bio je da se analiziraju osnovni

Correspondence to: Branko Srećković, Clinical Center "Bežanijska kosa", Bežanijska kosa bb, 11 000 Belgrade, Serbia.
E-mail: vesnadsendo@gmail.com

i pridruženi faktori rizika od MS kod bolesnika sa i bez MS i ustanovi korelacija siMS skora i siMS skora rizika sa osnovnim i pridruženim faktorima rizika od MS. **Metode.** Studijom su bila obuhvaćena 148 bolesnika sa prekomernom telesnom težinom [*body mass index* ((BMI) 25–30 kg/m^2) i gojazni (BMI > 30 kg/m^2), starosti 30–75 godina, podeljeni u dve grupe: I – sa MS (68 bolesnika) i II – bez MS (80 bolesnika). Korišćeni su siMS skor, kao metod za kvantifikaciju MS, i siMS skor rizika, kao indikator aterosklerotskih komplikacija. **Rezultati.** Bolesnici sa MS imali su statistički značajno više vrednosti OS, hipertenzije, triglicerida (p < 0,001), glikemije (p = 0,006), kao i pridruženih faktora rizika od MS [HOMA IR (p = 0,002) CRP (p = 0,01) mokraćne kiseline (p < 0,001), alanin aminotranferaze (ALT) (p = 0,007) i gama-glutamil transferaze (GGT) (p = 0,001)] i niže vrednosti HDL-holesterol, (p < 0,001) u odnosu na bolesnika bez MS. Skor siMS pokazao je korelaciju sa pridruženim faktorima MS [log HOMA IR, logCRP, mokraćnom kiselinom (p < 0,001) i fibrinogenom (p = 0,005), parametrima jetrene funkcije: logALT (p = 0,001), log GGT, (p < 0,001) i bubrežne funkcije: kreatininom (p = 0,013) i serumskim proteinima (p = 0,006)]. Skor siMS rizika je statistički značajno korelirao sa vrednostima homocisteina, trombocita, mokraćne kiseline, uree, albumina i proteina. **Zaključak.** Statistički značajno više vrednosti pridruženih faktora rizika od MS (HOMA-R, CRP, mokraćne kiseline, ALT, GGT) kod bolesnika sa MS potvrđene su i korelacijom sa siMS skorom. Skor siMS ukazuje na to da su insulinska rezisencija, CRP, fibrinogen, mokraćna kiselina, NAMBJ pridruženi faktori rizika od MS. Skor siMS rizika ukazuje na to da gojaznost i hiperproteinski unos povećavaju HHCi sa starenjem, te da povećavaju rizik od bubrežnih poremećaja i aterosklerotskih komplikacija.

Ključne reči:
biomarkeri; homocistein; metabolički sindrom; rizik, procena; faktori rizika.

Introduction

Hyperhomocysteinemia (HHcy) was found in some age-related clinical entities such as osteoporosis, hypothyroidism, cardiovascular diseases (CVD), cancer, end-renal stage disease and neurodegenerative diseases. Homocysteine (Hcy) is increased by several mechanisms as methionine enriched diets, defects in the methionine metabolism and B6, B12 and folate deficits [1].

Plasma Hcy directly correlates with age, waist circumference (WC), fasting glucose, triglyceride, uric acid, fibrinogen levels, insulin resistance, and inversely with creatinine clearance, and HDL-cholesterol [2].

Animal studies suggested HHcy as additional component of the metabolic syndrome (MS). Studies were based on theory that insulin might affect Hcy metabolism, in which hyperinsulinism caused increased levels of Hcy [3, 4]. Further studies have shown that MS and HHcy are established independent risk factors for CVD, and HHcy might be co-founding factor of MS [5, 6].

In our previous studies correlation of siMS score with Hcy indicated that Hcy is a co-founding factors of MS. [7] siMS score defined by Soldatović et al. [8] presents summary score of all MS factors [abdominal obesity, glycemia, systolic and diastolic blood pressure, triglycerides and high density lipoprotein (HDL)-cholesterol]. siMS score correlates with values of uric acid, microalbuminuria, fibrinogen, as well as with an inflammation parameter, C- reactive protein (CRP) [7]. Next clinical entity, nonalcoholic fatty liver disease (NAFLD) is also considered as a sign of MS. NAFLD is a chronic liver disease, which includes a spectrum of hepatic pathology from simple steatosis, steatohepatitis, to cirrhosis. Incresed Hcy may be associated with hepatic fat accumulation, both caused by hyperinsulinism [9]. Hcy induces endothelial cell injury and impairs vasodilatation by increased inactivation of nitric oxide and decreased generation of nitric oxide [10]. Hcy promote oxidative stress in vascular cells and tissues by reactive oxygen species (ROS), who have been shown to cause endothelial injury and the development of atherosclerosis [11]. Correlation between Hcy, hypertension and hyperlipoproteinemia indicated that Hcy could be promoting factor for atherosclerosis [12].

The aim of this study was to analyze and correlate MS cluster factors [WC, glycoregulation disorders, hypertension, hypertrigliceridemia, low HDL-cholesterol] and associated factors of MS [insulin resistance, CRP, uric acid, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, HHcy, NAFLD and microalbuminuria] in patients with MS and without MS. siMS score and siMS risk score correlation with basic cluster MS factors and associated factors were also examined.

Methods

The study included 148 overweight [body mass index (BMI) 25–30 kg/m^2] and obese (BMI > 30 kg/m^2) patients, aged 30–75 years, classified into two groups: I – with MS (68 patients), and II – without MS (80 patients). Measured anthropometric parameters were body weight (BW), body height (BH), BMI, and WC. BMI was calculated as BW in kilograms divided by the square of BH in meters. Blood pressure (BP) was measured in seating position using sphygmomanometer. Oral glucose tolerance test (OGTT) with 75 g glucose was used for estimation of glycoregulation early disorders. Values of glycemia and insulin were measured during OGTT in 0, 30 and 120 min. Lipid status was determined by total cholesterol, HDL-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides by spectrophotometer methods and apolipoprotein (Apo) A1, Apo B, Apo E and lipoprotein a [Lp(a)] by immunochemical methods. The Adult Treatment Panel (ATP) III classification was applied for diagnosing MS. A diagnosis of MS was confirmed if three out of five parameters were found as follows: WC > 102 cm for males and > 88 cm for females, BP > 135/85 mm/Hg, fasting blood glucose > 6.1 mmol/L, increased triglycerides (> 1.7 mmol/L), decreased HDL-C (< 1.03 mmol/L for males and HDL-C < 1.29 mmol/L for females). Patients who consumed more than 2 units of alcohol per day (for females), or 3 units per

day (for males), or more than 14 units per week (females) and 21 units per week (for males) were excluded from the study [one unit of alcohol (10 g) is equivalent to one glass of whiskey – 3 cL, or one glass of brandy – 3 cL, or one glass of wine – 20 cL, or one glass of beer – 25 cL) [13].

In this study we analyzed cluster factors of MS and associated factors such as insulin resistance, Hcy, CRP, PAI-1, fibrinogen, uric acid, liver and renal function parameters. Insulin was measured using radioimmunoassay method. Insulin resistance and insulin sensitivity was determined by Homeostatic Model Assessment Insulin Resistance (HOMA IR): HOMA-IR = insulinemia (mU/L) × glycaemia (mmol/L)/ 22.5 (cut off value is 3.2 μmol/mU/mL). Hcy as an independent marker of atherosclerosis was determined on Abbott´s Architect analyzer, using CMIA technology. Levels of CRP, as an inflammation marker, were determined by immunometric method. PAI-1, as an thrombogenic marker, was determined by plasminogen substrate essay. Liver function parameters determined were aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), albumin, total proteins. Renal function parameters, determined by immune-nephelometric method, were: urea, creatinine, creatinine clearance, microalbuminuria from 24-hour urine. Soldatovic et al. [8] established a new siMS score for MS quantification, simple for clinical use and scientific research.

The formula for siMS score using MS reference values is calculated as follows:

$$siMS\ score = \frac{2 \times Waist}{Height} + \frac{Gly}{5.6} + \frac{Tg}{1.7} + \frac{TA\ systolic}{130} - \frac{HDL}{1.03\ or\ 1.3\ (male\ or\ female)}$$

Age and positive family anamnesis were added to siMS score; siMS risk score, useful for cardio/cerebrovascular events risk evaluation, was thus obtained [14].

$$siMS\ risk\ score = siMS\ score \times \left(\frac{Age}{45\ or\ 50\ (males\ or\ females)}\right) \times \left(\frac{Family\ history\ of\ cardio\ or\ cerebro-vasular\ event}{(event = 1.2, else = 1)}\right)$$

Complete internist-cardiology examination: ECG, BP and other methods necessary or possible to determine the cardiac status were carried out.

Ethics

The Ethics Committee of the Faculty of Medicine, University of Belgrade approved the present study. All patients have given their consent.

Statistics

Data are presented as count (%) or mean ± standard deviation, depending on data type. Student's *t*-test and Mann-Whitney *U* test were used to assess significant differences between groups. Pearson's correlation was used to explore the significant relationship between Hcy and other parameters. All *p* values less than 0.05 were considered significant. All data were analyzed using SPSS 20.0 (IBM corp.) statistical software.

Results

Average age of 68 patients with MS was 46.69 ± 15.04 years, while average age of patients without MS was 47.73 ± 16.66 years ($p > 0.5$). MS was found in 45.95% of 80 patients. The gender distribution was as follows: in the MS group, there were 20.3% of male and 79.7% of female patients, while in the group of MS free patients, there were 5.6% of male and 94.4% of female patients.

Anthropometric parameters (BW, BMI, WC, systolic BP, diastolic BP, mean BP ($p < 0.001$) were statistically much higher in patients with MS than in patients without MS. Higher fasting glycemia ($p = 0.006$) and significantly higher values of triglycerides ($p < 0.001$) as well as lower HDL-cholesterol ($p < 0.001$) were also found in patients with MS (Table 1). The distribution of patients regarding to each criterion, showed that the increased WC had 88.0% of patients, 48.2% of patients had hypertension, 21.2% of patients had hyperglycemia, 45.9% of patients had increased triglyceride values and 38.1% of patients had decreased HDL-cholesterol.

Table 1

Anthropometrical and biochemical parameters in patients with metabolic syndrome (MS) and without MS

Parameter	With MS	Without MS	*p*-value
Age (years), mean ± SD	46.7 ± 15.0	47.7 ± 16.7	0.695
Gender (male), n (%)	21 (30.9)	15 (18.8)	0.086
Alcohol consumption, n (%)	13 (20.3)	4 (5.6)	0.009
BW (kg), mean ± SD	97.3 ± 20.1	82.7 ± 17.1	< 0.001
BMI (kg/m^2), mean ± SD	33.2 ± 6.1	29.5 ± 6.1	< 0.001
WC (cm), mean ± SD	105.8 ± 14.4	92.7 ± 14.2	< 0.001
sBP (mmHg), mean ± SD	135.8 ± 12.1	118.7 ± 11.2	< 0.001
d BP (mmHg), mean ± SD	88.1 ± 8.8	77.8 ± 8.8	< 0.001
BP mean (mmHg), mean ± SD	104.0 ± 8.9	91.4 ± 9-2	< 0.001
Cholesterol (mmol/L), mean ± SD	5.9 ± 1.2	5.8 ± 1.2	0.669
HDL-C (mmol/L), mean ± SD	1.22 ± 0.3	1.45 ± 0.3	< 0.001
LDL-C (mmol/L), mean ± SD	3.65 ± 1.2	3.7 ± 1.1	0.627
Triglycerides (mmol/L), mean ± SD	2.1 ± 0.9	1.3 ± 0.5	< 0.001
Glycemia (mmol/L), mean ± SD	5.4 ± 1.4	4.9 ± 0.8	0.006

BW – body weight; BMI – body mass index; WC – waist circumference; sBP – systolic blood pressure; dBP – diastolic blood pressure; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol; SD – standard deviation.

Table 2
Metabolic syndrome (MS) associated parameters in patients with metabolic syndrome (MS) and without MS

Parameter	With MS	Without MS	*p*-value
Homocysteine (μmol/L)	13.3 ± 3.6	12.9 ± 4.2	0.119
Insulin fasting (mlU/L)	31.2 ± 31.5	20.9 ± 16.8	0.007
Insulin at 120 minute (mlU/L)	61.6 ± 42.3	45.8 ± 45.4	0.014
Mean value insulin (mlU/L)	63.0 ± 52.8	52.8 ± 36.8	0.066
HOMA IR (μmol/mU/mL)	7.7 ± 8–6	4.5 ± 3.7	0–002
CRP (mg/dL)	5.5 ± 6.8	3.7 ± 5.4	0.00
Uric acid (μmol/L)	359.2 ± 85–6	307.3 ± 77.9	< 0.01
Fibrinogen (g/L)	268-5 ± 62.8	253.4 ± 63.1	0.153
Thrombocytes (10^9/L)	3.8 ± 0–8	3.7 ± 0–7	0.517
PAI-1 (U/mL)	5.98 ± 1.84	5.77 ± 1.78	0.776
AST (U/L)	25.2 ± 15.2	22.9 ± 7.6	0.227
ALT (U/L)	29.7 ± 20.3	23.0 ± 11.8	0.007
GGT (U/L)	28.7 ± 14.8	19.5 ± 11.9	< 0.01
Urea (mmol/L)	4.9 ± 1.3	4.7 ± 1.1	0.467
Creatinine (μmol/L)	76.9 ± 15.8	72.9 ± 15.1	0.115
Creatinine clearance (mL/min)	121.7 ± 57.3	111.4 ± 33.4	0.224
Microalbuminuria (mg/24 h)	81.9 ± 80.8	55.9 ± 57.4	0.225
ApoB (g/L)	1.64 ± 0.38	1.61 ± 0.27	0.603
ApoA1 (g/L)	1.12 ± 0.28	0.96 ± 0.26	0-004
Apo A2 (g/L)	357.9 ± 65.5	351.0 ± 66.6	0.674
Apo E (g/L)	47.0 ± 12.3	43.3 ± 14.5	0.255
Lp (a) (g/L)	0.166 ± 0.185	0.236 ± 0.290	0.223

The results are expressed as mean value ± standard deviation.
HOMA IR – Homeostatic Model Assessment of Insulin Resistance; CRP – C-reactive protein; PAI-1 – plasminogen activator inhibitor-1; AST – aspartate aminotransferase; ALT – alanine aminotrasferase; GGT – gamma glutamyl transferase; Apo – apolipoprotein; Lp – lipoprotein.

Insulin values (p = 0.007), insulin at 120 min during OGTT (p = 0.014), mean value insulin in OGTT (p = 0.066) and HOMA-IR (p = 0.002) were significantly higher in patients with MS. Higher Apo B (p = 0.01), CRP (p = 0.01), uric acid (p < 0.001) and liver enzymes ALT (p = 0.007) and GGT (p < 0.001) were also found in patients with MS. Thrombocytes, fibrinogen, PAI-1, urea, creatinine, creatinine clearance, microalbuminuria values were higher in patients with MS then in those without MS but without any significance (p > 0.5) (Table 2).

In order to determine the effect of MS on liver enzymes, a dual factorial analysis of variance was used, in which MS and alcohol consumption were independent variable, and ALT and GGT were dependent variables. Based on this analysis, it was found that MS correlates with ALT and GGT independently of alcohol consumption (p = 0.011; p < 0.001).

Presence of MS risk factors in patients with MS was as follows: 6.9% patients were with no MS factors, 17.2% had one MS factor, 31% had two MS factors, 26.9% had three MS factors, 15.2% had four MS factors and 2.8% had all five MS factors.

Hcy correlated significantly (Pearson's correlation) with values of thrombocites (p=0.046), urea (p = 0.002), creatinine (p = 0.006), creatinine clearance (p = 0.047) and siMS risk score (p = 0.015).

The siMS score confirmed significant correlation with log CRP, uric acid, log HOMA IR, log GGT (p < 0.001), log ALT (p = 0.001), thrombocytes (p = 0.01), fibrinogen (p = 0.005), proteins (p = 0.006), creatinine (p = 0.013). This risk score showed a statistically significant correlation with values of urea (p < 0.01), albumin (p = 0.003), total proteins (p = 0.057), thrombocytes (p = 0.046), uric acid (p = 0.038), and Hcy (0.015) (Table 3).

Table 3
Pearson' correlation analysis of siMS score and siMS risk score, and various parameters of metabolic syndrome (MS)

Parameter	siMS score	siMS risk score
Homocysteine	0.120 (0.177)	0.215 (0.015)
Log HOMA IR	0.457 (< 0.001)	0.130 (0.181)
Log CRP	0.333 (< 0.001)	-0.125 (0.189)
Uric acid	0.336 (< 0.001)	0.183 (0.038)
Fibrinogen	0.250 (0.005)	-0.099 (0.272)
Thrombocytes	0.281 (0.001)	-0.176 (0.046)
Log ALT	0.281 (0.001)	0.105 (0.237)
Log GGT	0.369 (< 0.001)	0.114 (0.211)
Total proteins	0.241 (0.006)	-0.168 (0.057)
Albumin	0.037 (0.681)	-0.265 (0.003)
Urea	0.040 (0.649)	0.388 (< 0.001)
Creatinine	0.218 (0.013)	-0.115 (0.191)

***Results are presented as correlation coefficient rho and *p*-value (in brackets).**
HOMA – Homeostatic Model Assessment; CRP – C-reactive protein; GGT – gamma glutamyl transferase.

Figure 1 shows the correlation between siMS score and log HOMA IR, log CRP, fibrinogen, log ALT, log GGT, and Hcy.

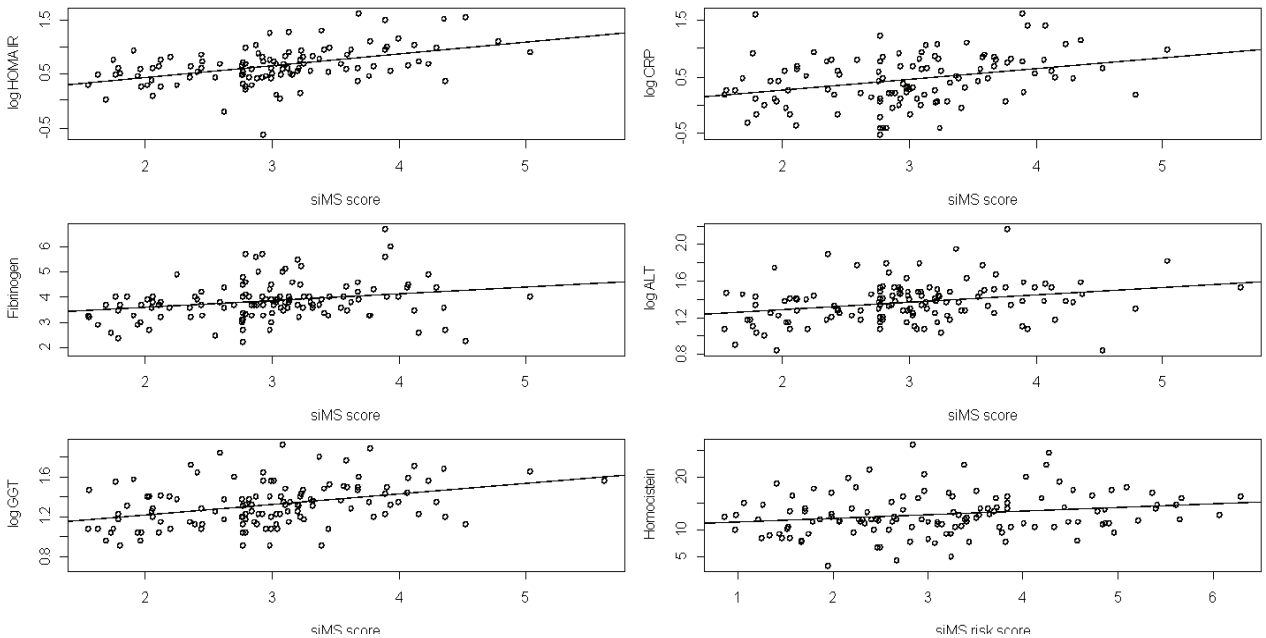

Fig. 1 – Correlation between siMS score and log Homeostatic Model Assessment Insulin Resistance (HOMA IR), log C-reactive protein, fibrinogen, log alanine aminotransferase (ALT), log gamma glutamyl transferase (GGT) and homocystein.

Discussion

Chronic diseases as diabetes, osteoporosis, hypothyroidism, as well as renal dysfunction and diet are considered to be associated with moderately elevated Hcy concentrations [15]. Hcy is amino acid formed in metabolism cycle of methionine to cysteine. HHcy is recognized as an independent risk factor for atherosclerosis [14]. Connection of Hcy and insulin resistance is explained by disruption of insulin signaling by Hcy interfering phosphorylation of insulin receptors. The result of this impaired insulin receptor signal cascade is lowered GLUT4 translocation to the plasma membrane and therefore reduced glucose uptake [16]. Catena et al. [2] showed that plasma Hcy was directly correlated with age, a factor of MS and insulin resistance, while inversely correlated with creatinine clearance and HDL-cholesterol, vitamin B12, and folate levels. A correlation of Hcy with hypertension and hyperlipoproteinemia in our previous studies indicates that Hcy can be an important indicator of risk for atherosclerotic complications and their progression [12]. Sheu et al. [17] found in their studies higher Hcy values in hypertensive patients than in normotensive ones, and significant correlation of plasma Hcy with insulin values in OGTT was also found. The latest results of our studies showed a positive correlation of Hcy with a long term glycoregulation parameter HbA1C, HOMA-IR, Apo B, and negative correlation with Apo E. The siMS score significantly correlated with Hcy, uric acid, microalbuminuria, a thrombosis factor – fibrinogen, an inflammation factor – CRP, and confirmed that these are metabolic syndrome assiciated factors [7]. Our study in patients with coronary heart disease showed correlation between Hcy and systolic BP, triglycerides and uric acid, which confirms association of Hcy with insulin resistance and MS as well as the further risk of atherosclerosis complications [18].

Patients with MS covered by the present study were characterized by statistically important much higher values of anthropometric parameters (BW, BMI, WC), BP, triglycerides, insulinemia in OGTT at 0 min and 120 min, mean value of insulin levels, HOMA-IR, CRP, uric acid, Apo B as well as liver function parameters ALT and GGT as markers of NAFLD, and statistically lower HDL-cholesterol. Summarized above mentioned, these results showed that patients with MS had higher values of basic cluster factors of MS (WC, hypertension, hyperlipoproteinemia type IV) as well as values of associated factors of MS such as hyperinsulinemia, insulin resistance, CRP, uric acid and NAFLD.

Abdominal obesity and insulin resistance have a significant role in MS development [19]. Recent studies have shown that patients with MS have significantly higher levels of high sensitive CRP, compared to the control group, which is a marker of chronic inflammation in patients with MS, whose values increased linearly with the increase number of factors for MS [20]. Obesity is characterized by elevated levels of inflammatory factors such as CRP and prothrombogenic factors such as fibrinogen, which occur before other MS disorders and are useful in the assessment of cardiovascular risk [21].

Results obtained by Dimitrijević-Srećković et al. [22] indicate the existence of NAFLD even in the youngest obese population: children (7.3%), adolescents (18.9%), and youth 20 to 30 years old (29%). NAFLD is a liver sign of MS, while youth with NAFLD manifested, besides increased ALT and GGT values, abdominal obesity, hyperinsulinism in OGTT, pronounced insulin resistance, increased triglyc-

erides, CRP and uric acid. The study of Čolak et al. [23] have also shown elevated liver enzymes in obese students with increased risk for CVD. Other studies of obese and adolescent population indicate the association of NAFLD with insulin resistance [24].

In the present study, siMS score showed a correlation with MS associated factors (log CRP, uric acid, log HOMA-IR, fibrinogen, thrombocytes), liver parameters (log ALT, log GGT) and hyperproteinemia, retention of nitrogen substances and increased risk for kidney damage. The correlation of siMS score with liver function parameters indicates that fatty liver is a MS associated factor. Hcy is an intermediate in methionine metabolism, which takes place mainly in the liver [25]. Impaired remethylation of Hcy to methionine leads to increased levels of Hcy promoting the liver damage from NAFLD to non alcoholic steatohepatitis [26].

Correlation of siMS score with renal function parameters, creatinine and total proteins, as shown in the present study, indicates that even initial renal function disorders can represent MS associated factors. Higher values of microalbuminuria in patients with MS, compared to patients without MS, indicate the initial stage of kidney damage in obese patients. Our previous study has shown the appearance of microalbuminuria in obese children, adolescents and young people, which is normalized after weight reduction [27]. Correlation of homocysteine with platelets, renal function parameters (urea, creatinine, creatinine clearance) and siMS risk score was also confirmed. Hyperprotein diet based on meat and dairy products, most frequent in MS obese people nutrition, can contribute to increased glomerular filtration with increased creatinine clearance and provoke HHcy and renal damage. Berstad et al. [28] have shown that higher intake of animal saturated fatty acids correlates positively with higher Hcy levels. Microalbuminuria as associated factor of MS is a strong indicator of CVD and renal dysfunction. It is suggested that HHcy enhances oxidative stress, inducing endothelial and mesangial cell dysfunction, resulting in microalbuminuria [29]. High animal-protein diet correlates positively with high Hcy levels, whereas high plant-protein diet inversely correlates with total Hcy levels [30]. A correlation of siMS score with liver and renal function parameters indicates that disorders of these systems could appear in obese MS patients as associated MS factors. HHcy can be caused by increased intake of proteins from dairy and meat products abounding in saturated fats of animal origin and reduced intake of vegetables rich in folic acid, which all contributes to progression of atherosclerotic complications, fatty liver and renal damage.

In the present study, siMS risk score correlated with Hcy, platelets, uric acid and renal function parameters (urea, total albumins and proteins). Our results also indicate that HHcy increases with age and represents a vascular complications risk indicator. Correlation with uric acid indicates that hyperproteinic intake could contribute considerably to vascular complications and values of total proteins and albumins.

Mediterranean diet, rich in dietary fibers and complex carbohydrates in fruits, vegetables and cereals, monounsaturated fats in olive oil, omega-3 polyunsaturated fats in fish and reduction of saturated fats and proteins of animal origin proved favorable effects on body mass reduction, glycoregulation, hypertension, lipid status, insulin resistance, inflammatory and thrombotic factors, and HHcy [30]. Han et al. [31] highlight the importance of increasing folic acid and vitamin B supplementation, diet which consists of daily fruit and vegetable intake, healthy lifestyle based on regular exercise and refraining from tobacco smoking and alcohol consumption for prevention of HHcy.

Conclusion

Patients with MS had statisticaly significant higher values of MS associated factors (HOMA-IR, CRP, uric acid, ALT, GGT) which correlated well with siMS score. siMS score correlation with fibrinogen, creatinine and proteins indicated that thrombosis factors, so as renal function parametars could be associated with MS. Used as a method of MS quantification, siMS score confirmed that MS patients have an increased risk for glycoregulation disorders – prediabetes and diabetes type 2 (abdominal obesity followed by hyperinsulinism and insulin resistance), hyperlipoproteinemia type IV (elevated triglycerides and low HDL-cholesterol), and hypertension. siMS score further indicated that insulin resistance, IR, CRP, fibrinogen, uric acid and NAFLD are associated factors of MS. siMS risk score is another score that indicated that obesity and hyperprotein diet aggravates HHCy with age, increasing the risk for renal dysfunction and promoting atherosclerotic complications.

REFERENCES

1. *Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN.* The metabolism and significance of homocysteine in nutrition and health. Nutr Metab (Lond) 2017; 14: 78.
2. *Catena C, Colussi G, Nait F, Capobianco F, Sechi LA.* Elevated Homocysteine Levels Are Associated With the Metabolic Syndrome and Cardiovascular Events in Hypertensive Patients. Am J Hypertens 2015; 28(7): 943–50.
3. *Fonseca V, Dicker-Brown A, Ranganathan S, Sing W, Barnard RJ, Fink L,* et al. Effects of high-fat-sucrose diet on enzymes in homocysteine metabolism in the rat. Metabolism 2000; 49(6): 736–41.
4. *Oron-Herman M, Rosenthal T, Sela BA.* Hyperhomocysteinemia as a component of syndrome X. Metabolism 2003; 52(11): 1491–5.
5. *Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK,* et al. Metabolic szndrome and risk incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 2007; 49(4): 403–14.
6. *Garcin JM, Cremades S, Garcia-Hejl C, Bordier L, Dupuy O, Mayaudon H,* et al. Is hyperhomocysteinemia an additional risk factor of the metabolic syndrome? Metab Syndr Relat Disord 2006; 4(3): 185–95.

7. *Srećković B, Soldatovic I, Colak E, Mrdovic I, Sumarac-Dumanovic M, Janeski H,* et al. Homocysteine is the confounding factor of metabolic syndrome-confirmed by siMS score. Drug Metab Pers Ther 2018; 33(2): 99–103.
8. *Soldatovic I, Vukovic R, Culafic D, Gajic M, Dimitrijevic-Sreckovic V*. siMS Score: Simple Method for Quantifying Metabolic Syndrome. PLoS One 2016; 11(1): e0146143.
9. *de Carvalho SC, Muniz MT, Siqueira MD, Siqueira ER, Gomes AV, Silva KA,* et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutr J 2013; 12: 37.
10. *Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA*. Hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation 1997, 95: 1119–21.
11. *Papatheodorou L, Weiss N*. Vascular oxidant stress and inflammation in hyperhomocysteinemia. Antioxid Redox Signal 2007; 9(11): 1941–58.
12. *Sreckovic B, Sreckovic VD, Soldatovic I, Colak E, Sumarac-Dumanovic M, Janeski H,* et al.. Homocysteine is a marker for metabolic syndrome and atherosclerosis. Diabetes Metab Syndr 2017; 11(3): 179–82.
13. *Abd El-Kader SM, El-Den Ashmawy EM*. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol 2015; 7(6): 846–58.
14. *McCully KS*. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969; 56(1): 111–28.
15. *Faeh D, Chiolero A, Paccaud F*. Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about? Swiss Med Wkly 2006; 136(47–48): 745–56.
16. *Li Y, Jiang C, Xu G, Wang N, Zhu Y, Tang C,* et al. Homocysteine upregulates resistin production from adipocytes in vivo and in vitro. Diabetes 2008; 57(4): 817–27.
17. *Sheu WH, Lee WJ, Chen YT*. Plasma homocysteine concentrations and insulin sensitivity in hypertensive subjects. Am J Hypertens 2000; 13(1Pt 1): 14–20.
18. *Srećković B*. Determination of homocysteine level as an independent risk factor for coronary heart disease [subspeciality thesis]. Belgrade: Faculty of Medicine, University of Belgrade; 2010. (Serbian)
19. *Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C*. American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109(3): 433–8.
20. *Gowdaiah PK, Mamatha TR, Nirgude D, Hosamani PB*. High sensitivity C-reactive protein in metabolic syndrome. Int J Adv Med 2016; 3(3): 607–10.
21. *Mauras N, Delgiorno C, Kollman C, Bird K, Morgan M, Sweeten S*, et al. Obesity without established comorbidities of the metabolic syndrome is associated with a proinflammatory and prothrombotic state, even before the onset of puberty in children. J Clin Endocrinol Metab 2010; 95(3): 1060–8.
22. *Dimitrijević Srećković V*. Metabolic syndrome, prediabetes and non alcoholic fatty liver diseases- treatment by Mediterranean diet. In: *Dimitrijević Srećković V, Vukašinović V,* editors. FASTING-A WAY TO LIFE- Christian fasting as a method for preventing and treating diabetes, atherosclerosis and cancer. Vrnjci: Interklima-grafika; 2013. p. 37–49.
23. *Čolak E, Pap D, Majkić Singh-N, Obradović I*. The Association of obesity and liver enzyme activities in a student population at Increased Risk for Cardiovascular Disease. J Med Biochem 2013; 32(1): 26–31.
24. *Ciba I, Widhalm K*. The Association between the non-alcoholic fatty liver disease and insulin resistance in obese children and 20 adolescents. Acta Pediatr 2007; 96: 109–12.
25. *Garcia-Tevijano ER, Berasain C, Rodriguez JA, Corrales FJ, Arias R, Martin-Duce A,* et al. Hyperhomocysteinemia in liver cirrhosis: mechanisms and role in vascular and hepatic fibrosis. Hypertension 2001; 38(5): 1217–21.
26. *Pacana T, Cazanave S, Verdianelli A, Patel V, Min HK, Mirshahi F,* et al. Dysregulated Hepatic Methionine Metabolism Drives Homocysteine Elevation in Diet-Induced Nonalcoholic Fatty Liver Disease. PLoS One 2015; 10(8): e0136822.
27. *Dimitrijević-Srećković V*. Metabolic sindrome in children and adolescents. In: *Nedeljković S,* editor. Yugoslav study of atherosclerosis precursors in school children, 20 years of follow-up. Belgrade: Faculty of Medicine, University of Belgrade; 2011. p. 731–45. (Serbian)
28. *Berstad P, Konstantinova SV, Refsum H, Nurk E, Vollset SE, Tell GS*, et al. Dietary fat and plasma total homocysteine concentrations in 2 adult age groups: the Hordaland Homocysteine Study. Am J Clin Nutr 2007; 85(6): 1598–605.
29. *Jager A, Kostense PJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM,* et al. Serum homocysteine levels are associated with the development of (micro)albuminuria: the Hoorn study. Arterioscler Thromb Vasc Biol 2001; 21(1): 74–81.
30. *Dimitrijević-Srećković V*. Mediterranean cardioprotective nutrition. In: *Nedeljković S,* editor. Yugoslav study of atherosclerosis precursors in school children, 20 years of follow-up. Belgrade: Faculty of Medicine, University of Belgrade; 2011. p.
31. *Han L, Liu Y, Wang C, Tang L, Feng X, Astell-Burt T,* et al. Determinants of hyperhomocysteinemia in healthy and hypertensive subjects: A population-based study and systematic review. Clin Nutr 2017; 36(5): 1215–30.

Received on June 26, 2018
Revised on August 17, 2018
Accepted on September 3, 2018
Online first September 2018.