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Serum uric acid, triglycerides and total bilirubin are associated with Hepatic Steatosis Index in adolescent population

Serumske vrednosti mokraćne kiseline, triglicerida i ukupnog bilirubina su povezane sa Indeksom masne jetre u adolescentnoj populaciji

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Summary

Objective: Given the fact that pathophysiological background of non-alcoholic fatty liver disease is not completely elucidated, we aimed to explore the relationship between dyslipidemia, inflammation and oxidative stress with hepatic steatosis index (HSI) in the cohort of adolescent population in Montenegro.
Patients and Methods: A total of 136 adolescents participated in this cross-sectional study. Anthropometric and biochemical markers were provided. HSI was calculated. Associations of biochemical parameters with HSI were tested using univariable and multivariable ordinal regression analysis for HSI tertiles as dependent variable.
Results: Adjusted odds for uric acid (OR=2.641, P=0.007) and for triglycerides, (OR=5.275, P=0.008) showed their highly significant positive associations with HSI. On the contrary, adjusted odds for bilirubin (OR=0.923, P=0.015), indicated its significant negative independent association with HSI. Although C-reactive protein correlated positively with HSI in univariable analysis (OR=1.962, P<0.001), it lost its independent prediction for HSI in multivariable analysis (OR=1.442, P=0.130).
Conclusion: Serum uric acid, triglycerides and total bilirubin are associated with HSI in adolescents. Longitudinal studies are needed to confirm the causal relationship between mentioned biomarkers and hepatic steatosis and to further explore its diagnostic potential in NAFLD in young population.

Key words: dyslipidemia, inflammation, hepatic steatosis, oxidative stress

Sažetak

Cilj: S obzirom na činjenicu da patofiziološki aspekt nealkoholne steatoze jetre nije u potpunosti razjašnjen, cilj studije je bio da se ispita povezanost dislipidemije, inflamacije i oksidativnog stresa sa Indeksom masne jetre (IMJ) u kohorti adolescentne populacije u Crnoj Gori.
Pacijenti i Metode: Ukupno 136 adolescenata je učestvovalo u studiji preseka. Antropometrijski i biohemijski marker su mereni. IMJ je izračunat. Povezanost biohemijskih parametara sa IMJ ispitana je univarijantnom i multivarijantnom ordinalnom regresionom analizom, sa tercilnim vrednostima IMJ kao zavisnom varijablom.
Rezultati: Prilagođene odds vrednosti za mokraćnu kiselinu (OR=2,641, P=0,007) i trigliceride (OR=5,275, P=0,008) pokazali su visoko značajnu pozitivnu povezanost sa IMJ. Suprotno tome, prilagođene odds vrednosti za bilirubin (OR=0,923, P=0,015) pokazale su značajnu negativnu povezanost sa IMJ. Premda su vrednosti C-reaktivnog proteina pozitivno korelirale sa IMJ u univarijantnoj (OR=1,962, P<0,001), isti je izgubio nezavisnu predikciju za IMJ u multivarijantnoj analizi (OR=1,442, P=0,130).
Zaključak: Serumske vrednosti mokraćne kiseline, triglicerida i ukupnog bilirubina su povezane sa IMJ u adolescenata. Longitudinalne studije su potrebne da potvrde uzročnu povezanost između pomenutih biomarkera i steatoze jetre, kao i da dalje ispitaju njihov dijagnostički potencijal za ovo oboljenje u mlađoj populaciji.

Ključne reči: *dislipidemija, inflamacija, steatoza jetre, oksidativni stres*

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Introduction

There is an increasing prevalence in NAFLD in general population during the recent years. However, not only adults but young population also suffers from this metabolic disorder (1). In parallel with the increasing of obesity, sedentary lifestyle and unhealthy eating behaviours, the increase in NAFLD is reported. If not properly treated NAFLD often tracks late in life, causing severe complications in adulthood (1, 2).
The NAFLD is defined as the accumulation of fat in the liver for more than 5% in hepatocytes, after excluding causes of other liver diseases and alcohol consumption. This disorder

exists in two clinical forms, i.e. simple steatosis being presented with liver fat accumulation only, and non-alcoholic steato-hepatitis (NASH) which is a progressive entity accompanied with lobular inflammation and severe changes (i.e. hepathocytes injury and fibrosis) proven at liver histology (2).

Children and adolescents with NAFLD often exhibit impaired glucose tolerance (3) and are at increased risk of diabetes mellitus type 2 in adulthood (4). Moreover, obstructive sleep apnoea, polycystic ovarian syndrome and cardiovascular diseases are also observed in those youngsters (2). Although the higher prevalence of NAFLD is observed in children with obesity compared with normal weight counterparts, about 5% of cases are shown to have normal body mass index (BMI) (5), thus presuming that central obesity, rather than overall obesity increases the risk for NASH (3, 6, 7).

The NAFLD represents complex and multifactorial disorder but it is assumed that oxidative stress and inflammation represent the major factors of its pathogenesis (8), similarly as it is in obesity (9, 10), metabolic syndrome (11, 12), polycystic ovarian syndrome (13, 14), diabetes type 2 (15, 16), cardiovascular diseases (17-19), which are all tightly associated with NAFLD. Namely, a wide spectrum of pro-inflammatory adipokines and cytokines modulate insulin signaling pathways and lead to insulin resistance (IR). The latter further promotes increased lypolysis in adipose tissue, with excess of free fatty acids (FFA) inflow in the liver. These prpcesses altogether with increased lipogenesis in the liver induce its steatosis (5, 8).

However, the pathophysiological mechanisms are not thoroughly explored, and quest for novel inflammation and oxidative stress biomarkers still continues (20, 21). Additionally, several algorithms that are widely used in adults are reported to be convenient in children and adolescent population, also (22). Among them, hepatic steatosis index (HSI) which includes sex, transaminases (i.e. alanine aminotransferase (ALT) / aspartate aminotransferase (AST) ratio), BMI and diabetes mellitus in its calculation was shown to be a reliable screening tool for NAFLD at an early stage in young population (22, 23). Importantly, if timely and properly diagnosed, the intervention programs of weight loss, nutritional habits and physical activity may be beneficial to reduce cardiometabolic risk factors and to avoid progression of further NAFLD complications in adulthood (2, 24-26).
Given the fact that studies that examined NAFLD in adolescent population using HSI are scarce, and that pathophysiological background of this metabolic disorder is not completely clear, we aimed to explore the relationship between dyslipidemia, inflammation and oxidative stress with HSI in the cohort of exclusively 16-19 years old adolescents in Montenegro.

Patients and Methods

Study population

A total of 136 adolescents from two suburban secondary schools in Podgorica, ages between 16-19 years were consecutively recruited in this study. The research was carried out according to the Declaration of Helsinki principles, after obtaining the approval of the Institutional Ethical Committee. All adolescents signed informed consent that are well informed about the aim of the study. Additionally, for participants <18 years parents' written approval was provided.
After completing a questionnaire consisted of inquiries about lifestyle habits, illnesses, and medications use, anthropometric measurements and blood collection were carried out in the morning between 7-9 hours a.m., after an overnight fast of at least 8 hours.
All normal weight and overweight/obese adolescents, free of known other diseases who voluntarily accepted to participate were included in the research.
Adolescents <16 years and >19 years old, with morbid obesity (i.e. BMI ≥40 kg/m^2), as well as those with a history of smoking, alcohol consumption or any medications use were excluded. Additionally, those with reported symptoms of acute inflammatory disease, as well as those with C-reactive protein (CRP) >10.0 mg/L were further excluded from the study.
Participants were asked not to perform any vigorous physical activity the day before the venipuncture procedure was performed.

Anthropometric measurements

Basic anthropometric measurements: body height and weight, and waist circumference (WC) were obtained and BMI was calculated, as described elsewhere (27).

Biochemical analyses

The blood samples were taken as previously reported (27). Serum levels of fasting glucose, creatinine, total proteins, AST, ALT, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, uric acid, CRP, total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), and triglycerides (TG) were measured using standardized procedures (Roche Cobas 400, Mannheim, Germany).

Statistical analysis

Prior to statistical analysis data distribution was tested using Shapiro-Wilk test. Due to the skewed data distribution Kruskal-Wallis and Mann-Whitney tests were used for three groups and two subgroups comparisons, respectively. Variables from those analyses were presented as median (interquartile range). Comparison of categorical data was performed by Chi-square test for contingency tables. Categorical data were presented as absolute frequencies. Correlation between HSI score and continuous data were tested by Spearman's correlation analysis and presented with correlation coefficient (ρ). In order to test deeper

associations between HSI score and examined markers, ordinal logistic regression analysis was employed. Markers which were significantly correlated with HSI in Spearman's correlation analysis were further tested by univariable ordinal regression analysis. Multivariable ordinal regression analysis was set for examining potential independent associations of predictors (independent variables) significant in univariable analysis and HSI (ordinal dependent variable). Also, multivariable ordinal regression analysis only included markers which showed no multicollinearity. Data from those analysis are presented as the estimated odds ratios (ORs) and 95% confidence intervals (CIs). Nagelkerke R^2 value presented the explained variation in HSI score. IBM® SPSS® statistical software version 22.0 (Chicago, Illinois, USA) was used for data examinations. Significance level was set at $P<0.05$.

Results

General characteristics and laboratory examinations of adolescents were given for HSI tertile groups and compared in Table 1.

Table 1. General and laboratory data of adolescents according to HSI tertiles

	The first HSI tertile **(HSI≤29)**	The second HSI tertile (29<HIS<34)	The third HSI tertile **(HSI≥34)**	P**
Adolescents No. Females Males	46 35 11	45 42 3	45 37 8	0.071
Age, years	17 (17-18)	18 (17-19)	19 (17-19)	0.449
BMI, kg/m^2	19.6 (18.6-21.6)	22.5 (21.1-25.5) ^{a*}	26.0 (24.6-26.9)^{a*,b*}	<0.001
WC, cm	74 (72-78)	82 (76-87)^{a†}	88 (84-97)^{a*,b*}	<0.001
Glucose, mmol/L	5.0 (4.6-5.2)	4.9 (4.9-5.1)	5.1 (4.8-5.2)	0.145
Creatinine, μmol/L	57 (52-60)	58 (55-64)	57 (46-66)	0.447
Total proteins, g/L	70 (68-72)	73 (70-76) ^{a†}	73 (71-75)	0.017
TC, mmol/L	3.88 (3.72-4.34)	4.29 (3.82-4.77)$^{a\#}$	4.34 (3.67-4.64)^{a†}	0.014
HDL-c, mmol/L	1.52 (1.39-1.66)	1.45 (1.29-1.77)	1.45 (1.17-1.82)	0.105
LDL-c, mmol/L	2.05 (1.79-2.31)	2.17 (2.02-2.72)	2.20 (1.99-2.85)^{a†}	0.006
TG, mmol/L	0.73 (0.54-0.96)	0.87 (0.72-0.99)$^{a\#}$	0.98 (0.74-1.32)$^{a*,b\#}$	<0.001
AST, U/L	15 (12-17)	15 (14-17)	17 (14-21) $^{a\#}$	0.106
ALT, U/L	10 (7-12)	11 (10-14)^{a*}	19 (13-30)^{a*,b*}	<0.001
GGT, U/L	9 (6-10)	10 (9-13)^{a†}	11 (9-14)^{a†}	<0.001
ALP, U/L	65 (53-85)	63 (60-78)	61 (46-67)	0.106
Bilirubin, μmol/L	11.0 (7.8-13.7)	8.4 (6.7-14.7)	6.5 (4.6-8.9)^{a*,b†}	<0.001
Uric acid, μmol/L	210 (192-248)	255 (220-303)	268 (225-308)^{a*,b†}	<0.001
CRP, mg/L	0.31 (0.30-0.42)	0.45 (0.30-0.97)^{a†}	0.71 (0.35-2.98)$^{a*,b\#}$	<0.001

Data are given as median (interquartile range) and compared by Kruskal-Wallis test with post hoc Mann-Whitney test.
***P for Kruskal-Wallis test*
a-significantly different when compared to the first HSI tertile group
b-significantly different when compared to the second HSI tertile group
*†P<0.01; # P<0.05;*P<0.001*

Equal number of males and females were in each HIS tertile group. There were significant differences in BMI, WC, total proteins, TC, LDL-c, TG, ALT, uric acid, bilirubin, and CRP across HSI tertile groups. BMI, WC, TG and CRP were significantly different between all three groups, being the highest in the third and lowest in the first HSI tertile group. Total proteins levels were higher in the second than in the first tertile group. TC level and GGT activity were higher in the second and third group compared to the first HSI tertile group. LDL-c level and AST activity were higher in the third than in the first HSI tertile group. Bilirubin and uric acid were the highest in the third compared to two other groups (Table 1).

Significant negative correlations were determined between HSI score and HDL-c and HSI score and bilirubin. HSI correlated significantly positively with most other examined markers such as TC, LDL-c, TG, GGT, uric acid and CRP. As expected HSI demonstrated significant positive correlation with BMI, AST, AST because those markers entered the HSI score calculation. Also, WC significantly positively correlated with HSI (Table 2).

Table 2. Correlation coefficients of HSI and examined markers

	ρ	P
Age, years	0.033	0.701
BMI, kg/m^2	0.670	<0.001
WC, cm	0.634	<0.001
Glucose, mmol/L	0.109	0.204
Creatinine, μmol/L	-0.058	0.505
Total proteins, g/L	0.096	0.299
TC, mmol/L	0.263	0.002
HDL-c, mmol/L	-0.177	0.039
LDL-c, mmol/L	0.294	<0.001
TG, mmol/L	0.340	<0.001
AST, U/L	0.176	0.040
ALT, U/L	0.680	<0.001
GGT, U/L	0.325	0.001
ALP, U/L	-0.119	0.329
Bilirubin, μmol/L	-0.383	<0.001
Uric acid, μmol/L	0.348	<0.001
CRP, mg/L	0.426	<0.001

Univariable ordinal regression analysis demonstrated significant positive associations of TC, LDL-c, TG, HDL-c, GGT, uric acid and CRP with HSI (Table 3). Explained variation in HSI obtained by each marker is given as Nagelkerke R^2 in Table 3.

Independent variables that were associated significantly with HSI in univariable analysis (Table 3) and that were showing no multicollinearity were further included in multivariable ordinal regression analysis. This statistical analysis showed independent associations between TG, bilirubin and uric acid and HSI in adolescents (Table 3). As given in the Model, adjusted odds for TG (OR=5.275, P=0.008) and for uric acid (OR=2.641, P=0.007) demonstrated their highly significant positive associations with HSI. On the contrary, adjusted odds for bilirubin (OR=0.923, P=0.015), indicated its significant negative independent association with HSI score. Nagelkerke R^2 for

the Model was 0.372 indicated that 37.2% variation in HSI score could be explained by the Model and its markers (Table 3).

Table 3. Estimated odds ratios after univariable and multivariable ordinal regression analysis for HSI tertiles as dependent variable

Predictors	Unadjusted OR (95% CI)	P	Nagelkere R^2
TC, mmol/L	1.896 (1.178-3.056)	0.008	0.061
HDL-c, mmol/L	0.413 (0.170-1.005)	0.051	0.031
LDL-c, mmol/L	2.392 (1.361-4.200)	0.002	0.080
TG, mmol/L	5.530 (2.305-13.277)	<0.001	0.135
GGT, U/L	1.165 (1.061-1.280)	0.001	0.117
Bilirubin, μmol/L	0.912 (0.862-0.966)	0.002	0.103
Uric acid, μmol/L	3.080 (1.808-5.254)	0.001	0.147
CRP, mg/L	1.962 (1.387-2.774)	<0.001	0.161
Model	Adjusted OR (95% CI)	P	Nagelkere R^2
LDL-c, mmol/L	1.765 (0.875-3.561)	0.113	0.372
TG, mmol/L	5.275 (1.573-18.011)	0.008	
GGT, U/L	1.085 (0.952-1.239)	0.219	
Bilirubin, μmol/L	0.923 (0.865-2.316)	0.015	
Uric acid, μmol/L	2.641 (1.266-5.366)	0.007	
CRP, mg/L	1.442 (0.899-2.316)	0.130	

Model consisted of LDL-c, TG, GGT, bilirubin, uric acid and CRP.

Discussion

As far as we are aware, this is the first study that examined the relationship of dyslipidemia, inflammation and oxidative stress with HSI in exclusively adolescent cohort and the first such study in Montenegro. We reported that higher uric acid and TG levels, as well as lower total bilirubin level are associated with higher HSI in youngsters.

Our results are in line with those shown in a cohort of Italian and Turkish children who have also reported uric acid and TG as independent predictors of NAFLD (28, 29). Above this, we have previously reported that uric acid was as an independent predictor of fatty liver index in large sample of adult female population (30). On the other hand, Ozsu et al. (31), showed that these two biomarkers remained poor predictors in children with NASH. Contradictory results in studies mentioned above may be explained by variability in age of children/adolescent cohorts, differencies in sample-size groups, ethnicity and different diagnostic methods used for NAFLD detection.

There are several proposed mechanisms of the association between uric acid and NAFLD. Namely, uric acid shows pro-inflammatory properties and can contribute tothe release of pro-inflammatory cytokines. Also, high levels of this biomolecule are closely related with hyperurinsulinemia and IR, which are the key points of NAFLD onset and progression (8). At last, the generation of uric acid is followed by reactive oxygen species (ROS) production causing increased oxidative stress in the liver and consequent NAFLD (32). We have also previously shown an independent association between increased xanthine oxidase (XO) activity (i.e. an enzyme which converts purine bases to uric acid) and BMI in overweight/obese population (9), suggesting that obesity may give significant contribution to such metabolic changes. Above this, enlarged adipose tissue secrets a wide range of adipokines and pro-inflammatory cytokines, accompanied with increased production of free radicals and decreased antioxidative defence system (9, 12, 27, 33, 34). All these mediators contribute to increased IR state (8, 12, 35). The latter promotes increased lipolysis of TG in adipocytes resulting in increased FFA in circulation. The FFA are being uptaken by the liver but were not β-oxidised, which enhance oxidative phosphorylation, leading to increase in ROS production and liver fat peroxidation. As well, concommitantly increased lipogenesis contributes to TG storage and hepatic steatosis (5, 8). Accordingly, TG are shown to be an independent predictor of many cardiometabolic disorders (36, 37). On the other hand, although we reported positive correlation between CRP and HSI, and also inverse correlation between HDL-c and HSI in univariable analysis, these two biomarkers did not remain independent predictor of HSI. This is contrary to findings of some previous studies (21, 31, 38).

It is established that HDL-c and bilirubin exert antioxidant properties (31, 40). Unlike HDL-c, total bilirubin independently correlated with NAFLD in our study which is in concordance with some other reports (39, 40). This might be explained by potential of bilirubin to inhibit lipid peroxidation and prevent the ROS production, thus exerting favourable properties in cardiometabolic distrurbances (40-42).

The strength of our study is that we included a cohort comprised of late adolescents with a narrow age range of (i.e. 16-19 years), and excluded confounding factors such as comorbidities, medications use, smoking, hormonal variations during childhood. Moreover, we used non-invasive test, such as HSI, which is proven to be convenient proxy of NAFLD in young population (22). Furthermore, we excluded patients with morbid obesity (i.e. BMI ≥40 kg/m^2), given the fact that HSI shows insufficient diagnostic accuracy for NAFLD in youngsters with severe obesity (43).

The limitations of the study are its small sample size, especially concerning male adolescents, since they were not reluctant to participate in the study, such as girls did. Moreover, cross-sectional nature of this study does not allow us to conclude causality between examined biomarkers and HSI. Above all, we did not used routine imaging method such as ultrasound due to the low sensitivity/specificity for the diagnosing of steatosis in children, especially in those with <33% of hepatocytes occupied with steatosis (5).

We also did not used magnetic resonance–based methods, due to its high cost. Computed tomography scans are not used given the radiation risks, while the gold standard for

NAFLD, i.e. liver biopsy due to its invasive nature is not recommended in such investigations.

Conclusion

This study is the first one that examined the relationship of dyslipidemia, inflammation and oxidative stress with HSI in exclusively adolescent cohort and the first such study in Montenegro. We reported that higher uric acid and TG levels, as well as lower total bilirubin level are associated with higher HSI in youngsters. These biomarkers are widely available, since they are cost-effective and routinely measured. Longitudinal studies are neccessary to further explore its diagnostic potential in NAFLD.

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