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INVERSE ASSOCIATION OF SERUM BILIRUBIN WITH METABOLIC SYNDROME AND INSULIN RESISTANCE IN POLISH POPULATION

ODWROTNY ZWIĄZEK STĘŻENIA BILIRUBINY W SUROWICY Z ZESPOŁEM METABOLICZNYM I INSULINOOPORNOŚCIĄ U OSÓB DOROSŁYCH W POLSCE

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STRESZCZENIE

WSTĘP. Bilirubina ma potencjalne właściwości antyoksydacyjne i cytoprotekcyjne. Stwierdzono, że jej stężenie odwrotnie koreluje z chorobami sercowo- metabolicznymi. Niedawno przeprowadzone badania wykazały związek stężenia bilirubiny w surowicy z zespołem metabolicznym (ZM) pośród dzieci i młodzieży w USA oraz u osób dorosłych w Korei . Celem pracy była ocena związku stężenia bilirubiny całkowitej we krwi z zespołem metabolicznym i insulinoopornością w Polsce

METODY. Zbadaliśmy 1568 osób w wieku od 18 do 93 lat. Badane osoby stanowiły reprezentatywną próbę dorosłych mieszkańców Polski i pochodziły z przekrojowego badania, w trakcie którego oznaczono stężenie bilirubiny całkowitej w surowicy oraz czynniki ryzyka chorób sercowo- naczyniowych.

WYNIKI. Rozpowszechnienie ZM w poszczególnych kwartylach stężenia bilirubiny od pierwszego do czwartego (95%CI w nawiasach) wynosiło odpowiednio 28.9% (24.5%-33.3%), 32.6% (28.3%-36.9%), 23.4% (19.0%-27.8%), 21.8% (17.5%-26.2%) (p=0.002). Analiza wieloczynnikowa wykazała, że iloraz szans dla posiadania ZM w trzecim i czwartym kwartylu bilirubiny wynosił odpowiednio 0.70 (0.50-0.99) i 0.68 (0.48-0.95), przy przyjęciu najniższego kwartyla stężenia bilirubiny jako referencyjnego. Ze wzrostem liczby spełnionych kryteriów ZM obserwowano zmniejszanie się średniego stężenia bilirubiny całkowitej w surowicy (p=0,012). W badanej grupie wykazano również silny, niezależny odwrotny związek stężenia bilirubiny z insulinemią na czczo i insulinoopornością (HOMA IR). Iloraz szans dla występowania insulinooporności w czwartym kwartylu bilirubiny wynosił 0.53 (0.38-0.74) przyjmując najniższy kwartyl bilirubiny jako referencyjny.

WNIOSKI. U dorosłych osób w Polsce poziom całkowitej bilirubiny w surowicy odwrotnie koreluje z rozpowszechnieniem ZM i insulinoopornością.

SŁOWA KLUCZOWE: *bilirubina w surowicy, zespół metaboliczny, insulinooporność*

ABSTRACT

BACKGROUND. Bilirubin has got a potential anti-oxidant, anti-inflammatory and cytoprotective effect. It has been shown that its concentration is inversely related to cardiometabolic diseases. Recent studies have revealed the association between serum bilirubin concentrations and metabolic syndrome (MS) among children and adolescents in U.S. and among Korean adults. The aim of this study was to evaluate the association of total serum bilirubin level with MS and insulin resistance in Poland.

METHODS. We examined 1568 patients aged 18 to 93 years. The tested population was a nationally representative sample of Polish adults. They were derived from cross-sectional study, when serum total bilirubin level and risk factors of cardiovascular diseases were determined.

RESULTS. The prevalence of MS in bilirubin level quartiles (95%CI in parentheses) was 28.9% (24.5%-33.3%), 32.6% (28.3%-36.9%), 23.4% (19.0%-27.8%), 21.8% (17.5%-26.2%) respectively for quartiles 1-4 (p=0.002). The multivariate analysis showed odds ratio for MS in third and fourth quartile of bilirubin level equal to 0.70

(0.50-0.99) and 0.68 (0.48-0.95) respectively in comparison to the lowest quartile. The more criteria of metabolic syndrome were fulfilled by the patient, the lower was mean total bilirubin level (p=0.012). In study group there was also a strong, independent association of bilirubin level with fasting insulin level and insulin resistance (HOMA-IR). The odds ratio of insulin resistance was 0.53 (0.38-0.74) for the fourth quartile in reference to the lowest quartile of bilirubin.

CONCLUSION. In Polish adults serum total bilirubin level is inversely related to the prevalence of MS and insulin resistance.

KEY WORDS: *serum bilirubin, metabolic syndrome, insulin resistance*

INTRODUCTION

Bilirubin, a metabolic end product of heme breakdown, has got potential anti-oxidant, anti-inflammatory and cytoprotective properties (1) that play a protective role in various cardiovascular and metabolic diseases. The studies have shown an inverse relationship of serum total bilirubin level and risk of coronary artery disease (CAD) (2,3). In these studies a protective effect of bilirubin on the risk of CAD was comparable to that of high-density lipoprotein cholesterol (HDL-C) (2). An inverse association of serum total bilirubin level with the severity of CAD and cardiovascular morbidity and mortality was confirmed (3). An inverse relationship between serum total bilirubin level and cardiovascular diseases (CVD), peripheral vascular disease (PVD), carotid intimal media thickness and stroke was also shown (4,5,6).

Metabolic syndrome (MS) is a collection of interrelated cardiometabolic risk factors that includes abdominal obesity, insulin resistance, hypertension and dyslipidemia. This set of risk factors is connected with an increased risk of cardiovascular diseases and type 2 diabetes (7). The precise pathogenesis of MS is still unknown. Insulin resistance , adipokines , oxidative stress and chronic inflammation are suggested to cause MS (7,8). Bilirubin itself or via enzymes taking part in metabolic process of its production may influence these pathogenetic mechanisms(9,10). Recent study has revealed an inverse association of bilirubin level with metabolic syndrome and insulin resistance among children and adolescents in U.S.(11). Similar inverse relationship between bilirubin and metabolic syndrome has been shown among the Korean adult population (12). Such study has never been performed in adult population of Caucasians. The aim of our study was to establish the association of serum bilirubin level with the prevalence of metabolic syndrome and insulin resistance in a nationally representative sample of adults from Poland which is a country representing a high risk for cardiovascular diseases (CVD) in Central-Eastern Europe region.

MATERIALS AND METHODS

Study design and population

In this study the data of country-representative sample of adult Polish inhabitants aged 18 to 93 years was used. Adult population of Poles consists of about 28 million people. The sample was derived from a cross--sectional study conducted in 2002, whose main aim was to evaluate the prevalence of cardiovascular diseases risk factors in Poland. Multi-stage random sampling scheme was planned so that each country inhabitant ≥ 18 years of age had an identical probability of being drawn to participate in this study. The respondents lived in 300 clusters drawn from the territory of the whole country. Precise algorithm of sampling was previously described in other reports (13).

Total study sample count was 3051 people. All records with incomplete laboratory data were excluded from our analysis. Also subjects with bilirubin level ≥ 34.2 μmol/L were excluded in order to rule out patients with impaired liver function. The final number of analyzed records was 1568 (683 men, *mean age* [±*SD*] 45,8±15,9 years and 885 women, *mean age* [±*SD*] 46,9±16,6 years). The examined sample was divided into groups according to particular bilirubin level quartiles.

Anthropometric and biochemical data

For each respondent a questionnaire was completed concerning risk factors of cardiovascular diseases including arterial hypertension (AH), diabetes, cigarette smoking.

Each respondent underwent the following anthropometric measurements: body mass, waist circumference, arm circumference and blood pressure.

Waist circumference was measured to the nearest 0.5 cm in a standing position in the widest point perpendicularly to the body axis.

Blood pressure was measured using automatic blood pressure measurement devices Omron M5I, validated by the Association for the Advancement of Medical Instrumentation (AAMI). The cuff's size was adapted

to the arm circumference of each person examined. Each respondent had three blood pressure readings taken during one visit. Mean systolic and diastolic blood pressure (SBP and DBP) values from the second and the third measurement were taken for analysis. People whose mean values were ≥ 140 mmHg or ≥ 90 mmHg respectively and who did not use hypotensive medication underwent another series of blood pressure measurements during two following visits in order to confirm the diagnosis of AH. However, data from the additional measurements were not used in our study.

Blood samples were collected from people who gave their consent for the procedure. Respondents were asked to avoid food and sweet drinks for 12 hours preceding blood sampling. The samples obtained were then transported to the local laboratories within 2 hours, where they were processed in order to obtain serum and plasma in separate secondary test-tubes.

Material obtained was used for the following analyses: total cholesterol, HDL-cholesterol, triglycerides, glucose, bilirubin, insulin and high-sensitive C-reactive protein (hs-CRP).

Laboratory tests were performed in a certified central laboratory with certificate of accreditation number AB 260 given by the Polish Center of Accreditation (Polskie Centrum Akredytacji).

Total bilirubin level was determined by a photometric method (Hitachi 911, DIASys Diagnostic Systems GmbH&Co.KG, Holzheim Germany), total cholesterol by an enzymatic photometric method (Hitachi 911, FS 10135023, DIASys Diagnostic Systems GmbH&Co. KG, Holzheim Germany), HDL-cholesterol by direct enzymatic (Hitachi 911, HDL-Cplus 2nd generation 3030024, Roche Diagnostic GmbH, D-68298 Mannheim, Germany), triglycerides by enzymatic colorimetric (FS 10576023, DIASys Diagnostic Systems GmbH&Co.KG, Holzheim Germany).

Plasma fasting glucose level was measured in central laboratory by enzymatic method (Hitachi 911, Glucose Hexokinase FS 10250023, DIASys Diagnostic Systems GmbH&Co.KG, Holzheim Germany). High-sensitive C-reactive protein (hs-CRP) was assessed by nephelometric method (Behring Nephelometer 100 Analyzer, N High Sensitivity CRP OQIY2, Dade Behring). Fasting insulin level was evaluated by immunoenzymatic method NEIA (Abbott Axsym System, Abbott Laboratories Diagnostics Division IL 30064, USA, Insulin Reagent Pack 2D01-20). Fasting insulin level and insulin resistance (HOMA) were regarded as elevated when values belonged to the fourth quartile for the population.

Insulin resistance status was estimated by homeostasis model assessment (HOMA-IR) as previously described (14).

In this study we selected the group of people affected with MS using its new unified definition that was published in 2009 (15).

Statistical analysis

Variables with positively skewed distribution underwent logarithmic transformation: bilirubin, triglycerides, HDL-cholesterol, hs-CRP, systolic blood pressure (SBP), diastolic blood pressure (DBP). Reported mean values were back-transformed.

We compared prevalence of MS and insulin resistance between serum bilirubin quartiles using analysis of covariance (ANCOVA) with gender included in model as confounder.

Multivariate analysis was performed to evaluate the independent relationship between particular MS components and bilirubin level. Multiple logistic regression model was used. The results were given as odds ratio of particular MS component occurrence depending on the actual bilirubin quartile group that the respondent belonged to. Statistical analysis was performed with use of SAS 9.1 system for Windows (SAS, Cary, NC).

RESULTS

Clinical and biological characteristics of the study population are presented in table I. Body mass index (BMI), waist circumference, triglycerides level, total cholesterol level, systolic and diastolic blood pressure (SBP and DBP), glucose level, fasting insulin level, HOMA-IR, hs-CRP and age were higher in patients with MS. However, HDL-cholesterol level was higher in people without MS. Bilirubin level was lower in patients with MS in comparison to those without MS. Univariate analysis revealed higher mean level of total bilirubin in men when compared to women (9.75 vs. 8.72 μmol/L, p<0,0001). There was no association of mean bilirubin level with subjects' age observed. Mean bilirubin level in smokers was lower than in non-smokers (8.55 vs. 9.40 μmol/L , p=0.0009).

According to distribution of bilirubin level in quartiles the prevalence of MS relatively decreased as bilirubin level quartiles increased and it was 28.9% (95%CI: 24.5%-33.3%), 32.6% (95%CI: 28.3%-36.9%), 23.4% (95%CI: 19.0%-27.8%), 21.8% (95%CI: 17.5%-26.2%) respectively, (p=0.002) – (Fig. 1).

The results of multivariate analysis of relationship between bilirubin level and MS are presented in table II. In the study group of people belonging to the third and the fourth bilirubin level quartile the risk of MS was lower with OR 0.70 (0.50-0.99) and 0.68 (0.48-0.95) for the third and fourth quartile respectively when the lowest quartile of bilirubin level was used as a reference.

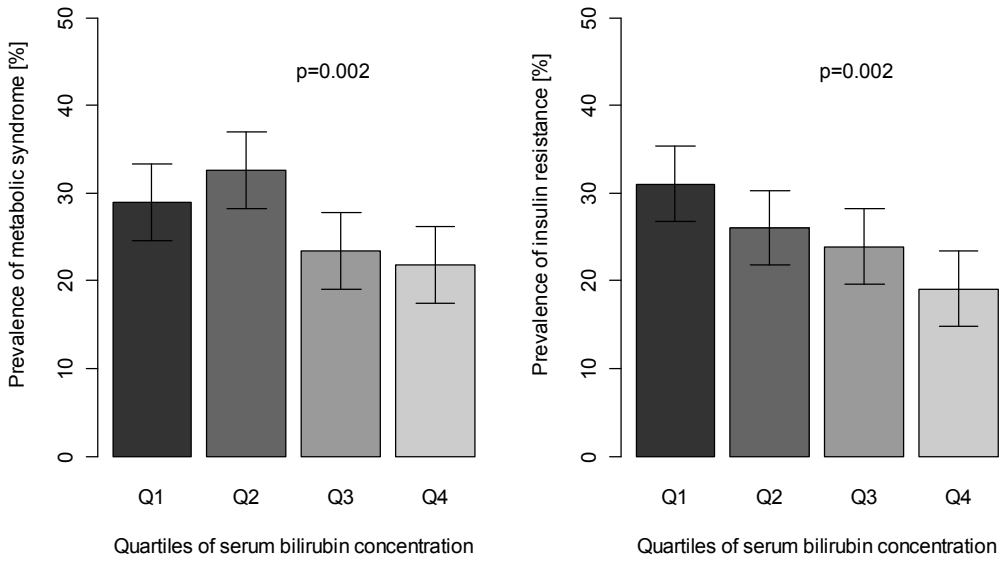

Fig. 1. Prevalence of MS and insulin resistance in relation to serum bilirubin concentration (adjusted for gender)
Ryc. 1. Rozpowszechnienie ZM i insulinooporności w zależności od stężenia bilirubiny w surowicy (skorygowane względem płci)

In the study population with an increase of MS criteria fulfilled the decrease of mean serum bilirubin level was observed (p=0.01) – (Fig. 2).

The results of multivariate analysis of relationship between bilirubin level and cardiometabolic risk factors are presented in table II. Among variables analyzed the strongest negative correlation with bilirubin level was revealed for triglycerides. The odds ratio of elevated triglycerides level was 0.39 (0.28-0.55) for the fourth quartile of bilirubin level in reference to the first one.

In the study population there was an independent association of bilirubin level with fasting insulin level and insulin resistance (HOMA-IR). Belonging to the highest quartile of bilirubin level was connected with almost half lower risk of having elevated fasting insulin level – OR=0.55 (0.39-0.76) and insulin resistance – OR=0.53 (0.38-0.74).

We observed also a strong negative influence of cigarette smoking on bilirubin level values (see table II).

Table I. Comparison of clinical and biological differences between patients with and without MS
Tabela I. Porównanie klinicznych i biologicznych różnic pomiędzy pacjentami z i bez ZM

Variable	Without MS (N=1149)			With MS (N=419)			p-value for difference
	Estimated mean	95% LCL	95% UCL	Estimated mean	95% LCL	95% UCL	
age [years]	42.1	41.1	43.0	51.9	50.3	53.4	<0.0001
percentage of men	43.4	40.6	46.3	43.9	39.2	48.7	0.8639
bilirubin* [µmol/L]	8.21	7.87	8.38	7.52	7.18	8.04	0.0349
BMI [kg / m^2]	24.8	24.5	25.0	29.1	28.7	29.6	<0.0001
waist circumference [cm]	85.2	84.5	85.9	98.5	97.5	99.6	<0.0001
Triglycerides * [mmol/L]	1.12	1.10	1.15	2.13	2.02	2.23	<0.0001
total cholesterol * [mmol/L]	5.11	5.05	5.17	5.69	5.57	5.81	<0.0001
HDL-cholesterol * [mmol/L]	1.46	1.45	1.48	1.17	1.14	1.19	<0.0001
SBP* [mmHg]	129.1	128.0	130.2	144.3	142.2	146.4	<0.0001
DBP * [mmHg]	80.2	79.5	80.9	88.9	87.7	90.2	<0.0001
fasting glucose * [mmol/L]	4.62	4.59	4.65	4.97	4.87	5.07	<0.0001
fasting insulin * [pmol/L]	42.4	40.3	43.7	66.7	62.5	71.5	<0.0001
HOMA IR*	1.24	1.19	1.29	2.13	1.98	2.29	<0.0001
hsCRP * [nmol/L]	10.6	9.9	11.3	18.4	16.7	20.4	<0.0001
Current smoker [%]	34,3	31,5	37,1	32,8	28,1	37,4	p=0.5795

*variables transformed logarithmically

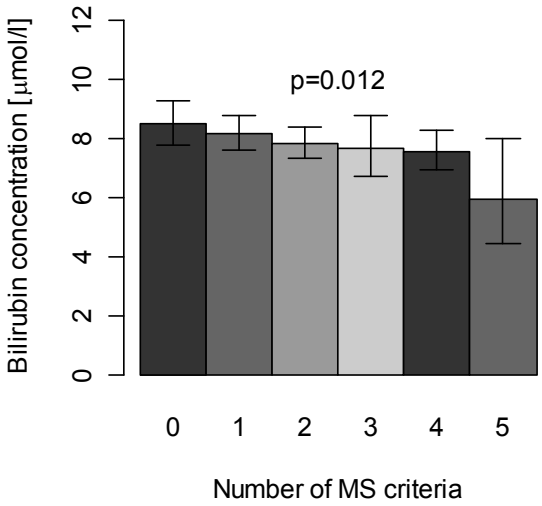

Fig. 2.. Mean serum bilirubin concentration in relation to the number of MS criteria (adjusted for gender)

Ryc. 2. Średnie stężenie bilirubiny w surowicy w zależności od liczby kryteriów ZM (skorygowane względem płci)

DISCUSSION

Our cross-sectional country-representative study has revealed an inverse relationship between serum total bilirubin level and MS. Our results are consistent with the report by Lin et al. which presented such a relationship for children and adolescents in the USA (11) and with the results of Jo et al. for the adult Korean population (12). In the latter report such association was observed both for total serum bilirubin and its fractions, however the strongest relationship was stated for direct bilirubin. Likewise, previous study performed among Koreans revealed the strongest correlation between MS and direct bilirubin (16). Our report is cross-sectional country-representative study conducted among European Caucasian adult population.

One of the elements characterizing MS is oxidative stress. In MS patients an elevation of oxidized LDL (oxLDL) concentration was found, which is connected with an increased risk of MS development. Coronary Artery Risk Development In Young Adults (CARDIA) study revealed that elevated oxLDL level was associated with an increased risk of MS to the same degree as MS components like abdominal obesity, hyperglycemia and hypertriglyceridemia (17). Bilirubin has got strong anti-oxidative and anti-inflammatory properties. It inhibits oxidation of LDL-cholesterol and other lipids, scavenges free oxygen radicals and counteracts oxidative stress (18).

Bilirubin itself or via molecules taking part in metabolic process of its production may also influence other pathogenetic mechanisms of MS. Heme oxygenase (HO) and biliverdin reductase (BVR) take part in metabolic pathway of bilirubin formation. HO converts pro-oxidative and pro-atherosclerotic heme into biliverdin and carbon monoxide (CO). BVR reduces biliverdin to bilirubin. Bilirubin is oxidized by reactive oxygen species (ROS) to biliverdin which protects cells from oxidative stress and then biliverdin is again reduced to bilirubin by BVR with use of NADPH (1). Experimental studies on mice and rats with type 2 diabetes and insulin resistance revealed that induction of HO causes an increase of adiponektins level, anti-inflammatory effect, reduction of insulin resistance and improvement of glucose tolerance (9).Up-regulating HO system also generates anti-oxidative and anti-atherosclerotic

Table II. Adjusted odds ratios of particular metabolic disorders or risk factors in relation to bilirubin level values (distributed in quartiles) for the whole study population

Tabela II. Skorygowane ilorazy szans występowania poszczególnych zaburzeń metabolicznych lub czynników ryzyka, w zależności od wartości stężenia bilirubiny (podział na kwartale) dla całej badanej populacji

Group	Q2 vs. Q1	Q3 vs. Q1	Q4 vs. Q1	p-value
Metabolic syndrome[1]	1.15 (0.84-1.58)	0.70 (0.50-0.99)	0.68 (0.48-0.95)	0.003
Waist circumference[2]	1.55 (1.11-2.15)	1.27 (0.91-1.76)	1.07 (0.77-1.48)	0.042
Triglycerides[2]	0.67 (0.49-0.92)	0.49 (0.35-0.69)	0.39 (0.28-0.55)	<.0001
HDL- cholesterol[2]	0.86 (0.61-1.22)	0.80 (0.56-1.14)	1.05 (0.74-1.50)	0.398
Elevated blood pressure[2]	0.86 (0.63-1.18)	0.85 (0.61-1.17)	0.95 (0.69-1.31)	0.709
Hyperglycemia[2]	1.15 (0.72-1.84)	1.06 (0.65-1.73)	0.76 (0.44-1.29)	0.441
Insulinemia[2]	0.76 (0.55-1.04)	0.66 (0.47-0.91)	0.55 (0.39-0.76)	0.003
HOMA[2]	0.81 (0.59-1.11)	0.70 (0.51-0.97)	0.53 (0.38-0.74)	0.003
hs-CRP[3]	0.77 (0.54-1.08)	0.70 (0.49-1.01)	0.61 (0.42-0.88)	0.054
Current smoking[4]	1.03 (0.76-1.39)	0.72 (0.53-0.98)	0.61 (0.44-0.83)	0.001

95% confidence intervals are given in parentheses.

1Adjusted to age, sex, hs-CRP and current cigarette smoking

2Adjusted to age, sex, hs-CRP, current cigarette smoking and other MS criteria

3Adjusted to age, sex, current cigarette smoking and MS criteria

4Adjusted to age, sex, hs-CRP and MS criteria

products like bilirubin, biliverdin and carbon monoxide (CO) (1). Oxidative stress is one of etiologic factors of insulin resistance (19), therefore inverse relationship between bilirubin and insulin resistance may result from anti-oxidative properties of these substances. There are also other hypotheses appearing.

Recent studies have revealed that apart from anti--oxidative effect human BVR together with substrates and products of its activity play a key role in insulin signal-transduction pathways and in regulation of gene expression (10). Insulin and insulin-like growth factor (IGF) act via activation of insulin receptor (IR/IGFR). Its activity as metabolism and growth factor regulator depends on protein tyrosine kinases (PTK). Combination of intracellular domain of insulin receptor (IR) kinase with its substrate is an initial step of signal cascade. Insulin receptor tyrosine kinase (IRK) activation after binding of insulin with extracellular domain of its receptor is a distinctive signal for proteins that are insulin receptor substrates (IRS). BVR is a cytoplasm soluble kinase with double affinity that has an ability of autophosphorylation and transfer of phosphate groups to both tyrosine and serine / threonine residues. These activities of BVR contribute to insulin effect and glucose uptake. There are three observations indicating the role of BVR in insulin resistance: 1. The presence of IRS intensifies BVR phosphorylation by IRK; 2. BVR directly phosphorylates IRS within serine residues; 3. Insulin-dependant glucose uptake increases when BVR expression is inhibited by si BVR mRNA. This concept is supported by the fact that BVR phosphorylates IRS-1 proteins in the regions that are responsible for a decrease of glucose uptake. There are two effector pathways for insulin: MAPK i PI3K pathways. MAPK pathway takes part mainly in the insulin effect on transcription and mitogenesis, while PI3K influences metabolic processes. Recent studies suggest the key role of BVR in both pathways as well as in regulation of protein kinase C (PKC) isoforms, which constitute a bridge between those two pathways (20).Our study proved strong inverse relationship between bilirubin and insulin resistance for the population of adults. Our results are consistent with the result obtained by Lin et al. in research conducted on children and adolescents (11). Similarly, inverse relationship between bilirubin and insulin resistance was observed by Hwang et al. in their study on adults (16). Those results may support the conclusion that inverse association of MS and bilirubin can be caused by its influence on insulin resistance reduction apart from its ant-oxidative effect. Further studies are necessary to explain this issue and to determine the role of bilirubin or molecules taking part in its production and precise molecular defects influencing insulin signal-transduction pathways that cause MS in people.

On the other hand, our research revealed that among all components of MS the strongest negative correlation was observed between bilirubin and hypertriglyceridemia. This finding supports the observation that serum bilirubin concentrations might be in association with serum triglycerides level as a risk factor for CVD (12,16, 21). Additionally, high triglyceride level in association with low concentration of bilirubin implies the possible relationship between bilirubin concentration and insulin resistance. However, some investigators observed no association between total bilirubin and triglyceride levels (11,22).

Apart from insulin resistance and triglycerides there was only an inverse correlation between serum bilirubin level and cigarette smoking observed out of all the other cardiovascular risk factors analyzed in our study. Whereas serum hs-CRP level reached the limits of statistical analysis (p=0.054), and there was no such association for arterial blood pressure and glucose level.

Previous reports differ in results concerning relationship between bilirubin and cardiometabolic risk factors. Results of some studies show an independent inverse association of serum total bilirubin level with cigarette smoking (23), obesity (11,16), arterial blood pressure, glucose (16), LDL-cholesterol and CRP (16,21). Findings of other researchers present no such correlation for arterial blood pressure, glucose , LDL--cholesterol and CRP (11,22).

CONCLUSION

This large country-representative study revealed that among Polish adults bilirubin level within normal limits or moderately elevated is inversely associated with prevalence of MS and insulin resistance. The mechanism of the association between MS and total bilirubin may be related to the insulin resistance status but further studies are needed for determination of the association between serum bilirubin and insulin resistance.

Study limitations

Our study has several limitations. First, transaminases activities, gamma-glutamyltransferase activity and viral hepatitis markers were not determined, which does not allow the precise assessment of potential liver injury influence on the results obtained. Second, although we collected data about alcohol drinking, the information received seems too incomplete and unreliable to be included in our analysis. Last, since levels of direct and indirect bilirubin were not determined, it is not possible to state which type of bilirubin is associated with MS.

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