An Evaluation of Thiol/Disulphide Homeostasis and HDL Values in Patients with Cholelithiasis: A Prospective Case-control Study

Guniz Yanik Ustuner[1], Fatma Ebru Akin[2], Suadiye Saglam[3], Gamze Gok[3], Ozcan Erel[3] and Osman Ersoy[2]

[1]*Department of Internal Medicine, Bursa Cuneyt Yildiz State Hospital, Bursa, Turkey*
[2]*Department of Gastroenterology, Ankara Yildırım Beyazit University, Ankara City Hospital, Ankara, Turkey*
[3]*Department of Biochemistry, Ankara Yildırım Beyazit University, Ankara City Hospital, Ankara, Turkey*

ABSTRACT

Objective: To determine the changes in thiol/disulphide homeostasis in patients determined with asymptomatic cholelithiasis and to investigate any potential correlation between these thiol-disulphide parameters and HDL cholesterol.
Study Design: A descriptive, comparative study.
Place and Duration of Study: Ankara City Hospital between 15 September and 31 December 2019.
Methodology: This study included 42 patients aged 18-70 years, who presented at the Gastroenterology Clinic and were diagnosed with cholelithiasis on ultrasound examination. A control group was formed of 51 healthy volunteers aged 18-70 years. Thiol/disulphide homeostasis and HDL cholesterol was noted and compared between the groups.
Results: The mean age was 44.16 ± 13.35 years in cholelithiasis patient group and 31.88±13.27 years in the control group. The triglyceride, VLDL, total cholesterol/HDL, and non-HDL levels were significantly higher and HDL level was significantly low in the patient group (both $p < 0.05$). Regarding thiol-disulphide balance, native thiol, total thiol, and albumin values were found to be statistically significantly low in the patient group ($p < 0.05$), and the IMA, index-1, index-2, and index-3 values were significantly high ($p < 0.05$).
Conclusion: The oxidant/antioxidant balance shifted towards oxidation in patients with asymptomatic gallstones. The non-HDL value was increased. There was a positive correlation between the thiol-disulphide parameters and the non-HDL value. The increasing non-HDL amount could be effective in the pathogenesis of gallstone disease by disrupting the oxidative balance.

Key Words: *Cholelithiasis, Thiol-disulphide homeostasis, Lipid profile, Non-HDL, HDL, Oxidative stress.*

How to cite this article: Ustuner GY, Akin FE, Saglam S, Gok G, Erel O, Ersoy O. An Evaluation of Thiol/Disulphide Homeostasis and HDL Values in Patients with Cholelithiasis: A Prospective Case-control Study. *J Coll Physicians Surg Pak* 2022; **32(04)**:424-429.

INTRODUCTION

Cholesterol is a lipid, which is synthesised in the liver and secreted *via* the biliary system.[1] When the reasons for the formation of gallstones are examined, although many metabolic abnormalities have been reported such as abnormal regulation of hepatic cholesterol, gallbladder dysfunction, oxidative stress, and imbalance of the pro-oxidant and anti-oxidant systems, the mechanism of gallstone formation has not been fully clarified.[2,3]

Thiols form bonds with disulphides by entering into an oxidative reaction with oxidants. İt is catalysed by specialised enzymes like oxidoreductases and thiol oxidases; and occurs mostly in the endoplasmic reticulum (ER) and the mitochondrial intermembrane space.[4,5]

Correspondence to: Dr. Guniz Yanik Ustuner, Department of Internal Medicine, Bursa Cuneyt Yildiz State Hospital, Bursa, Turkey
E-mail: guniz.yanik@gmail.com
Received: April 28, 2021; Revised: December 17, 2021; Accepted: January 10, 2022
DOI: https://doi.org/10.29271/jcpsp.2022.04.424

Disulphide bonds are covalent bonds, known as disulphide bridge, or SS-bonds. Under oxidative stress, oxidation of cysteine (cys) remnants leads to the formation of reversible mixed disulphides between thiols in protein structure and low-weight thiols. Disulphide bonds can be broken down again to the thiol group. Thus, there is dynamic homeostasis between thiol and disulphide bonds. According to this pathophysiology, in the presence of oxidative stress in the body, there is expected to be a regression in the amount of thiol and an increase in the amount of disulphide.[6,7]

Plasma thiols sweep up the physiological free-radicals and show an antioxidant property with several mechanisms. The thiol group constitutes 52.9% of all the antioxidants in the plasma in healthy individuals. Determination of plasma thiol and the thiol/disulphide balance has been shown to be a reflection of the formation of free-radicals in several diseases.[3,6-8]

Oxidative stress disrupts the oxidative balance because of an increase in reactive oxygen species, such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals, occurring during cellular metabolism, and insufficient antioxidants,

which detoxify these. Serum thiol groups are an important component of the plasma antioxidant system. It has been suggested that disruption of the thiol-disulphide balance plays a role in the pathogenesis of several diseases.[8]

One of the pathologies thought to be associated with oxidative stress is the formation of gallstones.[3] Since the later half of 2014, plasma thiol and disulphide levels can be more accurately measured with a new method described by Erel and Neselioglu.[8] According to one theory, when there is a reduction in the level of the antioxidant enzyme paraoxonase-1 (PON1), which is found in HDL structure and is protective against HDL and LDL oxidation, there is an increase in the thiol/disulphide mechanism, which is the other antioxidant system.[9,10]

The aim of this study was to evaluate the changes in thiol-disulphide parameters in patients with asymptomatic gallstones, compared to a healthy control group; and to determine any relationship between HDL cholesterol and the thiol-disulphide parameters in the patient group.

METHODOLOGY

This descriptive, comparative study included 42 patients aged 18-70 years, who presented at the Gastroenterology Clinic of Ankara City Hospital between 15 September 2019 and 31 December 2019 and were diagnosed with cholelithiasis on ultrasound examination. A control group was formed of 51 healthy (no gallstones on ultrasound) volunteers aged 18-70 years. Inclusion criteria were age in the range of 18-70 years and a diagnosis of asymptomatic cholelithiasis. Exclusion criteria were pregnant patients, those with infection, a history of pulmonary embolism, the presence of deep vein thrombosis, a rheumatological disease, cholestasis, cholangitis, cirrhosis, inflammatory bowel disease, acute/chronic renal failure, a history of acute pancreatitis, and those using lipid-reducing medicines.

Data collection/laboratory tests: Peripheral venous blood samples were taken from all the study subjects for the examination of urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total and direct bilirubin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin, protein, hemogram (full blood count), triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and serum thiol/disulphide levels.

The blood samples taken for the serum thiol/disulphide level were centrifuged at 1600g for 15 mins, then stored at -80°C until assay. All the blood samples were transferred to the laboratory of the same hospital within an average of one hour. The serum analyses were performed with an automatic spectrometer, newly designed by Erel and Neselioglu.

Data obtained in the study were analysed statistically using IBM SPSS Statistics version. 21.0 software (IBM Corporation, Armonk, NY, USA). The numerical variables were stated as mean ± standard deviation (SD) or median (minimum-maximum) values for non-normal distribution. The conformity of continuous variables to normal distribution was tested with the Kolmogorov-Smirnov test. The differences between groups of continuous variables were determined with the Independent Samples t-test or the Mann-Whitney U-test for non-normal distribution, as appropriate. Correlations between variables were examined with Pearson analysis or Spearman correlation analysis, as appropriate. To assess the association between cholelithiasis and thiol/disulfide status, and plasma lipids, correlation analysis was performed. A value of $p < 0.05$ was accepted as statistically significant.

Approval for the study was granted by the Ethics Committee of the University Medical Faculty (Decision No. 81, dated: 17/07/2019).

Table I: General characteristics of the patient group and control group; and the comparisons between the groups of biochemical and hemogram parameters.

Variables	Control group (n=51)	Patient group (n=42)	p-value
Age	28 (18 - 70)	45 (19 - 69)	<0.001
Sex (FM/M)	30/21	27/15	0.590
Urea (mg/dL)	26 (16 - 60)	24.5 (14- 89)	0.286
Creatinine (mg/dL)	0.8 (0.4 - 1.2)	0.7 (0.5 - 1.2)	0.159
Total protein (g/L)	69.43 ± 3.14	67.24 ± 6.64	0.096
Albumin(g/L)	47 (46 - 54)	44 (32- 52)	<0.001
AST (U/L)	20 (12 - 53)	23 (13 - 49)	0.041
ALT (U/L)	17 (10 - 59)	31 (9 - 225)	<0.001
ALP (U/L)	59 (12 - 185)	84.5 (42 - 267)	<0.001
GGT (U/L)	15 (4 - 85)	43 (4 - 253)	<0.001
LDH (U/L)	185 (57 - 330)	188 (25 - 355)	0.274
Amylase (U/L)	72.65 ±22,01	84.34±25.43	0.059
Lipase (U/L)	37 (24- 72)	44 (28 - 122)	0.006
Total bilirubin (mg/dL)	0.6 (0.3 - 2,12)	0.7 (0.32 - 6,4)	0.592
Direct bilirubin (mg/dL)	0.2 (0.1 – 0.71)	0.2 (0.05 – 4.4)	0.481
WBC (x10^9/L)	6656.56 ±1449.11	7417.95 ±2860.95	0.135
Hb (g/dL)	13.99 ± 1.53	13.46 ± 1.46	0.098
Platelet (x10^9/L)	264340.00 ±50620.88	275538.46±86754.60	0.477
ESH (mm/saat)	5.5 (3 - 20)	17 (3 - 40)	0.048
CRP (mg/dL)	0.77 (0.77-4.15)	3.74 (0.75- 16.7)	<0.001

F: Female, M: Male, WBC: White blood cells, Hb: Hemoglobin, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase. Moreover, t-test and the Mann-Whitney U-test were also used.

RESULTS

Evaluation was made of 42 patients diagnosed with cholelithiasis and 51 healthy control subjects, all in the age range of 18 - 70 years. The patient group comprised 26 (64.3%) females and 16 (35.7%) males with a median age of 45 (19 - 69) years. The control group comprised 30 (58.8%) females and 21 (41.1%) males with a median age of 28 (18 - 70) years. In the

patient group, hypertension (HT), diabetes mellitus (DM), HT + DM, and HT, DM + coronary artery disease (CAD) were determined in one patient each. In the comparisons of biochemical parameters, the albumin value of the patient group was found to be statistically significantly lower than that of the control group ($p < 0.001$), and the AST, ALP, GGT, lipase ESR and CRP values were significantly higher ($p < 0.05$, Table I).

In the comparisons of the lipid profiles of the patient group and control group, the triglyceride, VLDL, total cholesterol/HDL, and non-HDL levels were determined to be statistically significantly higher in the patient group than in the control group ($p < 0.05$); whereas, the HDL level was significantly low ($p < 0.027$). The total cholesterol and LDL levels were found to be higher in the patient group but this increase was not statistically significant (Table II).

Table II: Comparisons of the lipid profiles and thiol/disulphide balance in the patient group and the control group.

Variables	Control group (n = 51)	Patient group (n = 42)	p-value
Total cholesterol (mg/dL)	167.35±29.41	179.54±38.84	0.094
Triglyceride (mg/dL)	84.00 (43.00 - 253.00)	126.00 (23.00 - 351.00)	0.002
HDL (mg/dL)	49.00 (29.00 - 98.00)	43.00 (22.00 - 74.00)	0.027
LDL (mg/dL)	97.37 ± 27.83	105.43 ± 29.17	0.183
VLDL (mg/dL)	17.00 (9.00 - 51.00)	25.00 (5.00 - 121.00)	0.001
Total cholesterol/HDL	3.00 (2.00 - 6.00)	4.00 (2.00 - 10.00)	0.001
Non-HDL (mg/dL)	115.96 ± 31.32	134.51 ±37.79	0.015
Native thiol	421.28 ± 33.53	363.39 ± 57.57	<0.001
Total thiol	472.15 (362.35 - 535.20)	425.05 (275.40 - 489.00)	<0.001
Disulphide	22.37 ± 7.06	23.25 ± 9.66	0.623
IMA	0.60 ± 0.12	0.67 ± 0.13	0.007
Albumin	4.38 (3.84 - 4.76)	4.05 (2.84 - 4.70)	<0.001
Index 1	5.40 ± 1.92	6.56 ± 2.93	0.03
Index 2	4.82 ± 1.57	5.69 ± 2.28	0.04
Index 3	90.36 ± 3.14	88.62 ± 4.56	0.04

HDL: High-density lipoprotein, LWQDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, Non-HDL: Lipoproteins other than high-densiity lipoproteins.

In the comparisons of the parameters providing native thiol, total thiol, and albumin values were found to be statistically significantly low in the patient group ($p < 0.05$), and the IMA ($p = 0.007$), index-1 (p:0.03), index-2 (p:0.04), and index-3 (p:0.04) values were significantly high. The increase in the disulphide level in the patient group was not determined to be statistically significant (Table II).

In the control group, no significant correlation was determined between the parameters of the lipid profile (total cholesterol, triglycerides, HDL, LDL, VLDL, total cholesterol/HDL, non-HDL) and those providing thiol-disulphide balance ($p > 0.05$).

In the patient group, when the the lipid profile parameters (total cholesterol, triglycerides, HDL, LDL, VLDL, total cholesterol / HDL, non-HDL) and those providing thiol-disulphide balance were compared, a statistically significant positive correlation was determined between total cholesterol and total thiol ($p = 0.028$) and albumin ($p = 0.033$), between triglycerides and albumin ($p = 0.027$), total thiol ($p = 0.036$), disulphide ($p = 0.032$), and albumin ($p = 0.025$), between total cholesterol/HDL and albumin($p = 0.047$), total thiol ($p = 0.032$) and albumin ($p = 40.011$). No significant correlation was determined between HDL and the parameters providing thiol-disulphide balance (Tables II, III).

DISCUSSION

The studies, which have examined the link between gallstones and oxidative stress, have shown that the contributions of an impairment of the balance between the pro-oxidant and antioxidant systems, oxidative stress and free-radicals to the formation of gallstones could be significant.[2,3] Antioxidant defence systems regulate the levels of endogenous and exogenous reactive oxygen products in the human body.[11,12] Serum thiol groups are important members of the plasma antioxidant system. The thiol-disulphide balance has a critical role in antioxidant protection, detoxification, signal transmission, apoptosis, the regulation of enzymatic activity and transcription factors, and intracellular signal mechanisms.[13,14] With the new method of Erel and Neselioglu, plasma thiol and disulphide levels can be measured separately and in total.[8]

Ischemia-modified albumin (IMA) is an oxidatively modified form of albumin and elevates depending on oxiadative stress (OXS) after acute ischemia and returns to its normal levels in hours after reperfusion.[15]The authors used IMA because it is a marker associated with oxidative stress.

Patients with asymptomatic gallstones were evaluated in the current study; and the results showed that in this patient group, the amount of total thiol and native thiol was reduced, and there was a statistically significant increase in the ratio of disulphide to native thiol and total thiol (index-1 and index-2, $p < 0.05$). These findings suggest that a change in the thiol/disulphide balance could have a role in the pathogenesis of cholelithiasis.

Ozyazici *et al*. reported that the amount of disulphide in thiol/disulphide homeostasis in appendicitis led to an increase in the disulphide/native thiol and disulphide/total thiol ratios.[16] Ercan *et al*. showed that the thiol level fell and the disulphide level increased in acute pancreatitis; and stated that the thiol/disulphide balance could have a role in the pathogenesis of acute pancreatitis.[17]

Moreover, from another perspective, just as an impairment in thiol/disulphide homeostasis could play a role in the pathogenesis of cholelithiasis, it could be considered that cholelithiasis may disrupt the thiol/disulphide homeostasis.

Table III: Comparisons of the parameters providing thiol/disulphide balance and the lipid profiles in the patient group and the control group.

Variables		Native thiol	Total thiol	Disulphide	IMA	Albumin	Index 1	Index 2	Index 3
Total Cholesterol	Correlation coefficient	0.302	0.352	0.177	.120	.343	.111	.111	-.111
	p-value	0.062	.028	.280	.468	.033	.500	.500	.500
Triglyceride	Correlation coefficient	0.115	0.175	0.201	-0.04	0.358	0.134	0.134	-0.134
	p-value	0.493	0.293	0.225	0.81	0.027	0.424	0.424	0.424
HDL	Correlation coefficient	0.058	0.059	-0.033	0.103	-0.099	0	0	0
	p-value	0,726	0.722	0.844	0.534	0,55	1	1	1
LDL	Correlation coefficient	0.34	0.333	0.335	0.066	0.354	0.189	0.189	-0.189
	p-value	0.032	0.036	0.035	0.685	0.025	0.244	0.244	0.244
VLDL	Correlation coefficient	0.175	0.233	0.208	0.063	0.359	0.134	0.134	-0.134
	p-value	0.28	0.147	0.198	0.701	0.023	0.409	0.409	0.409
Total cholesterol / HDL	Correlation coefficient	0.165	0.193	0.095	-0.184	0.328	0.004	0.004	-0.004
	p-value	0.328	0.253	0.578	0.275	0.047	0.98	0.98	0.98
Non HDL	Correlation coefficient	0.344	0.344	0.318	0.175	0.403	0.17	0.171	-0.171
	p-value	0.032	0.032	0.048	0.286	0.011	0.3	0.298	.298

Index 1: Disulphide/native thiol, Index 2: Disulphide/total thiol, Index 3: Native thiol/total thiol, Dısulphıde:total thiol-native thiol/2, IMA: İschaemia modified albümin. Moreover, t-test and the Mann-Whitney U-test were also used.

In a study by Aydin *et al*., the preoperative levels of native thiol, total thiol, and disulphide in symptomatic cholelithiasis patients were found to be lower than those of a control group. No statistically significant difference was found in the disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios; and it was thought that the antioxidant capacity could have been reduced by the oxidative stress originating from cholelithiasis. The postoperative native thiol, total thiol, and disulphide levels were again found to be lower than those of the control group, but a decrease was observed in the disulphide/native thiol and disulphide/total thiol ratios and an increase in the native thiol/total thiol ratio.[2] In the current study, the disulphide/native thiol, disulphide/total thiol, and native thiol/-total thiol ratios were observed to be statistically significantly higher in the asymptomatic cholelithiasis patients than in the control group.

While strong relationships between elevated triglycerides and low HDL values and cholelithiasis have been shown in some studies, the relationship of total cholesterol and LDL with cholelithiasis has been found to be weak.[18]

Ataman *et al.* claimed that high cholesterol and a high LDL level were effective in the formation of gallstones, but a low HDL cholesterol level had no effect.[1] In the current study, when the lipid profiles of the patient group and control group were compared, the triglyceride, VLDL, total cholesterol/HDL, and non-HDL levels were determined to be statistically significantly higher in the patient group than in the control group, while the HDL level was significantly low ($p < 0.05$).

Simsek *et al*. showed that in familial hypercholesterolemia, the total thiol and native thiol levels, and the native thiol/-total thiol ratio decreased, and the disulphide level increased. They stated that when the antioxidant system was impaired, it led to atherosclerosis by increasing LDL oxidation. The total cholesterol, triglycerides, HDL, and LDL levels were compared with the native thiol, total thiol, disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios in a patient group, but no significant correlation was determined ($p > 0.05$).[19] In the current study, when the lipid profiles of the patient group and control group were compared, the triglyceride, VLDL, total cholesterol/HDL, and non-HDL levels were determined to be statistically significantly higher in the patient group than in the control group, while the HDL level was significantly low ($p < 0.05$). A positive correlation was observed between non-HDL and disulphide ($p = 0.048$). No statistically significant relationship was determined between HDL and the parameters providing the thiol/disulphide balance in the patient group.

In theory, when there is a decrease in enzyme PON1 level, which is protective against HDL and LDL oxidation, there will be an increase in the thiol/disulphide mechanism, which is the other antioxidant system.[10] In the current study, a positive correlation was observed between LDL and native thiol ($p = 0.032$) and total thiol ($p = 0.036$), and between non-HDL and total thiol ($p = 0.032$), native thiol ($p = 0.032$) and disulphide ($p = 0.048$), but no statistically significant relationship was determined between HDL and the parameters providing thiol/disulphide balance.

Parlak *et al*. investigated the relationship between pulmonary embolism and the thiol/disulphide redox system and HDL. The total thiol, native thiol, and HDL levels were found to be significantly lower in the patient group compared to the control group ($p = 0.001$). In correlation analysis, a significant positive correlation between HDL and native thiol and total thiol, and a significant negative

correlation between HDL and SH/SS (native thiol/total thiol) was observed.[20]

When selecting the control group, care was taken not to include subjects with comorbidities. However, as the frequency of chronic diseases increase with age and participation in the study was on a voluntary basis, the mean age of the control group was lower than that of the patient group. Although the study was prospective, it was conducted in a single centre and the number of patients was relatively low.

CONCLUSION

In this study of patients with asymptomatic gallstones, the oxidant/antioxidant balance was seen to be shifted towards oxidation, the non-HDL value was increased and a positive correlation was determined between the thiol-disulphide parameters and the non-HDL value. This suggests that the increasing non-HDL amount could be effective in the pathogenesis of gallstone disease by disrupting the oxidative balance. Further multi-centre prospective studies are needed on this subject.

ETHICAL APPROVAL:

Approval for the study was granted by the Ethics Committee of the University Medical Faculty (Decision No. 81, dated: 17/07/2019).

PATIENTS' CONSENT:

Written informed consents were obtained from all the patients.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

GYU: Study design, literature search.
FEA: Literature review, initial drafting.
SS, GG: Statistical analysis, data analysis.
OE: Manuscript editing and review.
OE: Critical review of the manuscript for final approval.

REFERENCES

1. Atamanalp SS, Keles MS, Atamanalp RS, Acemoglu H, Laloglu E. The effects of serum cholesterol, LDL, and HDL levels on gallstone cholesterol concentration. *Pak J Med Sci* 2013; **29(1)**:187. doi.org/10.12669/pjms.291.2798.
2. Aydın O, Pehlivanlı F, Karaca G, Daphan CE, Aydınuraz K, Boyunaga H, *et al.* The effect of cholelithiasis and cholecystectomy on thiol/disulfide homeostasis in patients undergoing cholecystectomy. *KU Tıp Fak J* 2019; **21(1)**: 95-101. -doi.org/10.24938/kutfd.531638.
3. Sanikidze T, Chikvaidze EJRpd. Role of the free radicals in mechanisms of gallstone formation: An EPR study. *Radiat Prot Dosimetry* 2016; **172(1-3)**:317-24. doi: 10.1093/rpd/ncw237.
4. Ulrich K, Jakob U. The role of thiols in antioxidant systems. *Ree Radical Biol Med* 2019, **140**:14-27. doi: 10.1016/j. freeradbiomed.2019.05.035.
5. Herrmann JM, Dick TP, Redox biology on the rise. *Biol Chem* 2012; **393(9)**:999-1004. doi: 10.1515/hsz-2012-0111.
6. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. *J Biol Chem* 2013; **288(37)**: 26489-96. doi: 10.1074/jbc.R113.462929.
7. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med* 2009; **47(10)**: 1329-38. doi: 10.1016/j.freeradbiomed.2009.08.021.
8. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochemistry* 2014; **47(18)**:326-32. doi: 10.1016/j.clinbiochem.2014.09.026.
9. Baskol G, Kose K. Paraoxanase: Biochemical features, functions and clinical importance. *Erciyes Med J* 2004; **26(2)**:75-80.
10. Go YM, Jones DP. Cysteine/cystine redox signaling in cardiovascular disease. *Free Radical Biol Med* 2011; **50(4)**:495-509. doi:10.1016/j.freeradbiomed.2010.11.029
11. Erol S, Arslan Z, Isik D. The effects of bilirubin and phototherapy on neonatal thioldisulfide homeostasis. *J Coll Physicians Surg Pak* 2019; **29(9)**:843-7. doi: 10.29271/jcpsp.2019.09.843.
12. Awad KH, Elmorsy WA. Impact of phototherapy on oxidative stress ındices in preterm neonates with unconjugated hyperbilirubinemia. *Egyptian J Hos Med* 2020; **81(4)**: 1815-21. doi: 10.21608/EJHM.2020.120450.
13. Biswas S, Chida AS, Rahman I. Redox modifications of protein–thiols: Emerging roles in cell signaling. *Biochemical Pharmacol* 2006; **71(5)**:551-64. doi: 10.1016/j.bcp.2005.10.044.
14. Circu ML, Yee Aw T. Medicine. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radical Biology Med* 2010; **48(6)**:749-62. doi: 10.1016/j.freeradbiomed.2009.12.022.
15. Gulpamuk B, Tekin K, Sonmez K, Inanc M, Neselioglu S. The significance of thiol/disulfide homeostasis and ischemia--modified albumin levels to assess the oxidative stress in patients with different stages of diabetes mellitus. *Scandinavian J Clin Laboratory Investigation* 2018; **78**: 136-42. doi: 10.1080/00365513.2017.1422540.
16. Ozyazici S, Karateke F, Turan U, Kuvvetli A, Kilavuz H, Karakaya B, *et al*. A novel oxidative stress mediator in acute appendicitis: Thiol/disulphide homeostasis. *Mediators Inflamm* 2016; 6761050. doi: 10.1155/2016/6761050.
17. Haydar FGE, Otal Y, Şener A, Günaydın GP, İçme F, Temrel TA, *et al*. The thiol- disulphide homeostasis in patients with acute pancreatitis and its relation with other blood parameters. *Turkish Association of Trauma and Emergency Surgery* 2020; **26(1)**:37-42. doi: 10.14744/tjtes.2019. 38969.
18. Faisal N,Saeed M, Lodhi MFB. Association of dieting, physical activity and lipid profile with cholelithiasis in young women. *APMC* 2019; **13(2)**:169-72.
19. Simsek O, Carlıoglu A, Alısık M, Edem E, Bicer C. Thiol/disulfide balance in patients with familial hypercholes-terolemia. *Cardiol Res Practice* 2018; 9042461.

doi: 10.1155/2018/9042461.

20. Parlak ES, Alisik M, Karalezli A, Sayilir AG, Bastug S, Er M, *et al.* Are the thiol/disulfide redox status and HDL cholesterol levels associated with pulmonary embolism? Thiol/disulfide redox status in pulmonary embolism. *Clin Biochemistry* 2017; **50(18)**:1020-4. doi: 10.1016/j.clinbiochem.2017.07.018.