ORIGINAL ARTICLE

LOW SERUM BILIRUBIN LEVELS IN WOMEN WITH THE ANTIPHOSPHOLIPID SYNDROME

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ABSTRACT

The aim: To investigate the relationship between serum bilirubin level and the presence of the APS in women with a history of spontaneous miscarriages.

Materials and methods: Fifty six women aged 22-38 (median 27) years with a history of spontaneous miscarriages were divided into two groups: 33 women with the APS and 23 without. Patients were tested for the presence of lupus anticoagulant, anticardiolipin, anti-β2-glycoprotein 1, antiphospholipid antibodies and genetic thrombophilic defects.

Results: Groups were comparable by age, blood pressure, BMI, co-morbidity (anemia, heart abnormality, thyroid disease, overweight). Median serum total bilirubin levels were 7,2 μmol/L (interquartile range [5,8-9,7]) in women with the APS and 10,5 μmol/L (interquartile range [7,5-15,1]) in control group, p=0.005. The chance of detecting a total bilirubin level of less than 8 μmol/L is 4.1 times higher in the APS patients than in the control group (OR 4,1; 95% CI 1,274-13,213). Logistic regression analysis found a statistically significant association between total bilirubin and the presence of the APS (odds ratio, 0.856; 95% CI, 0.734-0.997, p=0.046). Patients with the APS had elevated serum C-reactive protein (medians 2,3 vs 1,1 mg/L, p=0.01) and fibrinogen (medians 2,8 vs 2,5 g/L, p=0.006) levels compared with controls. Correlation analysis revealed a significant correlation between all types of bilirubin and inflammatory markers.

Conclusions: All types of serum bilirubin (total, direct and indirect) are significantly reduced in women with APS, associated with higher inflammatory markers and lower levels of 25-hydroxyvitamin D, which may be the result of oxidative stress.

KEY WORDS: antiphospholipid syndrome, serum bilirubin, oxidative stress, inflammatory markers

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INTRODUCTION

In recent years, researchers have paid considerable attention to the effects of bilirubin on organ systems depending on its level in the serum [1]. Serum bilirubin possesses protective effects in various diseases including cardiovascular, autoimmune, oncologic, neuronal diseases, venous thromboembolism, chronic kidney disease [1-3]. Bilirubin might exert anti-inflammatory, anti-oxidative, anti-atherosclerotic, anti-thrombotic, immunosuppressive effects via inhibition of lipid peroxidation, oxidation of low density lipoproteins, pro-inflammatory cytokines, inflammatory cell proliferation, platelet activation.

According to the literature data, bilirubin as an antioxidant and immunomodulator may be a protective factor for autoimmune disease. Low serum bilirubin levels are reported to be related to systemic lupus erythematosus, rheumatoid arthritis, Takayasu arteritis, Crohn's disease, primary Sjögren's Syndrome [1, 4-7]. Yang Z. and others described lower serum bilirubin levels in SLE patients without liver diseases than in healthy controls ($P = 0.000$) and this is associated with inflammatory process and lupus renal involvement [4]. According to the EULAR definition, antiphospholipid syndrome (APS) is a systemic autoimmune disorder with vascular and obstetric manifestations associated with thrombotic and inflammatory mechanisms.

THE AIM

The aim of the study was to investigate the relationship between serum bilirubin level and the presence of the APS in women with a history of spontaneous miscarriages.

MATERIALS AND METHODS

The study had been carried out at the clinical bases of the Department of Internal Medicine No 2 Danylo Halytsky Lviv National Medical University. After obtaining a written consent in accordance with the principles of the Declaration of Helsinki, European Convention on Human Rights and Biomedicine, and relevant laws of Ukraine, 56 women aged 22-38 (median 27) years with a history of one or more spontaneous miscarriages in the first trimester of pregnancy were included into the study. This study was

Table I. Baseline patient characteristics (medians [interquartile ranges] and % (n))

Varible	APS patients	Control group	p-value
Age, years	27 [25-31]	28 [24-30]	>0.05
BMI, kg/m^2	21,4 [20,2-23,8]	21,6 [19,8-23,1]	>0.05
Systolic BP, mmHg	100 [95-120]	100 [100-110]	>0.05
Diastolic BP, mmHg	65 [60-80]	60 [60-70]	>0.05
Heart rate, beats/min	78 [70-90]	75 [69-80]	>0.05
Screening indicators of the hemostasis and tests for the detection of LA			
Prothrombin time, sec	14,1 [13,6-14,7]	13,3 [12,9-13,8]	0.0001
Quick prothrombin test, %	93,8 [86,0-98,0]	100,1 [95,1-107,5]	0.001
INR	1,1 [1,0-1,1]	1,0 [0,97-1,02]	0.001
APTT, sec	37,0 [33,9-38,7]	34,0 [32,3-36,0]	0.009
Platelet aggregation, sec	18,2 [15,4-21,0]	18,4 [16,2-21,0]	>0.05
DVVT	1,2 [1,15-1,22]	1,0 [1,0-1,1]	0.000001
APTT-lupus anticoagulant	1,2 [1,15-1,24]	1,05 [1,0-1,1]	0.000001
Prothrombin time diluted to 50 times	1,16 [1,1-1,25]	1,0 [1,0-1,0]	0.000003
Prothrombin time diluted to 500 times	1,19 [1,1-1,33]	1,0 [1,0-1,0]	0.000001
Comorbidities – % (n)			
Anemia	18 (n=6)	22 (n=5)	>0.05
Valvular abnormalities	27 (n=9)	17 (n=4)	>0,05
Thyroid disease	42 (n=14)	26 (n=6)	>0.05
Overweight	15 (n=5)	22 (n=5)	>0.05

conducted to diagnose APS. The main group included 33 (59%) women with the APS. Another 23 (41%) women without APS were recruited as a control group. All patients underwent history taking and physical examination, including measurement of heart rate, blood pressure (BP), anthropometric parameters. Exclusion criteria were as follows: age over 40 years, pregnancy, chronic renal or liver disease, hematology diseases, diabetes, malignancies, smoking and antithrombotic treatment before.

Diagnosis of antiphospholipid syndrome is based on clinical and laboratory data according to the Sydney criteria [8]. The most commonly used tests to detect APS include lupus anticoagulant (LA), anticardiolipin, anti-β_2-glycoprotein 1, antiphospholipid antibodies IgG and IgM. LA was performed using clotting time with phospholipid dependent diluted viper venom time (DVVT), activated partial thromboplastin time (APTT) with lupus anticoagulant-sensitive reagent, prothrombin time with diluted to 50 and 500 times thromboplastin tests in three steps (screening, mixing and confirmatory) [9, 10, 11]. LA was confirmed in plasma on two or more occasions for at least 12 weeks. The frequency of low-titre LA was 24%, medium and high titers – 76%. In addition to APS, the patients were tested for the presence of genetic thrombophilic defects: factor V Leiden mutation, factor II (Prothrombin) mutation, plasminogen activator inhibitor (PAI-1) mutation, platelet fibrinogen receptor (ITGB3) mutation.

Complete blood count, general urine test, coagulation tests (fibrinogen, prothrombin time, quick prothrombin test, international normalization ratio (INR), platelet aggregation), blood lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride), 25-hydroxyvitamin D, thyroid hormones, liver and renal function tests were performed. The indexes of liver function included: total, direct, indirect bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, alkaline phosphatase, gamma-glutamyltransferase (GGT). Patients were examined for the presence of viruses (human herpesvirus, Epstein-Barr virus, human cytomegalovirus, parvovirus B19, hepatitis B and C virus). The instrumental stage of the investigations included ECG, transthoracic echocardiography (left ventricular ejection fraction, heart chambers sizes, valvular abnormalities), ultrasound of the abdominal cavity, kidneys, and thyroid gland.

Statistical analysis was done using Statistica 5.0; data are presented as the median [lower-upper quartiles], comparisons between groups were made using the Mann-Whitney U-test; correlative analysis was provided by Kendall (τ) and Spearman rank (r, when one of the variables is discrete dichotomous variable such as 0 and 1). Categorical data were assessed using the Fisher's exact test. Multivariate logistic regression was used to analyse all potential influencing factors associated with APS. A p value of < 0.05 was considered significant.

RESULTS

The baseline patient characteristics are shown in Table I. Age was comparable between APS patients and healthy controls

Table II. The results of laboratory tests in the examined patients

Characteristics	APS patients	Control group	p-value
	Median [interquartile range]		
White blood cells, 109/L	6,5 [4,9-7,6]	5,5 [4,9-7,0]	>0.05
Hemoglobin, g/L	127 [120-135]	129 [120-137]	>0.05
Erythrocytes, 1012/L	4,5 [4,2-4,6]	4,6 [4,4-4,7]	>0.05
MCH, pg	28,3 [26,6-29,3]	29,1 [27,9-30,0]	>0.05
MCV, fl	85,4 [80,0-87,1]	85,6 [81,0-87,9]	>0.05
Hematocrit, %	38,5 [36,3-39,9]	39,4 [35,4-40,4]	>0.05
Platelets, 109/L	256 [219-314]	262 [218-317]	>0.05
Neutrophils, %	56,2 [51,8-62,3]	52,7 [49,8-65,8]	>0.05
Lymphocytes, %	33 [27-38]	35 [27-40,0]	>0.05
Erythrocyte sedimentation rate, mm/H	7,0 [5,8-14,0]	7,0 [5,0-10,5]	>0.05
C-reactive protein, mg/L	2,3 [1,8-3,6]	1,1 [0,9-2,6]	0.01
Fibrinogen g/L	2,8 [2,6-3,4]	2,5 [2,3-2,7]	0.006
Total cholesterol, mmol/L	4,7 [4,0-5,3]	4,9 [4,4-5,5]	>0.05
LDL-C, mmol/L	2,6 [2,2-3,0]	2,9 [2,3-3,2]	>0.05
HDL-C, mmol/L	1,5 [1,3-1,8]	1,6 [1,4-1,8]	>0.05
Triglyceride, mmol/L	0,8 [0,7-1,4]	0,9 [0,8-1,2]	>0.05
Beta lipoproteins, units	46 [40-52]	40 [38-50]	>0.05
Total bilirubin, µmol/L	7,2 [5,8-9,7]	10,5 [7,5-15,1]	0.005
Direct bilirubin, µmol/L	2,5 [2,0-3,4]	3,4 [3,0-5,4]	0.01
Indirect bilirubin, µmol/L	4,7 [4,1-5,7]	7,0 [4,8-12,5]	0.03
AST, IU/L	15,6 [13,1-17,0]	17,0 [14,2-19,7]	>0.05
ALT, IU/L	11,8 [9,0-16,0]	13,4 [10,8-17,4]	>0.05
Alkaline phosphatase, IU/L	54,5 [46,0-62,0]	45,0 [44,0-68,0]	>0.05
GGT, IU/L	12,5 [9,0-15,0]	11,0 [10,0-16,0]	>0.05
Total protein, g/L	73,4 [70,5-76,8]	77,4 [73,4-80,7]	0.09
Albumin, g/L	48,0 [42,8-50,9]	47,3 [44,8-50,7]	>0.05
Creatinine, µmol/L	60,0 [54,3-70,0]	76,0 [69,0-84,0]	0.06
Urea, mmol/L	3,9 [3,3-4,7]	3,6 [3,3-4,7]	>0.05
Uric acid, µmol/L	251,5 [182,4-299,0]	276,5 [220,0-320,0]	>0.05
Glucose, mmol/L	5,1 [4,7-5,6]	5,2 [4,7-5,5]	>0.05
25-hydroxyvitamin D, ng/ml	19,9 [13,9-27,3]	25,2 [17,2-33,6]	>0.05
Thyroid-stimulating hormone, mU/L	1,59 [0,87-2,3]	1,7 [0,9-2,8]	>0.05

(p>0.05). Although both groups did not significantly differ by the body mass index (BMI), systolic or diastolic BP, heart rate and comorbidity (anemia, heart abnormality diagnosed with echocardiography, thyroid disease, overweight (BMI 25-29.9 kg/m^2)).

Those comorbidities such as obesity, arterial hypertension, and diabetes mellitus were not detected. Anemia was defined as hemoglobin level <120g/L. It was diagnosed as a microcytic character of anaemia according to mean corpuscular volume (MCV) and hypochromic according to mean corpuscular hemoglobin (MCH). Some heart abnormality was found often enough - mitral valve prolapse without regurgitation in 27% persons with the APS, it can be a sign of connective tissue malformation (Table I).

The APS patients significantly differed from the control group by the standart coagulation tests (prothrombin time, quick prothrombin test, INR, APTT, platelet aggregation) and screening tests for the detection of LA (APTT-lupus anticoagulant, DVVT, prothrombin time diluted to 50 and 500 times) (Table I).

A comparison of laboratory parameters between the APS patients and control group showed that inflammatory markers, such as C-reactive protein, fibrinogen were higher while total, direct, indirect bilirubin were lower in the APS patients. Median serum total bilirubin levels were 7,2 µmol/L [5,8-9,7] in women with the APS and 10,5 µmol/L [7,5-15,1] in control group, p=0.005 (Table II).

Table III. Statistically significant Kendall Tau correlation between bilirubin and laboratory parameters in the examined patients

	Bilirubin		Direct bilirubin		Indirect bilirubin	
	τ	p	τ	p	τ	p
Heart rate	-0,21	0.03	-0,30	0.01	-	-
DVVT	-0,24	0.01	-0,37	0.0009	-0,29	0.01
APTT-lupus anticoagulant	-0,42	0.00001	-0,38	0.0004	-0,33	0.003
Prothrombin time diluted to 50 times	-0,26	0.006	-0,29	0.008	-0,20	0.08
Prothrombin time diluted to 500 times	-0,27	0.004	-0,32	0.004	-0,21	0.05
C-reactive protein	-0,37	0.001	-0,53	0.0001	-0,43	0.001
Fibrinogen	-0,28	0.005	-0,24	0.03	-	-
Hemoglobin	+0,34	0.0006	+0,29	0.006	+0,24	0.03
Hematocrit	+0,39	0.0001	+0,39	0.0009	+0,32	0.007
MCH	+0,27	0.01	+0,29	0.01	+0,24	0.04
MCV	+0,30	0.005	+0,37	0.002	+0,30	0.01
Erythrocyte sedimentation rate	-0,29	0.01	-0,29	0.01	-	-
Thyroid-stimulating hormone	-0,27	0.05	-	-	-	-
25-hydroxyvitamin D	+0,30	0.04	-	-	+0,36	0.03

Table IV. Multivariate logistic analysis of the relationship between total bilirubin and existing antiphospholipid syndrome

	Antiphospholipid syndrome		
	OR	95% CI	p
Total bilirubin	0.856	0.734-0.997	0.046
Fibrinogen	4.980	0.787-31.495	0.088
Hemoglobin	1.036	0.955-1.124	0.391
C-reactive protein	1.395	0.633-3.075	0.407
Erythrocyte sedimentation rate	1.006	0.882-1.146	0.926

Additionally, patients with the APS displayed a decrease in the serum creatinine levels compared to the control group, but the difference between the groups was borderline (60,0 [54,3-70,0] vs 76,0 [69,0-84,0], p=0.06) (Table II).

Spearman's correlation analysis also revealed the statistically significant correlation between discrete dichotomous variable (APS is present (1) or absent (0)) and C-reactive protein (r=0,38, p=0.01), fibrinogen (r=0,39, p=0.004), total bilirubin (r=-0,38, p=0.006), direct bilirubin (r=-0,37, p=0.02), indirect bilirubin (r=-0,34, p=0.03).

Furthermore, the frequency of low total bilirubin (less 8 μmol/L) was significantly greater in the APS patients (61% vs 26%, p=0.014 by Fisher's exact test). Normal results for a total bilirubin test are 8-21 μmol/L and bilirubin above the upper limit was observed in two cases only (22,4 μmol/L and 23,4 μmol/L).

We also put our focus on a possible relationship between bilirubin concentrations and clinical manifestations and laboratory tests. Total bilirubin, including direct and indirect bilirubin were significantly negative correlated with screening tests for the detection of LA (APTT-lupus anticoagulant, DVVT, prothrombin time diluted to 50 and 500 times) (Table III).

According to Kendall Tau correlative analysis, a significant negative correlation was found between all types of bilirubin and inflammatory marker, such as C-reactive protein, between total, direct bilirubin and fibrinogen, erythrocyte sedimentation rate, between total bilirubin and thyroid-stimulating hormone. A significant positive correlation was found between all types of bilirubin and red blood cell parameters (hemoglobin, hematocrit, mean corpuscular hemoglobin and mean corpuscular volume); between total, indirect bilirubin, and 25-hydroxyvitamin D.

We performed a multivariate logistic regression analysis, including in the model bilirubin, fibrinogen, hemoglobin, C-reactive protein and erythrocyte sedimentation rate. Logistic regression analysis found a statistically significant association between total bilirubin and present of the APS (odds ratio, 0.856; 95% confidence interval, 0.734-0.997, p=0.046) (Table IV).

We used an odds ratio (OR) and confidence interval (CI) to determine if there is a relationship between the total bilirubin and APS patients. The result showed that the chance of detecting total bilirubin level less 8 μmol/L is 4.1 times higher in APS patients than in the control group (OR 4,1; 95% CI 1,274-13,213).

DISCUSSION

A number of studies found that bilirubin is a biomarker of systemic autoimmune diseases, but its mechanism remains largely unknown. Antiphospholipid syndrome is also a

systemic autoimmune disease. Lupus anticoagulant, anti-cardiolipin, anti-β2-glycoprotein-1 and antiphospholipid antibodies play a key role in the induction of oxidative stress and autoimmune-mediated atherothrombosis in patients with the APS [12]. Various studies have shown that higher levels of bilirubin can protect against diseases associated with increased oxidative stress [1, 4-7].

We were also interested, whether there is a link between serum bilirubin and the APS. In the present study, we report the clinical and laboratory findings of 56 women with a history of spontaneous miscarriages who were screened for antiphospholipid syndrome. We found that serum bilirubin levels and its fractions were significantly lower in patients with the APS and were closely associated with inflammatory markers. Some laboratory indices such as C-reactive protein, fibrinogen, and erythrocyte sedimentation rate were inversely correlated with total, direct/indirect bilirubin. In a very recent study, Xiaojing Zhao et al. [13] found significant negative relationship between total bilirubin and C-reactive protein, erythrocyte sedimentation rate in patients with inflammatory bowel disease (ulcerative colitis and Crohn's disease). It has been suggested that low-level total bilirubin is the result of increased oxidative stress-mediated by an inflammatory response. Our study also showed that lower levels of bilirubin are significantly associated with lower hemoglobin, 25-hydroxyvitamin D levels, and an increase in thyroid-stimulating hormone. Vitamin D is also a powerful antioxidant [14], and the significant association with bilirubin can be explained by the mechanism related to oxidative stress.

Interestingly, in patients with the APS there is a decrease in serum creatinine compared with the control group. Yang D. et al. [15] described significantly lower levels of bilirubin, uric acid, albumin, and creatinine in patients with myasthenia gravis who had low antioxidant status. However, the obtained results need to be confirmed.

CONCLUSIONS

Our findings demonstrated that serum levels of total, direct and indirect bilirubin are significantly reduced in women with the APS, which may be due to oxidative stress. Lower levels of serum bilirubin are significantly associated with inflammatory markers, such as C-reactive protein, fibrinogen and erythrocyte sedimentation rate, as well as lower levels of 25-hydroxyvitamin D and hemoglobin.

Perspective is the further study of whether anticoagulant therapy and the use of vitamin D, C, E can be aimed at suppressing oxidative stress in patients with the APS and increasing the level of total bilirubin in the blood in such patients.

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The Authors declare no conflict of interest.

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