Relationship between Serum Bilirubin Levels and Metabolic Syndrome in Patients with Schizophrenia Spectrum Disorders

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Objective: We investigated the relationship between serum bilirubin levels and metabolic syndrome (MetS), and the longitudinal effects of baseline serum bilirubin concentrations on MetS in patients with schizophrenia spectrum disorders undergoing atypical antipsychotics.

Methods: The sample of this study consisted of 131 patients with schizophrenia spectrum disorders. Waist circumference, blood pressure, and levels of triglycerides, high–density lipoprotein cholesterol, fasting glucose, and insulin were evaluated at baseline and at month six. Serum bilirubin levels were measured at baseline. Serum bilirubin levels of the patients with and without MetS criteria were compared. We also compared patients with high and low bilirubin levels (upper and lower 50th percentiles of serum bilirubin levels) in terms of MetS criteria, MetS frequency, and course of MetS.

Results: Serum direct bilirubin levels were more consistently related to MetS and MetS–related variables. The waist circumference and triglyceride criteria for MetS were significantly related to low serum direct bilirubin at baseline; waist circumference and fasting glucose criteria, and insulin resistance were associated with low serum direct bilirubin at follow–up. MetS diagnosis and the presence of the waist circumference criterion were more frequent at the baseline and the follow–up in low bilirubin group. At the end of the follow–up period, the rate of reverse MetS was significantly higher in the high bilirubin group.

Conclusion: Our results have suggested that serum direct bilirubin levels showed a more reliable and stable relationship with abdominal obesity for MetS components.in patients with schizophrenia spectrum disorders using antipsychotics. Further studies are required.

KEY WORDS: Schizophrenia; Metabolic syndrome; Bilirubin; Atypical antipsychotics.

INTRODUCTION

Metabolic syndrome (MetS) is a combination of metabolic dysfunctions including abdominal or visceral adiposity, hypertension, glucose, and lipid abnormalities that increase the risk for cardiovascular disease and diabetes.[1] MetS is a growing concern for routine psychiatric care when treating patients with schizophrenia because these metabolic abnormalities are regarded as a major risk factor for cardiovascular diseases and mortality.[2-4] Factors predisposing people with schizophrenia to MetS are complicated. Particularly second generation anti-

psychotics have been shown to greatly influence metabolic risk factors leading to weight gain and impairing glucose and lipid metabolism.[5] The other risk factors among patients with schizophrenia are attributed to poor dietary habits, unhealthy lifestyle and physical inactivity, possibly related to negative symptoms of schizophrenia.[2] Weight gain and metabolic side effects of atypical antipsychotics also leads to non-adherence or rejection of treatment by the schizophrenia patients.[6]

Bilirubin, the end product of heme metabolism, is an endogenous antioxidant with anti-inflammatory properties. High serum bilirubin concentrations are associated with increased total antioxidant capacity and offer protection against oxidative stress-induced diseases.[7] MetS is characterized by enhanced low-grade systemic inflammation and oxidative stress. During the past few years, studies have shown that serum bilirubin levels are

inversely associated with MetS and systemic inflammation in adults,[8-11] children and adolescents.[11] It have been reported that especially, abdominal obesity has been correlated with low serum bilirubin levels.[11-13] Low serum bilirubin has been proven to be associated with increased carotid intima media thickness[8,14] and peripheral arterial disease.[15-17] In one study, low prevalence of ischemic heart disease in patients with Gilbert syndrome, a genetic disorder causing mild to moderate elevations of unconjugated bilirubin, was detected.[18] Bilirubin serum levels are determined by genetic factors (intrinsic activity of enzymatic steps in bilirubin homeostasis), serum albumin concentration and its bilirubin-binding properties, and external factors such as dietary status, fasting, tobacco smoking, intake of drugs or plant products, living at altitude, age, fitness level, and general health status.[8] However, it has been reported that antipsychotic drugs do not cause significant changes in serum bilirubin levels.[19]

To our knowledge, the relationship between the bilirubin levels and MetS has not been studied in patients with schizophrenia or schizoaffective disorder using antipsychotics. In this naturalistic follow-up study, the relationship between the bilirubin levels and MetS-associated parameters, such as the frequency and course of MetS, was investigated in patients with schizophrenia spectrum disorders using antipsychotics. We also planned to investigate, if there is a relationship as we expected, the longitudinal effects of baseline serum bilirubin concentrations on incident MetS during six-month follow-up period. We hope that our results will provide preliminary information on whether or not bilirubin levels are a potential predictor for MetS in patients with schizophrenia spectrum disorders using antipsychotics. In the follow-up period, we examined whether serum bilirubin levels were associated with the development or reversal of MetS in these patients.

METHODS

Study Design and Population

The sample of the present study consisted of 151 patients who were diagnosed with schizophrenia or schizoaffective disorder according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria, aged 18-65 years, and receiving atypical antipsychotics for at least 12 weeks. The study conducted on a subgroup of patients who enrolled in another prospective naturalistic study investigating the association between antipsychotic medication use and MetS in patients with schizophrenia spectrum disorders.[20] This study supported by the Committee of Scientific Research Projects of Pamukkale University (Project Number: 2008TPF029). The patients were recruited from two treatment settings (Pamukkale University, Psychotic Disorder Outpatient Clinics and Denizli State Hospital, Turkey). Patients with the following exclusion criteria were not recruited: psychotic disorders or mood disorders due to a general medical condition, dementia, substance abuse, or known hepatic dysfunction according to medical records.

The patients were evaluated at baseline and at month six. Antipsychotic medications and the severity of positive and negative symptoms were also recorded.[21,22] At baseline visit, all patients received a full clinical examination, metabolic screening for MetS parameters and measurement of serum bilirubin levels. Following the exclusion of 8 patients with serum direct bilirubin values above the normal range (0.1-0.3 mg/dl) and 12 patients with indirect bilirubin levels above the normal range (0.2-0.7 mg/dl), the baseline sample included 131 patients (78 male, 53 female) and the follow-up sample included 121 patients (72 male, 49 female).

The Medical Ethics Committee of Pamukkale University approved the study protocol (protocol no: 4837, 21.10.2008). Objectives and procedures of this study were explained to all patients; patients were informed that their ongoing treatments would not be affected by study procedures and written informed consent were obtained from all subjects prior to participation of the study.

All patients informed about their baseline measurement, and relevant recommendations were given. For example, patients with triglyceride (TG) and glucose levels meeting the MetS criteria were informed of the associated cardiovascular risk, advised to seek an endocrinology referral, and recommended to avoid high-calorie foods, to lose weight, to exercise, and to quit smoking.

Anthropometric Measurements

Weight was measured with a spring balance that was kept on a firm horizontal surface. Subjects wore light clothing and stood upright without shoes, and weight was recorded to the nearest 0.5 kg. Height was measured with a tape to the nearest 0.1 cm. Subjects were requested to stand upright without shoes with their backs against the wall, heels together, and eyes directed forward. Waist circumference was measured in a standing position halfway between the costal edge and iliac crest. Body mass index (BMI) was calculated as the body weight in kilograms divided by the height in meters squared.[23] The blood pressure

measurements were obtained from the right arm with the participant in the sitting position using a mercury sphygmomanometer after 15 minutes of rest.

Serum Measurements

Overnight fasting blood samples were drawn and analyzed. Serum was immediately separated and stored at $-20^{o}C$ until subsequent analysis. Serum concentrations of direct (conjugated with glucuronic acid), indirect (unconjugated), and total bilirubin (includes both direct and indirect bilirubin), fasting glucose, TG, and high-density lipoprotein (HDL) cholesterol were measured on an AU 680 Chemistry System analyzer (Beckman Coulter, Nyon, Switzerland) using commercial kits. Serum insulin concentrations were analyzed on an ARCHITECT i2000SR analyzer (Abbott Diagnostics, Abbott Park, IL, USA) using commercial kits. The homeostasis model assessment (HOMA) was used as a measure of insulin resistance (HOMA-IR). HOMA-IR was calculated using the formula: HOMA-IR=[glucose (mmol/L)×insulin (μU/ml)/22.5] and insulin resistance was defined as HOMA-IR >2.7.[19] All body and biochemical measurements were repeated at follow-up visit.

Definition of Metabolic Syndrome

MetS was defined according to ATP III A (Adult Treatment Panel III A) criteria,[20] which takes into account the following five components: Fasting triglycerides (FTG) >150 mg/dl; HDL <40 mg/dL (men) or <50 mg/dl (women); blood pressure (systolic blood pressure/diastolic blood pressure) $>130/85$ mmHg or on antihypertensive medication; fasting glucose >100 mg/dl or on insulin or hypoglycemic medication; and waist circumference >102 cm (men) or >88 cm (women). Patients having three or more of the criteria were identified as having a MetS diagnosis.

Statistical Analysis

Initially, in order to explore which bilirubin components would be associated with MetS, the means of serum bilirubin levels (direct, indirect, and total) were compared between the patients with and without MetS criteria. As bilirubin values did not show a normal distribution, mean bilirubin levels of the groups were compared using the Mann-Whitney U test.

These analyses revealed a consistent relationship between direct serum bilirubin levels and MetS criteria, therefore we performed subsequent analyses based on a recent report on the lower risk of MetS in patients with se-

rum bilirubin levels at upper percentiles.[10] The patients within the lower 50th percentile of serum direct bilirubin levels (low bilirubin group) were compared to those within the upper 50th percentile (high bilirubin group) in terms of MetS criteria using the chi-square test.

The stability of the relationship between serum bilirubin levels and MetS was tested by examining the relationship between the baseline serum bilirubin levels and MetS associated parameters at follow-up.

The relationship between bilirubin levels and MetS criteria was also investigated using partial correlation analysis while controlling for the effect of age. $p < 0.05$ was accepted to be statistically significant. Statistical analyses were performed using SPSS software 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Bilirubin levels were measured in 151 patients at the beginning of study. For baseline sample, average age, mean duration of disease, and mean number of hospitalizations were determined to be 38.98±12.21 years, 13.32±9.74 years, and 2.84±3.57 respectively. Frequency of MetS diagnosis was 45.8% at the baseline and 46.6% at follow-up (Table 1). Frequency of MetS diagnosis was not significantly different between men and women in either visit. Criteria for MetS diagnosis were met by 47.2% of

Table 1. Sample characteristics

Characteristic	Data
Gender	
Female	53 (40.5)
Male	78 (59.5)
MetS diagnosis	
Baseline MetS present	60 (45.8)
Free of MetS	71 (54.2)
Follow-up MetS present	61 (46.6)
Free of MetS at follow-up	60 (45.8)
Course of MetS	
Continued MetS at both visits	46 (75.4)
Newly developed MetS at follow-up visit	15 (24.6)
Free of MetS at both visits	51 (85.0)
Reverse MetS at follow-up visit	9 (15.0)
Medications	
Atypical antipsychotics	63 (48.1)
Atypical combinations	36 (27.5)
Atypical-typical combinations	32 (24.4)
Smoking	
Yes	65 (49.6)
No	66 (50.4)

Values are presented as number (%).
MetS, metabolic syndrome.

women and 49.9% of men at baseline and 49.9% of women and 52.8% of men at follow-up ($p > 0.05$). At the baseline evaluation, average age of the patients with MetS (43.95±11.01 years) was significantly higher than those without MetS (34.78±11.65 years) ($p=0.000$).

Most of the patients with MetS (75.4%) at follow-up visit were patients who met criteria for MetS in both visits and 24.6% were patients who developed MetS within the six months between visits. Most of the patients (85%) without MetS at follow-up visit were free of MetS at baseline and 15% were reverse MetS patients. Most of our patients were treated with single or combined atypical antipsychotics (Table 1).

The Relationship between Serum Bilirubin Levels and MetS Diagnosis, and Its Stability

The mean serum direct bilirubin and total bilirubin levels of the patients with MetS diagnosis were significantly lower than those free of MetS. This relationship was also evident at follow-up. Regarding the course of MetS, the patients who met the diagnosis of MetS in both visits had significantly lower direct bilirubin levels compared to patients with reversed MetS diagnosis at the end of the follow-up period (Table 2).

Serum direct bilirubin levels were also significantly lower in patients who met the MetS criterion for waist circumference at baseline as compared to patients who were free of this criterion. The presence of TG level criteria for

Table 2. The relationship between serum bilirubin levels and MetS diagnosis at baseline and follow-up

Variable	Number	Direct bilirubin level	*p* value	Indirect bilirubin level	*p* value	Total bilirubin level	*p* value
Baseline							
MetS present	60	0.075±0.066		0.264±0.127		0.347±0.142	
Free of MetS	71	0.121±0.093	0.002	0.290±0.178	0.193	0.421±0.203	0.011
Individual MetS criteria							
BP criterion present	35	0.105±0.081		0,267± 0.128		0.369±0.166	
Free of BP criterion	96	0.103±0.087	0.958	0.292±0.166	0.339	0.397±0.198	0.429
FG criterion present	46	0.091±0.082		0.267±0.137		0.358±0.154	
Free of FG criterion	85	0.102±0.083	0.341	0.291±0.165	0.399	0.397±0.201	0.249
WC criterion present	80	0.085±0.075		0.248±0.141		0.333±0.161	
Free of WC criterion	51	0.126±0.090	0.006	0.337±0.163	0.001	0.464±0.198	0.000
HDL criterion present	96	0.095±0.082		0.281±0.144		0.377±0.178	
Free of HDL criterion	35	0.115±0.087	0.193	0.287±0.186	0.843	0.4034±0.212	0.488
TG criterion present	57	0.076±0.068		0.277±0.116		0.353±0.136	
Free of TG criterion	74	0.120±0.090	0.003	0.287±0.181	0.707	0.408±0.216	0.081
Insulin resistance present	33	0.087±0.083		0.276±0.151		0.363±0.165	
No insulin resistance	98	0.105±0.084	0.176	0.285±0.158	0.934	0.391±0.194	0.683
Follow-up visit							
MetS present	61	0.077±0.061		0.289±0.141		0.366±0.1622	
Free of MetS	60	0.112±0.091	0.016	0.286±0.161	0.930	0.399±0.197	0.314
Individual MetS criteria							
BP criterion present	45	0.092±0.079		0.264±0.142		0.356±0.171	
Free of BP criterion	76	0.097±0.076	0.627	0.304±0.151	0.220	0.399±0.185	0.206
FG criterion present	53	0.078±0.067		0.290±0.132		0.369±0.162	
Free of FG criterion	68	0.107±0.085	0.039	0.285±0.164	0.846	0.394±0.194	0.459
WC criterion present	66	0.084±0.076		0.281±0.16		0.366±0.180	
Free of WC criterion	55	0.106±0.081	0.124	0.295±0.13	0.629	0.403±0.181	0.266
HDL criterion present	87	0.090±0.075		0.286±0.140		0.378±0.169	
Free of HDL criterion	34	0.105±0.088	0.377	0.291±0.176	0.876	0.396±0.208	0.621
TG criterion present	55	0.084±0.072		0.303±0.125		0.388±0.159	
Free of TG criterion	66	0.103±0.084	0.063	0.274±0.169	0.293	0.379±0.198	0.78
Insulin resistance present	49	0.072±0.068		0.286±0.142		0.365±0.167	
No insulin resistance	72	0.105±0.084	0.055	0.288±0.157	0.824	0.395±0.189	0.506
Course of MetS							
MetS at both visits	46	0.063±0.054		0.276±0.126		0.339±0.146	
Reverse MetS at follow-up	9	0.115±0.088	0.022	0.264±0.111	0,795	0.380±0.175	0.163
Free of MetS at both visits	51	0.123±0.127		0.317±0.177		0.439±0.046	
Newly developed MetS at follow-up	16	0.111±0,092	0.621	0.290±0.169	0.611	0.403±0.028	0.530

Values are presented as mean±standard deviation.
MetS, metabolic syndrome; BP, blood pressure; FG, fasting glucose; WC, waist circumference; HDL, high density lipoprotein; TG, triglyceride.
By Mann-Whitney *U* test.

Table 3. Relationship of serum bilirubin levels according to the number of MetS components

Number of MetS criteria	Number (%)	Direct bilirubin level (mg/dl)	Indirect bilirubin levels (mg/dl)
Baseline			
None	10 (7.6)	0.140±0.078	0.395±0.210
Having 1 criteria for MetS	31 (23.7)	0.113±0.087	0.292±0.185
Having 2 criteria for MetS	29 (22.1)	0.117±0.099	0.273±0.147
Having 3 criteria for MetS	29 (22.1)	0.089±0.065	0.272±0.135
Having 4 criteria for MetS	24 (18.3)	0.072±0.082	0.252±0.128
Having 5 criteria for MetS	8 (6.1)	0.075±0.052	0.266±0.104
Follow-up			
None	11 (8.4)	0.105±0.093	0.267±0.148
Having 1 criteria for MetS	27 (20.6)	0.118±0.087	0.307±0.187
Having 2 criteria for MetS	22 (16.8)	0.103±0.099	0.270±0.133
Having 3 criteria for MetS	26 (19.8)	0.089±0.061	0.293±0.149
Having 4 criteria for MetS	25 (19.1)	0.084±0.065	0.304±0.143
Having 5 criteria for MetS	10 (7.6)	0.040±0.023	0.240±0.110

Values are presented as number (%) or mean±standard deviation.
MetS, metabolic syndrome.

MetS was significantly related to low serum direct bilirubin levels at the baseline, but this relationship was not evident at follow-up. Interestingly, at the follow-up visit, low serum direct bilirubin levels showed a significant relationship with the presence of fasting glucose level criteria and were associated with insulin resistance, nearly to a significance level, despite no association at baseline (Table 2).

The patients with MetS diagnosis and the patients who met the waist circumference criterion had lower total bilirubin levels compared to their counterparts. Indirect bilirubin levels showed only significant association with baseline waist circumference (Table 2). Serum total and indirect bilirubin did not show any significant association with the course of MetS.

Mean serum direct and indirect bilirubin levels of the patient based on the number of present MetS criteria are provided in Table 3. Linear regression analysis showed a significant relationship between direct bilirubin levels and the number of met criteria at baseline (B=−3,876, adjusted R square=0.048, p=0.007) and at follow-up evaluation (B=−3,734, adjusted R square=0.033, p=0.026). This relationship was not observed for indirect bilirubin levels ($p > 0.05$).

Low versus High Bilirubin Groups

These initial analyses revealed a consistent relationship between direct serum bilirubin levels and MetS, therefore we performed subsequent analyses based on a recent report on the lower risk of MetS in patients with serum bilirubin levels at upper percentiles.[10] We divided the patient into two groups: The patients within the first 50th percentile of serum direct bilirubin levels (low bilirubin group)

and within the second 50th percentile (high bilirubin group).

MetS diagnosis was significantly more frequent in the low bilirubin group compared to the high bilirubin group at baseline (respectively 58.8% vs. 32.3%) and follow-up (respectively 60.3 % vs. 37.7 %) (Table 4). In the high bilirubin group, significantly more patients were free of the waist circumference criterion compared to the low bilirubin group at baseline (53.2% vs. 26.1%) and follow-up (56.6% vs. 36.8%). In this group, significantly more patients were also free of TG level criteria at baseline and fasting glucose level criteria at follow-up (Table 4). However, more patients were free of blood pressure criteria at baseline and TG level criteria at follow-up in the high bilirubin group, but the differences between groups did not prove significant. Low and high bilirubin groups were not different in respect to the presence of insulin resistance at baseline or follow-up.

Regarding the course of MetS at the end of six months, MetS diagnosis was still present in 90% of the patients with low bilirubin and 66.7% of patients in the high bilirubin group. The ratio of patients with reverse MetS was significantly higher in the high bilirubin group compared to the low bilirubin group (33.3 % vs. 10%) (Table 4).

Correlations between Bilirubin Levels and MetS Associated Parameters

Consistent with the above findings, direct bilirubin levels showed a significant negative correlation with waist circumference (r=−0.178, p=0.042 for both visits) and TG levels (r=−0.243, p=0.005 for baseline; r=−0.185, p=0.042 for follow-up). In addition, fasting blood glucose at follow-up showed a significant negative correlation

Table 4. Comparison of MetS and its individual criteria in low and high bilirubin groups at baseline and follow-up visit

Variable	Low bilirubin group (0.01-0.09 mg/dl)	High bilirubin group (0.10-0.30 mg/dl)	χ^2	*p* value
Female	29 (42.0)	24 (38.7)	0.149	0.699
Male	40 (58.0)	38 (61.3)		
Baseline MetS present	40 (58.0)	20 (32.3)	8.698	0.003
Free of MetS	29 (42.0)	42 (67.7)		
Follow-up MetS present	41 (60.3)	20 (37.7)	6.063	0.014
Free of MetS	27 (39.7)	33 (62.3)		
Baseline individual MetS criteria				
Blood pressure				
Present (n=35)	20 (44.1)	15 (28.3)		
Free of (n=76)	38 (55.9)	38 (71.7)	3.191	0.074
Fasting glucose				
Present (n=46)	28 (40.6)	18 (29.0)	1.911	0.167
Free of (n=85)	41 (59.4)	44 (71.0)		
Waist circumference				
Present (n=60)	51 (73.9)	29 (46.8)	10.117	0.001
Free of (n=51)	18 (26.1)	33 (53.2)		
HDL levels				
Present (n=96)	54 (78.3)	42 (67.7)	1.846	0.174
Free of (n=35)	15 (21.7)	20 (32.3)		
Triglyceride levels				
Present (n=57)	37 (51.5)	20 (37.7)	6.065	0.014
Free of (n=74)	32 (48.5)	42 (62.3)		
Insulin resistance present (n=33)	21 (30.4)	12 (19.4)	2.127	0.145
No insulin resistance (n=98)	48 (69.6)	50 (80.6)		
Follow-up individual MetS criteria				
Blood pressure				
Present (n=45)	30 (44.1)	15 (28.3)	3.190	0.074
Free of (n=76)	38 (55.9)	38 (71.7)		
Fasting glucose				
Present (n=53)	36 (52.9)	17 (32.1)	5.268	0.022
Free of (n=68)	32 (47.1)	36 (67.9)		
Waist circumference				
Present (n=66)	43 (63.2)	23 (43.4)	4.728	0.030
Free of (n=55)	25 (36.8)	30 (56.6)		
HDL levels				
Present (n=87)	51 (75.0)	36 (67.9)	0.738	0.390
Free of (n=34)	17 (25.0)	17 (32.1)		
Triglyceride levels				
Present (n=55)	35 (51.5)	20 (37.7)	2.266	0.093
Free of (n=66)	33 (48.5)	33 (62.3)		
Insulin resistance present (n=49)	32 (47.1)	17 (32.1)	2.775	0.096
No insulin resistance (n=72)	36 (52.9)	36 (67.9)		
Course of MetS				
Continued MetS at both visits	36 (90.0)	10 (66.7)	4.340	0.037
Reverse MetS at follow-up visit	4 (10.0)	5 (33.3)		
Free of MetS at both visits	23 (82.1)	28 (71.8)	0.960	0.327
Newly developed MetS at follow-up visit	5 (17.9)	10 (28.2)		

Values are presented as number (%).
MetS, metabolic syndrome; HDL, high-density lipoprotein.
By Pearson chi-square analysis.

with direct bilirubin levels (r=−0,222, *p*=0.014).

Direct bilirubin levels did not show any significant correlations with weight, BMI, HOMA, or fasting insulin levels. No significant correlation was found between indirect bilirubin levels and MetS parameters. Serum bilirubin levels of smoking and non-smoking patients were not significantly different.

DISCUSSION

Our study has found that direct bilirubin levels are associated with the diagnosis and the course of MetS in patient

with schizophrenia spectrum disorders. Our results are similar to those of previous studies conducted in general populations, overweight individuals, and patients with cardiovascular disorder.[9-17,24-29] In our study, the main MetS parameters associated with bilirubin levels were waist circumference and TG levels. Low serum direct bilirubin levels were found to be associated with baseline and follow-up MetS diagnosis, with the presence of waist circumference and TG level criteria at baseline, and with fasting glucose level criteria at follow-up. Serum indirect bilirubin levels only showed significant association with the presence of waist circumference criteria at baseline. Recently, it was demonstrated that bilirubin is a potent endogenous antioxidant and also plays a protective role in several stages of atherosclerotic inflammation. In cases of obesity and MetS where oxidative stress is increased, consumption of bilirubin increases, leading to a reduction in serum bilirubin levels which results in an increased risk of cardiovascular diseases by causing endothelial dysfunction.[7,8,26,30]

Relationship between Direct and Total Bilirubin and Metabolic Syndrome

The relationship between serum direct bilirubin levels and MetS, as demonstrated in our study, has also been shown in many previous studies.[8-13] Jo et al.[10] have reported the bilirubin subtype that showed the most consistent relationship with MetS diagnosis as direct bilirubin in a sample of 5,321 patients. The relationship between direct bilirubin and MetS diagnosis observed in both visits in our study also supports this finding. We also observed that the more lower direct bilirubin levels were related to the more number of criteria for MetS were present as reported by Jenko-Pražnikar et al.[26] Recent studies suggest that the risk of MetS is increased two- to five-fold in patients with serum direct bilirubin levels that fall within the lower interquartile percentile (0th-75th percentile) as compared to patients within the uppermost percentile (75th-100th percentile) and an increase of 1 standard deviation in total bilirubin level reduces the risk of MetS by 17%. Likewise, our study revealed that MetS diagnosis was significantly lower in the high bilirubin group. In these patients, reversed MetS rate was also found to be significantly higher than the low bilirubin group. In conclusion, these findings, consistent with the literature, suggest that high direct serum bilirubin levels may be associated with a lower risk of MetS in schizophrenia patients undergoing antipsychotic treatment.

Relationship between Serum Bilirubin and MetS Parameters

Abdominal obesity

While waist circumference was significantly associated with all bilirubin forms at baseline, it was associated with only direct bilirubin levels at follow-up. Direct bilirubin levels were significantly lower in patients who met the MetS criterion for waist circumference at baseline and follow-up evaluation as compared to patients who did not meet this criterion. In addition, the presence of waist circumference criteria was significantly higher in the low bilirubin group as compared to the high bilirubin group at the baseline and follow-up visits. Correlation analyses also showed a significant negative correlation between waist circumference and direct bilirubin levels. Consistent with our results, the literature reports that abdominal obesity alone has been correlated with low serum bilirubin levels.[8,11-13,24,25] Choi et al.[8] have reported an inverse relationship between the high levels of bilirubin and abdominal obesity. Serum bilirubin was found to be inversely associated with the amount of visceral adipose tissue in 2,450 subjects examined in a Swedish Obese Subjects trial and this finding was considered a reflection of MetS in obese individuals.[31] Kwon et al.[13] have examined the bilirubin levels in women by dividing them into four categories by interquartile ranges and reported a significant relationship between high levels of bilirubin and lower waist circumference. Jenko-Pražnikar et al.[26] have also reported serum bilirubin levels were negatively associated with abdominal obesity in overweight asymptomatic middle-aged individuals. Distinct from above mentioned studies, Andersson et al.[32] have reported in the results of their study that weight loss causes an increase in serum bilirubin concentration. Abovementioned studies and our study have pointed out the relationship between serum bilirubin levels and obesity.

Dyslipidemia and abnormal glucose metabolism

In our study, TG levels were associated with lower direct bilirubin concentrations. However, baseline and follow-up data were partially inconsistent. Correlation analyses showed that the negative relationship was maintained between direct bilirubin and TG levels at both baseline and follow-up. However, direct bilirubin levels were significantly lower in patients who met the MetS criterion for TG levels compared to patients who didn't meet this criterion at baseline; the differences between these two groups was not present at follow-up. Similarly, the presence of the

MetS criterion for TG levels at baseline was significantly lower in the high bilirubin group, but this relationship was less apperent at follow-up. The relationship between bilirubin and lipid profile was first reported by Breimer *et al*.[28] in a large cohort of middle-aged, British men. This study indicated lower concentrations of TG and higher concentrations of HDL in individuals with higher bilirubin concentrations. A number of subsequent studies described negative relationships between bilirubin and TG levels[8,24,26,29] as well as bilirubin showed a positive association with HDL concentrations.[8,13,24,27,30] Chang *et al*.[24] reported that serum direct bilirubin levels were inversely associated with total cholesterol, low-density lipoprotein (LDL) cholesterol, and TG, and positively associated with HDL cholesterol levels. Yoshino *et al*.[30] reported a positive correlation between total bilirubin and HDL levels in an overweight group. Onat *et al*.[27] reported a significant linear relationship between bilirubin levels and HDL cholesterol in men. Jenko-Pražnikar *et al*.[26] also reported that serum bilirubin levels were negatively associated with FTG, total cholesterol, and LDL cholesterol in overweight individuals.

In our study, lipids other than TG showed no relationship with bilirubin levels, which may be due to several reasons. Most of the previously published studies were conducted with large sample sizes, whereas the relatively small sample size in this study may limit the validity of our results. Additionally, atypical antipsychotics have been shown to have negative effects on lipid profile (levels of TG, total cholesterol, HDL cholesterol, etc.).[6,33] Most of our patients were treated with atypical antipsychotics, which may have acted as a confounding factor on our results. Some studies also suggest that the relationship between bilirubin levels and serum lipids may vary based on sex.[26,27] Further well-controlled studies on the relationship between serum lipid profile and bilirubin levels in patients undergoing antipsychotic treatment, controlling for the effect of confounding factors, may provide more information about this issue. Nevertheless, our findings are consistent with the literature in that there is a relationship between low levels of bilirubin and high levels of triacylglycerol, which is the most remarkable result of most studies examining this matter in healthy subjects.[34]

In our study, serum direct bilirubin levels were significantly lower in patients who met the MetS criterion for fasting glucose levels only at follow-up visit as compared to patients who were free of this criterion. At follow-up, low serum direct bilirubin levels tended to accompany the presence of insulin resistance. There were no relationships

observed between the bilirubin levels and fasting insulin levels or HOMA-IR values. Although our results suggest a limited relationship between direct bilirubin levels and glucose metabolism in patients using antipsychotics, the results do not allow for a conclusion to be made on this matter. Previous studies have reported that serum total bilirubin concentrations were inversely associated with hyperinsulinemia, insulin resistance, and systemic inflammation.[12,26] The absence of a consistent relationship between bilirubin levels and glucose metabolism in our study, distinct from previous studies, may be attributed to characteristics of our patient group, such as the limited sample size and use of antipsychotics. A study conducted in a general population with 1,052 Turkish adult patients showed that total bilirubin levels are associated with insulin resistance but not associated with abdominal obesity.[27] This, as in our study, suggests that the relationship of bilirubin levels with MetS and its parameters in patients using antipsychotics, may have different characteristics than those observed in the general population. However, more studies are required on this issue.

Oxidative stress is believed to play a pathophysiological role in schizophrenia. Studies addressing the relationship between bilirubin levels and schizophrenia or psychosis report conflicting results.[35,36] Studies also suggest that oxidative stress is involved in the pathogenesis of MetS and as a natural antioxidant, bilirubin may play a protective role against oxidative stress and atherosclerotic processes in healthy individuals.[7,8,30] Our results suggest that bilirubin may also involve in MetS accompanied to schizophrenia. However, as our research is the first study on this issue, these conclusions must be supported with future studies.

The most important result of this study may be summarized as follows: Firstly, direct bilirubin levels are associated with several MetS associated parameters, including MetS diagnosis, abdominal obesity, and TG levels. Secondly, high levels of direct serum bilirubin were associated with a lower risk of MetS in patients with schizophrenia spectrum disorders using antipsychotics. Our study is the first to investigate the association of bilirubin with MetS and its parameters in patients with schizophrenia spectrum disorders. The key strength of our study is that it covers the longitudinal effects of baseline serum bilirubin concentrations on incident MetS for six-month follow-up period. The relationships of MetS diagnosis and abdominal obesity with direct bilirubin levels remained stable during the six-month follow-up period. This suggests that the direct bilirubin value measured at any given

time may be a useful predictor for abdominal obesity. Jenko-Pražnikar et al.[26] also reported that a low level of bilirubin in non-symptomatic individuals may be an early biomarker of MetS. We could not find a study that prospectively investigates the stability of the relationship between bilirubin levels and MetS parameters in the literature. Most of the atypical antipsychotics currently used in the treatment of schizophrenia increase the risk of MetS and worsen metabolic parameters, beginning soon after treatment initiation.[6,33] A factor with a stable relationship with MetS over time, may be used as a predictor to reduce the risk of MetS. High levels of direct bilirubin in patients with schizophrenia spectrum disorders may be a cost-effective indicator to demonstrate lower risk of MetS. In addition, antipsychotics that have strong metabolic side effects may be avoided in patients with low serum direct bilirubin levels, as to reduce the interference when evaluating for the risk of MetS. However long-term studies with a larger sample are required on this matter. Important limitations of our study include our limited sample size and the absence of a control group. Another limitation of our study is the widespread use of antipsychotics in our patient population. Although it was reported that antipsychotics do not affect the levels of serum bilirubin,[19] we can not exclude the possible confounding effect of antipsychotics. An investigation on the effects of antipsychotics on MetS and bilirubin levels starting from the initiation of therapy would better clarify this matter.

Our study suggests that the limited, yet stable relationship of bilirubin with abdominal obesity, the key characteristic of MetS, may be worth further investigating.

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