Gastroenterology and Hepatology From Bed to Bench.
2024 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

ORIGINAL ARTICLE

p-ISSN: 2008-2258 e-ISSN: 2008-4234

Dynamic thiol/disulfide homeostasis and myeloperoxidase levels in Gilbert's syndrome with mild hyperbilirubinemia

Burak Furkan Demir[1], Canan Topcuoglu[2], Turan Turhan[2], Emin Altıparmak[3], Nisbet Yılmaz[1], İhsan Ateş[1]

[1]Ankara City Hospital, Internal Medicine Department, Ankara, Turkey
[2]Ankara City Hospital, Department of Biochemistry, Ankara, Turkey
[3]Ankara City Hospital, Department of Gastroenterology, Ankara, Turkey

ABSTRACT

Aim: This study aimed to compare dynamic thiol/disulfide homeostasis and myeloperoxidase (MPO) levels in patients with Gilbert's syndrome (GS) and healthy controls.

Background: Thiol/disulfide homeostasis and MPO levels are both associated with increased progression of atherosclerosis.

Methods: The study included a total of 130 voluntary participants comprising 65 patients with GS and 65 healthy controls. These patients were selected randomly and dynamic thiol/disulfide homeostasis, MPO, complete blood count results, and biochemistry and lipid parameters were evaluated. Patients with known chronic diseases, medication usage, and acute infections were excluded from the study. Serum total thiol and native thiol levels were measured using the fully automated colorimetric method, while serum MPO levels were measured using the sandwich ELISA method.

Results: We found that patients with GS had significantly higher total thiol (352.3±38.6 vs. 317.9±47.9, p<0.001) and native thiol (386.6±42.6 vs. 348.0±51.1, p<0.001) and significantly lower disulfide (15.7±4.0 vs. 17.3±4.0, p=0.022) and MPO (130.7 vs. 166.3, p=0.006). In patients with bilirubin of <1 mg/dL, total thiol and native thiol levels were lower and disulfide, disulfide/native thiol (DNT) and disulfide/total thiol (DTT) ratios, and MPO levels were higher. Patients with bilirubin of <1 mg/dL also had higher total cholesterol.

Conclusion: In these patients with GS, the thiol/disulfide balance shifted towards thiols and proinflammatory MPO levels were lower. When bilirubin was <1 mg/dL, disulfide, DNT and DTT ratios, and MPO were higher. Bilirubin levels affected all parameters of thiol/disulfide homeostasis and MPO levels independently of other risk factors. In light of our results, we suggest that mild hyperbilirubinemia in cases of GS has an anti-inflammatory and antioxidant effect and may be protective against atherosclerosis.

Keywords: Disulfides, Gilbert's syndrome, Hyperbilirubinemia, Myeloperoxidase, Thiols

(Please cite as: **Demir BF, Topcuoglu C, Turhan T, Altıparmak E, Yılmaz N, Ateş İ. Dynamic thiol/disulfide homeostasis and myeloperoxidase levels in Gilbert's syndrome with mild hyperbilirubinemia. Gastroenterol Hepatol Bed Bench 2024;17(3):270-278. https://doi.org/10.22037/ghfbb.v17i3.2968).**

Introduction

Gilbert's syndrome (GS) is a condition that is usually associated with mild hyperbilirubinemia while other biochemical tests are normal. In individuals with GS, UGT1A1 activity, which is involved in bilirubin conjugation, decreases to 10-35% of the normal range. The literature suggests that bilirubin conjugation and hepatocyte bilirubin uptake are impaired in GS. Studies on patients with GS with elevated bilirubin have shown that the prevalence of atherosclerotic diseases such as coronary artery disease, myocardial infarction, and peripheral artery disease is lower than that of the normal population. This finding is ascribed to the antioxidant effect of bilirubin, which inhibits low-density lipoprotein (LDL) oxidation, neutralizes oxygen radicals, and reduces oxidative stress. Studies have shown that bilirubin has a more prominent protective effect on ventricular monocytes than either vitamin C or vitamin E and is directly related to total antioxidant capacity (1-7).

Atherosclerosis is a progressive, chronic, and potential fatal disease characterized by fatty fibrous

Received: 01 April 2024 *Accepted*: 03 June 2024
Reprint or Correspondence: **Burak Furkan Demir**, *Ankara City Hospital, Internal Medicine Department, Ankara, Turkey*.
E-mail: brkfrkndmr@gmail.com
ORCID ID: 0000-0001-9679-8042

plaques and chronic inflammation in large and medium-sized arteries (8).

Myeloperoxidase (MPO) is an enzyme that generates strongly reactive oxygen radicals. MPO increases the synthesis of proinflammatory cytokines interleukin-6 and interleukin-8 from the vascular endothelium and inhibits nitric oxide-mediated vasodilation. It also increases macrophage activation via LDL oxidation and thus triggers plaque formation and endothelial dysfunction in the atherosclerotic endothelium (9-12).

Thiols are an important part of the antioxidant system. They reduce, neutralize, and react reversibly with oxidants to form disulfide (R–S–S–R) bonds. These disulfides can later react with hydrogen to produce native thiols. Those reactions form the basis of the active and reversible thiol/disulfide homeostasis that provides protection against oxidative stress (13, 14).

We propose that mild hyperbilirubinemia may serve to shift the thiol/disulfide equilibrium towards thiol and that MPO, which is involved in the generation of oxygen radicals and the pathogenesis of atherosclerosis, may be reduced in cases of GS. Accordingly, we aimed to investigate thiol/disulfide homeostasis and MPO in GS and their determinants and to compare our results with those of a healthy population. Our study is the first in the literature to investigate thiol/disulfide homeostasis and MPO levels in cases of GS and their associations with hyperbilirubinemia. Previous studies have not established a clear relationship among hyperbilirubinemia, thiol/disulfide homeostasis, and MPO.

Methods

Study design

This study was conducted between March 2019 and June 2019 in the Ankara Numune Training and Research Hospital Internal Medicine Clinic.

The study included patients diagnosed with GS (ICD code E80.4) who presented to the Internal Medicine, Gastroenterology, and Family Medicine Outpatient Clinics of XXX for follow-up. A total of 65 patients and 65 healthy volunteers, aged 18-65 years, were enrolled. All participants signed informed voluntary consent forms.

The exclusion criteria were as follows for each group: comorbidities (anemia, hypertension, diabetes mellitus, acute/chronic kidney failure, liver failure, congestive heart failure, coronary artery disease, arrhythmia, cerebrovascular disease, chronic obstructive pulmonary disease, asthma, thyroid disease, cancer, autoimmune hemolytic diseases), the use of medication (acetylsalicylic acid, nonsteroidal anti-inflammatory drugs, warfarin, low-molecular-weight heparin, new-generation anticoagulants), smoking, and the use of vitamin supplements. We selected and enrolled individuals in the healthy control group randomly. Sample size calculations were performed using G*Power 3.1.9.6 for macOS.

The study was granted ethical approval by the relevant local ethics committee (date: 07.03.2019, number: 2585/2019).

Biochemical parameters

Serum samples were collected from all participants. Serum albumin, alkaline phosphatase (ALP), total bilirubin, and direct bilirubin levels were measured by colorimetry; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured enzymatically; gamma-glutamyl transferase (GGT), total cholesterol, triglyceride, and uric acid were measured using the enzymatic colorimetric method; and high-density lipoprotein (HDL) cholesterol was measured using the homogeneous enzymatic colorimetric method with an autoanalyzer (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). C-reactive protein (CRP) levels were measured via immunoturbidimetry with an autoanalyzer (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). LDL values were calculated using the equation suggested by Friedewald et al. Hematological tests were performed using the Sysmex XN-1000 device (Sysmex Corporation, Kobe, Japan).

Dynamic thiol/disulfide parameters

Serum total thiol and native thiol levels were measured using the fully automated colorimetric method developed by Erel et al. (13). First, the dynamic disulfide bonds in the samples were reduced to functional thiol by adding NaBH4. Subsequently, total and native thiol levels were measured by 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) reduction. The difference between total and native thiol was divided by two to obtain the amount of disulfide bonds in each sample. For this test, the range of detection is 2.8-400 µmol/L. %CV values are 4%, 5%, and 13% for

concentrations of 29.1, 16.0, and 7.15 µmol/L, respectively.

Myeloperoxidase measurement

Serum MPO levels were measured using the sandwich ELISA method with a commercial SinoGeneClon kit (SinoGeneClon Biotech Co., Ltd., Hangzhou, China; Ref. No. SG10664, Lot 201902). The sensitivity of this method is 0.5 pg/mL and intra- and inter-study CV values are <8% and <10%, respectively.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 20 for Windows (IBM Corp., Armonk, NY, USA). The normal distribution of the data was confirmed with the Kolmogorov-Smirnov test. Normally distributed numerical variables (age, white blood cells, neutrophils, hemoglobin, platelets, total cholesterol, HDL, LDL, albumin, lactate dehydrogenase, uric acid, native thiol, total thiol, and disulfide) were presented as mean ± standard deviation and non-normally distributed numerical variables (total/direct/indirect bilirubin, monocytes, triglycerides, ALT, AST, GGT, ALP, CRP, and sedimentation) were presented as median (minimum-maximum). Categorical variables were presented as numbers and percentages and were compared using chi-square and Fisher exact tests. The Student t-test was used for the pairwise comparison of normally distributed numerical variables and the Mann-Whitney U test was used for the pairwise comparison of non-normally distributed numerical variables. Analysis of variance (ANOVA) with post hoc Bonferroni correction was used to compare numerical variables with normal distribution, and the Kruskal-Wallis H test with post hoc Dunn correction was used to compare numerical variables with non-normal distribution between more than two groups. The correlations between numerical variables were evaluated using Pearson or Spearman correlation analyses depending on the distribution of the relevant data. Robust regression analysis was performed to identify independent determinants of native thiol, total thiol, disulfide, and MPO levels. Values of $p<0.05$ (*) were accepted as statistically significant.

Results

Participants' demographic data

This study included a total of 130 participants: 65 patients with GS and 65 healthy control subjects. The mean age of all participants was 27.3±10.2 (range: 18-60) years. Table 1 presents the detailed demographic characteristics of the study population. There was no significant difference in the ages of the participants in the GS and control groups. There were also no significant difference between sexes or age groups according to bilirubin levels (Table 2).

Among laboratory parameters, GS patients had significantly higher mean hemoglobin (15.4±1.5 vs. 14.8±1.8 g/dL, p=0.024), median total bilirubin (1.9 vs. 0.5 mg/dL, p<0.001), median direct bilirubin (0.5 vs. 0.2 mg/dL, p<0.001), and median indirect bilirubin (1.4 vs. 0.3 mg/dL, p<0.001), while they had significantly lower mean total cholesterol (149.2±32.9 vs. 161.3±35.6 mg/dL, p=0.046) and mean lactate dehydrogenase (178.1±33.0 vs. 188.0±23.6 mg/dL, p=0.048).

Bilirubin levels were <1 mg/dL in 93.8% (n=61) of the control group, while they were 1-2 mg/dL in 53.8% (n=35) and >2 mg/dL in 44.6% (n=29) of the patients with GS (Table 3).

Compared to the control group, GS patients had significantly higher mean total thiol levels (352.3±38.6 vs. 317.9±47.9 µmol/L, p<0.001) and mean native thiol

Table 1. Demographic characteristics

Variables	Overall n=130	Gilbert's syndrome n=65	Control group n=65	p
Age (years)	27.3±10.2	26.5±10.5	28.0±9.9	0.416
<20, n (%)	54 (41.5)	28 (43.1)	26 (40.0)	0.779
20-40, n (%)	59 (45.4)	30 (46.2)	29 (44.6)	
>40, n (%)	17 (13.1)	7 (10.8)	10 (15.4)	
Sex, n (%)				
Female	35 (26.9)	12 (18.5)	23 (35.4)	
Male	95 (73.1)	53 (81.5)	42 (64.6)	0.047*

Normally distributed numerical data are presented as means ± standard deviations. Categorical variables are presented as numbers (%). *: p<0.05, statistically significant.

Table 2. Demographic characteristics and bilirubin levels

Variables	Bilirubin levels (mg/dL)			p
	<1	1-2	>2	
	n=62	n=39	n=29	
Age, years	27.6±9.6	27.7±11.5	26±9.7	0.767
<20, n (%)	26 (41.9)	17 (43.6)	11 (37.9)	0.929
20-40, n (%)	28 (45.2)	16 (41.0)	15 (51.7)	
>40, n (%)	8 (12.9)	6 (15.4)	3 (10.3)	
Sex, n (%)				
Female	20 (32.3)	12 (30.8)	3 (10.3)	0,071
Male	42 (67.7)	27 (69.2)	26 (89.7)	

Normally distributed numerical data are presented as means ± standard deviations. Categorical variables are presented as numbers (%).*: p<0.05, statistically significant.

Table 3. Distribution of laboratory findings

Variables	Overall n=130	Gilbert's syndrome n=65	Control group n=65	p
White blood cells (3900-10200 µL)	7204.6±1647	7252.3±1665	7156.9±1640.3	0.743
Neutrophils (1500-7700 µL)	4142.3±1364	4160±1357.7	4124.6±1380.6	0.883
Monocytes (100-900 µL)	500 (100-1800)	500 (100-1800)	500 (200-900)	0.774
Hemoglobin (13.5-17.2 g/dL)	15.1±1.7	15.4±1.5	14.8±1.8	0.024*
Platelets (150000-400000 µL)	256976.9±57404.4	253800±58634.8	260153.8±56422.3	0.530
Total cholesterol (<200 mg/dL)	155.3±34.7	149.2±32.9	161.3±35.6	0.046*
HDL (>40 mg/dL)	49.6±11.3	49.6±11.3	49.5±11.4	0.975
LDL (<100 mg/dL)	83.6±28.3	79±28.3	88.1±27.8	0.067
Triglycerides (<150 mg/dL)	93 (35-518)	91 (35-332)	101 (37-518)	0.216
Albumin (g/dL)	4.9±0.5	5.0±0.4	4.9±0.5	0.161
ALT (<50 U/L)	15 (5-89)	15 (6-57)	15 (5-89)	0.801
AST (<35 U/L)	17 (9-56)	16 (9-56)	17 (11-42)	0.938
GGT (<73 U/L)	16 (6-169)	16 (6-169)	16 (6-47)	0.814
Lactate dehydrogenase (120-246 U/L)	183.0±29.0	178.1±33.0	188.0±23.6	0.048*
ALP (56-119 U/L)	70.5 (11-160)	72 (11-160)	70 (36-139)	0.751
Sedimentation (0-20 mm/h)	2 (2-37)	2 (2-37)	2 (2-33)	0.111
CRP (0-5 mg/L)	1 (0.3-22)	1 (0.3-22)	1 (0.3-9)	0.831
Uric acid (3.7- 9.2 mg/dL)	4.2±1.2	4.1±1.3	4.2±1.2	0.510
Bilirubin (mg/dL)				
Total (0.3-1.2 mg/dL)	1.1 (0.1-6.1)	1.9 (0.7-6.1)	0.5 (0.1-1.1)	<0.001*
Direct (<0.3 mg/dL)	0.3 (0.1-3.5)	0.5 (0.2-3.5)	0.2 (0.1-0.4)	<0.001*
Indirect (<0.9 mg/dL)	0.8 (0-3.1)	1.4 (0.5-3.1)	0.3 (0-0.8)	<0.001*
Bilirubin, n (%)				
<1 (mg/dL)	62 (47.7)	1 (1.5)	61 (93.8)	<0.001*
1-2 (mg/dL)	39 (30.0)	35 (53.8)	4 (6.2)	
>2 (mg/dL)	29 (22.3)	29 (44.6)	-	

Normally distributed numerical data are presented as means ± standard deviations. Non-normally distributed numerical data are presented as medians (minimum-maximum ranges). Categorical variables are presented as numbers (%). *: p<0.05, statistically significant.

levels (386.6±42.6 vs. 348.0±51.1 µmol/L, p<0.001), while they had significantly lower mean disulfide levels (15.7±4.0 vs. 17.3±4.0 µmol/L, p=0.022), mean disulfide/native thiol (DNT) ratios (4.5±1.0% vs. 5.6±1.6%, p<0.001), mean disulfide/total thiol (DTT) ratios (4.1±0.9% vs. 5.1±1.4%, p<0.001), and median MPO levels (130.7 vs. 166.3 ng/mL, p=0.006) (Table 4).

In the GS group, native thiol and total thiol levels were negatively correlated with age (r=-0.334, p=0.007 and r=-0.393, p=0.001, respectively), total cholesterol (r=-0.265, p=0.049 and r=-0.268, p=0.031, respectively), LDL (r=-0.278, p=0.025 and r=-0.292, p=0.018, respectively), and sedimentation (r=-0.258, p=0.048 and r=-0.250, p=0.044, respectively), while they were

Table 4. Distribution of oxidative stress parameters

Variables	Overall n=130	Gilbert's syndrome n=65	Control group n=65	p
Native thiol (μmol/L)	335.1±46.6	352.3±38.6	317.9±47.9	<0.001*
Total thiol (μmol/L)	367.3±50.7	386.6±42.6	348.0±51.1	<0.001*
Disulfide (μmol/L)	16.5±4.0	15.7±4.0	17.3±4.0	0.022*
NTT, %	91.1±4.0	91.0±2.0	91.2±6.0	0.676
DNT, %	4.6±1.3	4.5±1.0	5.6±1.6	<0.001*
DTT, %	5.0±1.5	4.1±0.9	5.1±1.4	<0.001*
Myeloperoxidase ng/mL	142.6 (59.5-585.3)	130.7 (59.5-336.1)	166.3 (82.1-585.3)	0.006*

Normally distributed numerical data are presented as means ± standard deviations. Non-normally distributed numerical data are presented as medians (minimum-maximum ranges). *: p<0.05, statistically significant.

Table 5. Independent determinants of oxidative stress

Variables	β±SE	95% CI		p
		Lower limit	Upper limit	
Native thiol				
Total bilirubin	11.6±3.7	4.4	18.8	0.002*
Albumin	20.7±8.1	4.6	36.9	0.012*
LDL	-0.45±0.13	-0.71	-0.19	0.001*
	R^2=0.228, p<0.001*			
Total thiol				
Total bilirubin	12.5±3.9	4.7	20.2	0.002*
Albumin	29.7±8.7	12.4	46.9	0.001*
LDL	-0.46±0.14	-0.74	-0.19	0.001*
	R^2=0.275, p<0.001*			
Disulfide				
Age	-0.11±0.04	-0.18	-0.04	0.002*
Sex	1.9±0.8	0.32	3.54	0.019*
Total bilirubin	-0.95±0.3	-1.59	-0.31	0.004*
	R^2=0.291, p<0.001*			
Myeloperoxidase				
Age	1.9±0.9	0.11	3.72	0.048*
Albumin	-36.6±18.4	-72.99	-0.31	0.043*
Total bilirubin	-25.7±8.4	-42.3	-9.2	0.003*
	R^2=0.250, p<0.001*			

*: p<0.05, statistically significant. Abbreviations: β, regression coefficient; SE, standard error; CI, confidence interval.

positively correlated with hemoglobin (r=0.299, p=0.016 and r=0.336, p=0.006, respectively) and albumin (r=0.455, p<0.001 and r=0.503, p<0.001, respectively).

The disulfide levels of the GS patients were not significantly correlated with demographic characteristics or laboratory results, but their MPO levels correlated with age (r=0.334, p=0.007).

Independent determinants of oxidative stress were investigated via retrospective multivariate regression analysis. The results are presented in Table 5. Independently of other risk factors, a 1-unit increase in total bilirubin, albumin, or LDL resulted in an 11.6-fold increase, a 20.7-fold increase, or a 0.45-fold decrease in native thiol levels, respectively. Independently of other risk factors, a 1-unit increase in total bilirubin, albumin, or LDL resulted in a 12.5-fold increase, a 29.7-fold increase, or a 0.46-fold decrease in total thiol, respectively. Independently of other risk factors, a 1-unit increase in total bilirubin, a 1-year increase in age, or being male resulted in a 0.95-fold decrease, a 0.11-fold decrease, or a 1.9-fold increase in disulfide levels, respectively. Independently of other risk factors, a 1-year increase in age, a 1-unit increase in albumin, or a 1-unit increase in total bilirubin resulted in a 1.9-fold increase, a 36.6-fold decrease, or a 25.7-fold decrease in MPO, respectively.

Total cholesterol was significantly higher for patients with a bilirubin level of <1 mg/dL but not

Table 6. Distribution of oxidative stress parameters according to bilirubin levels

Variables	Bilirubin level (mg/dL)			p
	<1	1-2	>2	
	n=62	n=39	n=29	
Native thiol (µmol/L)	315.7±47.3	348.7±39.8	358.2±36.6	<0.001*
Total thiol (µmol/L)	345.9±50.8	383.1±44.5	391.8±39.7	<0.001*
Disulfide (µmol/L)	17.5±4.1	15.5±3.9	15.8±3.9	0.026*
NTT, %	91.4±0.6	91.1±0.2	91.4±1.6	0.922
DNT, %	5.7±1.6	4.5±1.0	4.4±1.0	<0.001*
DTT, %	5.2±1.4	4.1±1.0	4.0±0.9	<0.001*
Myeloperoxidase (ng/mL)	167.7 (82.1-585.3)	130.7 (62.7-423.7)	127.8 (59.5-275)	0.003*

Normally distributed numerical data are presented as means ± standard deviations. Non-normally distributed numerical data are presented as medians (minimum-maximum ranges). Values given in bold indicate significant intergroup differences. *: p<0.05, statistically significant.

significantly different between patients with bilirubin levels of 1-2 mg/dL and >2 mg/dL (162.7±35.6 vs. 148.3±32.2 vs. 143.4±32.9, respectively; p=0.037).

Patients with a bilirubin level of <1 mg/dL had significantly lower mean native thiol (315.7±47.3 vs. 348.7±39.8 vs. 358.2±36.6 µmol/L, respectively; p<0.001) and mean total thiol (345.9±50.8 vs. 383.1±44.5 vs. 391.8±39.7 µmol/L, respectively; p<0.001) and significantly higher mean disulfide (17.5±4.1 vs. 15.5±3.9 vs. 15.8±3.9 µmol/L, respectively; p<0.001), mean DNT ratio (5.7±1.6 vs. 4.5±1.0 vs. 4.4±1.0 µmol/L, respectively; p<0.001), mean DTT ratio (5.2±1.4 vs. 4.1±1.0 vs. 4.0±1.0 µmol/L, respectively; p<0.001), and median MPO (167.7 vs. 130.7 vs. 127.8 ng/mL; p=0.003) compared to patients with bilirubin levels of 1-2 mg/dL and >2 mg/dL. Oxidative stress parameters did not differ significantly between patients with bilirubin levels of 1-2 mg/dL and >2 mg/dL (Table 6).

Laboratory findings and oxidative stress parameters were not significantly different between GS patients with bilirubin levels above and below 2 mg/dL.

GS patients aged >40 years had significantly lower mean native thiol (361.3±38 vs. 355.1±38.3 vs. 325.3±32.0 µmol/L, respectively; p=0.048), significantly lower mean total thiol (400.6±40.1 vs. 381.3±42.4 vs. 353.6±33.0 µmol/L, respectively; p=0.019), and significantly higher median MPO (107 vs. 116.8 vs. 179.1 ng/mL, respectively; p=0.045). Other oxidative stress parameters were not significantly different between different age groups. Mean native thiol (328±34.2 vs. 357.7±37.6 µmol/L, p=0.015) and mean total thiol (355.7±37.3 vs. 393.6±40.8 µmol/L, p=0.005) were significantly lower among female versus male GS patients. Mean disulfide and median MPO levels were not significantly different between the sexes.

Discussion

In our study, for all participants (65 GS patients and 65 control subjects), total thiol, native thiol, and disulfide levels were negatively correlated with age. Dröge reported that aging shifted the thiol/disulfide homeostasis towards oxidation and was correlated with a decrease in the intracellular thiol pool and, consequently, with disordered cellular function (15). Ateş et al. reported a negative correlation between total thiol and age (16). In our sub-analysis of patients with GS, we also found age to be negatively correlated with total thiol and native thiol levels, but not with disulfide levels.

Son et al. demonstrated that MPO levels increased with age, which may be associated with increased inflammatory cells and protein oxidation in aging (17). In our study, we found that MPO increased with age for all control subjects and in the GS group.

Bulmer et al. reported that GS patients had increased plasma antioxidants, erythrocyte antioxidant enzymes, and circulating antioxidant capacity (18). Copur et al. similarly found increased total antioxidant status in patients diagnosed with GS (19). Vitek et al. reported reduced ischemic heart disease in GS patients (5). Zhang et al. found MPO levels to be higher in patients with coronary artery disease than in the control group and concluded that elevated MPO may be a risk factor for coronary artery disease (20).

In our study, bilirubin and hemoglobin were higher and total cholesterol and lactate dehydrogenase were

lower in the GS group compared to the control group. Furthermore, mean total thiol and mean native thiol were higher while mean disulfide, DNT ratio, DTT ratio, and median MPO were lower in GS patients than controls. These results are consistent with the protective effect of hyperbilirubinemia and the findings reported in previous studies. Reduced levels of MPO as a pro-inflammatory and atherogenic mediator and the shift in dynamic thiol/disulfide homeostasis towards thiols in the GS group suggest that GS patients are more resistant to oxidative stress and atherosclerosis due to the protective effect of bilirubin.

Şimşek et al. argued that a shift in thiol/disulfide homeostasis towards disulfide in patients with familial hypercholesterolemia is an important step in the progression to atherosclerosis, and they found a negative correlation between total and native thiol and total cholesterol, LDL, and triglyceride levels (21). In our study, native and total thiol were negatively correlated with total cholesterol, LDL, and sedimentation rate and positively correlated with albumin and hemoglobin in the GS group.

We found that, independently of other risk factors, a 1-unit increase in total bilirubin, albumin, or LDL resulted in an 11.6-fold increase, a 20.7-fold increase, or a 0.45-fold decrease in native thiol levels, respectively. Moreover, we established total bilirubin, albumin, and LDL as independent determinants of total thiol. Independently of other risk factors, a 1-unit increase in total bilirubin, albumin, or LDL resulted in a 12.5-fold increase, a 29.7-fold increase, or a 0.46-fold decrease in total thiol, respectively.

In light of our results, we suggest that bilirubin, an antioxidant, shifts the thiol/disulfide homeostasis towards thiol. We ascribe the positive correlation between thiol compounds and albumin to the fact that albumin thiols constitute the majority of the thiol pool. Our finding that LDL, a prominent factor in the pathogenesis of atherosclerosis, shifted the thiol/disulfide homeostasis towards disulfide is consistent with the results reported by Şimşek et al. (21).

We further suggest that the negative correlations between bilirubin and disulfide and MPO are important due to demonstrating bilirubin's antioxidant potential. Choi et al. found that reduced bilirubin was associated with higher age, triglycerides, and CRP, while higher bilirubin was associated with higher HDL, GGT, uric acid, hemoglobin, and albumin (22).

In our study, we divided patients into 3 groups according to total bilirubin levels of <1 mg/dL, 1-2 mg/dL, and >2 mg/dL. These three groups were similar in terms of age and sex. This may be due to the relatively young and small study population. In patients with bilirubin of <1 mg/dL, we found significantly higher total cholesterol and insignificantly higher LDL and triglyceride levels. Lactate dehydrogenase levels were reduced in patients with bilirubin of 1-2 mg/dL. Moreover, we found that hemoglobin was significantly higher in the GS group than in the control group (15.4±1.5 vs. 14.8±1.8 g/L, p=0.024). Similarly, Choi et al. demonstrated that hemoglobin had the strongest influence on total bilirubin levels (22). Among oxidative stress parameters, patients with bilirubin of <1 mg/dL had significantly lower total thiol and native thiol and higher disulfide, DNT ratios, DTT ratios, and MPO.

We also divided the GS patients into two groups using a bilirubin level of 2 mg/dL as the cut-off and did not find a significant difference in demographic, laboratory, or oxidative stress parameters. In their study of 124 infants, Topal et al. compared two groups with mean total bilirubin levels of 15.93±4.28 mg/dL and 8.21±2.41 mg/dL and found that bilirubin was negatively correlated with total and native thiol, while it was positively correlated with disulfide and bilirubin. They concluded that elevated bilirubin shifted the dynamic thiol/disulfide homeostasis towards disulfide, thus leading to oxidative stress in infants (23). In contrast, we found that mild hyperbilirubinemia shifted the thiol/disulfide homeostasis towards thiol. This is further supported by our finding that patients with bilirubin of <1 mg/dL had elevated disulfide, DNT ratios, and DTT ratios and reduced MPO. These findings suggest that only mild hyperbilirubinemia may be protective against oxidative stress (24).

In this study, we investigated dynamic thiol/disulfide homeostasis and MPO levels and their determinants in 65 healthy individuals and 65 patients with GS. The limitations of our study include its relatively small sample size (n=130). The imbalanced distribution of sexes between the groups is also one of the limitations of the study. However, to the best of our knowledge, this study is the first to investigate dynamic thiol/disulfide homeostasis and MPO levels in cases of GS with mild

hyperbilirubinemia. We consider this to be key in terms of the contributions of our study to the literature.

Conclusion

We conclude that GS patients have elevated total thiol and native thiol and reduced disulfide, DNT and DTT ratios, and MPO compared to healthy individuals. Bilirubin levels were found to affect all parameters of thiol/disulfide homeostasis and MPO levels independently of other risk factors. In light of our findings concerning dynamic thiol/disulfide homeostasis and MPO, mild hyperbilirubinemia may be associated with lower risk for oxidative stress and atherosclerosis.

Conflict of interests

The authors declare that they have no competing interests.

References

1. Fretzayas A, Moustaki M, Liapi O, Karpathios T. Gilbert syndrome. Eur J Pediatr 2012;171:11-5.

2. Kobayashi T, Sleeman JE, Coughtrie MW, Burchell B. Molecular and functional characterization of microsomal UDP-glucuronic acid uptake by members of the nucleotide sugar transporter (NST) family. Biochem J 2006;400:281-9.

3. Wolkoff AW, The hyperbilirubinemias. In: Loscalzo J, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson L, editors. Harrison's Principles of Internal Medicine 19th Edition. New York: Mc Graw-Hill; 2015.P.1999-2004.

4. Djoussé L, Levy D, Cupples LA, Evans JC, D'Agostino RB, Ellison RC. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. Am J Cardiol 2001;87:1196-200.

5. Vitek L, Jirsa M, Brodanova M, Kalab M, Marecek Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002;160:449-56.

6. Wu T-W, Fung K, Wu J, Yang C-C, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochem Pharmacol 1996;51:859-62.

7. Ukibe NR, Onwe CT, Ukibe COEG, Ukibe BC, Ukibe VE, Obeagu EI. Advances in laboratory diagnosis and clinical management of Gilbert disease: a comprehensive review. Pathophysiology 2024;17:24.

8. Mallika V, Goswami B, Rajappa M. Atherosclerosis pathophysiology and the role of novel risk factors: a clinicobiochemical perspective. Angiology 2007;58:513-22.

9. Van der Veen BS, de Winther MP, Heeringa P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid Redox Signal 2009;11:2899-937.

10. Eriksson EE, Xie X, Werr J, Thoren P, Lindbom L. Importance of primary capture and l-selectin–dependent secondary capture in leukocyte accumulation in inflammation and atherosclerosis in vivo. J Exp Med 2001;194:205-18.

11. Lefkowitz DL, Roberts E, Grattendick K, Schwab C, Stuart R, Lincoln J, et al. The endothelium and cytokine secretion: the role of peroxidases as immunoregulators. Cell Immunol 2000;202:23-30.

12. Van Der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite a potential additional mechanism of nitric oxide-dependent toxicity. J Biol Chem 1997;272:7617-25.

13. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.

14. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. J Biol Chem 2013;288:26489-96.

15. Dröge W. Aging-related changes in the thiol/disulfide redox state: implications for the use of thiol antioxidants. Exp Gerontol 2002;37:1333-45.

16. Ateş İ, Özkayar N, Yılmaz FM, Bayrakçı N, Neşelioğlu S, Erel Ö, et al. Oxidative stress level in patients with chronic kidney disease. Ortadogu Tıp Derg 2018;10:45-50.

17. Gen Son T, Zou Y, Pal Yu B, Lee J, Young Chung H. Aging effect on myeloperoxidase in rat kidney and its modulation by calorie restriction. Free Radic Res 2005;39:283-9.

18. Bulmer AC, Blanchfield JT, Toth I, Fassett RG, Coombes JS. Improved resistance to serum oxidation in Gilbert's syndrome: a mechanism for cardiovascular protection. Atherosclerosis 2008;199:390-6.

19. Copur B, Yilmaz N, Topcuoglu C, Kiziltunc E, Cetin M, Turhan T, et al. Relationship between elevated bilirubin level and subclinical atherosclerosis as well as oxidative stress in Gilbert syndrome. Gastroenterol Hepatol Bed Bench 2020;13:133.

20. Zhang R, Brennan M-L, Fu X, Aviles RJ, Pearce GL, Penn MS, et al. Association between myeloperoxidase levels and risk of coronary artery disease. Jama 2001;286:2136-42.

21. Şimşek Ö, Çarlıoğlu A, Alışık M, Edem E, Biçer CK. Thiol/disulfide balance in patients with familial hypercholesterolemia. Cardiol Res Pract 2018;2018.

22. Choi S, Yun K, Choi H. Relationships between serum total bilirubin levels and metabolic syndrome in Korean adults. Nutr Metab Cardiovasc Dis 2013;23:31-7.

23. Topal I, Mertoglu C, Surucu Kara I, Siranli G, Gok G, Erel Ö. Thiol-disulfide homeostasis, serum ferroxidase activity, and serum ischemia modified albumin levels in neonatal jaundice. Fetal Pediatr Pathol 2019;38:138-45.

24. Vítek L, Tiribelli C. Gilbert´ s syndrome revisited. J Hepatol 2023;79:1049-1055.