The Relation of Oxidative Stress and Antioxidant Biomarkers in Serum Non-Alcohol Fatty Liver Disease adult and Obese Kids

Fatima J. Abdul Latif[1], Abeer G. Al Sawafi[2], Dawood S. Ali[3], Asmaa T. Mawla[4], Talal H. Noon[5]

[1,2]Medical Laboratory Technology Dept., College of Health and Medical Technology, Southern Technical University, Basrah, Iraq

[3]chemistry Dept., College of Science, University of Basra, Basrah, Iraq.

[4]Basra Children's Specialist Hospital, Basra Health Department/Iraq

[5]Basra GIT Hospital, Al- Zehra 'a Medical College, University of Basra, Basra, Iraq

*Corresponding author:
Abeer G. Al Sawafi
dr.abeer@stu.edu.iq

Abstract

Background and objective: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function and is characterized by hepatic steatosis in individuals with little or no alcohol consumption. The purpose of the study was to find the relationship between enzymatic and non-enzymatic antioxidants, lipid damage (MDA) and some biochemical parameters in 60 adult and 20 kids - patients with NAFLD.

Methods: This study included 160 patients and healthy control group, aged range from 5 to 75 years while BMI range from 15.9 to 50.9 Kg/m2, enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)); non-enzymatic antioxidants (GSH, vit E and direct and total bilirubin); and lipid damage (malondialdehyde (MDA)), and biochemical markers (liver enzyme (ALT, AST & ALP), glucose, Albumin and lipid profile were determined in the serum samples.

Results: The activity of SOD, CAT, GPx, GSH and MDA and liver enzyme Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP)), lipid profile increase excepted HDL were significantly elevated in adult and kids of NAFLD compared to controls (p<.001). Whereas decrease level of Vitamin E and ALB.

Conclusions: Our findings show the anti- oxidative stress-related parameters are markedly altered in the liver of NAFLD patients exhibiting high alterations in liver function tests, also dyslipidemia appear in total NAFLD adult and obese kids' patient groups.

Keywords: Oxidative stress, antioxidant biomarkers, NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the health problems with great burden on the liver that may end with liver cirrhosis and hepatocellular carcinoma [1]. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function and is characterized by hepatic steatosis in individuals with little or no alcohol consumption. Fatty liver is closely related to other components of the metabolic syndrome and type 2 diabetes, including dyslipidemia. Excess liver fat had been considered benign but has recently been recognized as the metabolic syndrome hepatic component. NAFLD subjects also display peripheral insulin resistance [2, 3]. In recent years, the prevalence of NAFLD has increased and affects up to 30% of the global population. NAFLD is accompanied by increases in serum alanine aminotransferase, Aspartate Aminotransferase, as well as Alkaline Phosphatase. However, some patients may have also normal liver parameters [4]. Although obesity, particularly central (abdominal) obesity, is a well-recognized risk factor for it, NAFLD has been also reported in lean individuals (body mass index < 30 kg/ m2) [5]. Furthermore, the prevalence of NAFLD differs depending on the gender, ethnicity, and race as a proof of probable involvement of genetic and epigenetic factors in the pathogenesis of the disease. Insulin resistance (IR) is the major pathophysiological factor implicated in NAFLD, as well as metabolic syndrome (MS), a cluster of cardiovascular risk factors comprising visceral obesity, blood hypertension, glucose intolerance, and dyslipidemia [6]. In this way, NAFLD has been considered as the liver expression of MS, not only burdened with high cardiovascular risk but also responsible of a progressive metabolic, cardiovascular, and/or kidney disease, even without an overt MS [7]. IR is thought to play a pivotal role both to the initiation of the disease and the pathogenic switch of fatty liver to advanced forms of NAFLD, even if the mechanisms underlying this process are still partially unknown [8]. The main suites of enzymatic antioxidants are superoxide dismutase (SODs), catalase (CAT), and glutathione peroxidase and reductase (GSH.Px).These enzymes are working simultaneously as SOD converts superoxide anions into hydrogen peroxide and then another both enzymes convert hydrogen peroxide into water. Non-enzymatic antioxidants are also an important against the ROS and could involve different kinds of

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molecules like Vit.A, Vit E, Vit C, Bilirubin, and glutathione [9]. The SODs are group of metalloenzymes, which protecting from oxidative harm by stimulating the dismutation of the superoxide anion radical to hydrogen peroxide (H2O2) plus water [10], whereas Catalase is an antioxidant enzyme that acts as a catalyst for the conversion of hydrogen peroxide to oxygen and water [11]. Glutathione peroxidase and glutathione reductase are enzymes that act as antioxidants. The reduced form of glutathione is defensive in nature. The oxidized form is not protective. Reduced glutathione helps to neutralize hydrogen peroxide produced inside the cell. These enzymes are key players in preventing increased levels of oxidative stress. This repeated oxidization and reduction of glutathione makes it a free radical's scavenger [12]. Glutathione can prevent damage to important cellular components caused by ROS and their derivatives, such as free radicals, peroxides, lipid peroxides, or by organic pollutants and heavy metals [13].

Therefore, the aim of our research was to evaluate the enzymatic and non-enzymatic antioxidants, oxidative damage to the lipids, and biochemical markers (glucose, liver function tests and lipid profile contents) in the serum of NAFLD patients.

2. Materials and Methods

Patient characteristics

Sixty adult patients with NAFLD (29 men and 31 women) and 20 obese children (13 boy and 7 girl) were enrolled whom visited Al-Fayha Teaching Hospital, Al-Basra city, Iraq for ultrasonography (USG) was performed as a screening test for the NAFLD during the period of (11 December 2020 to 14 May 2021). The control group consisted of 60 adult generally healthy volunteers (38 men and 22 women) and 20 healthy children (10 boy and 10 girl). The registered subjects were aged between 5 to 75 years old who were referred by a physician or surgeon, BMI range from (15.9- 50.9 Kg/m2). All necessary diagnosis tests were taken upon oral approval (patient consent) in the questionnaire.

Those patients who were diagnosed graded by ultrasound as having a fatty liver disease based on WHO (2015) guidelines and aged range (5-75) years old. All subjects included in the study (both in the study and control group) were negative for (liver cancer, hepatitis (by a history of subjects and the virology test results were negative (alcoholism, any acute or chronic liver disease, and pregnant women).

Blood samples

Peripheral venous blood was taken into clot tube to obtain serum and. The blood was centrifuged at 4000 RPM for 10 min. The resulting serum were collected and stored at 20- C until were analysis.

Enzymatic and non-enzymatic antioxidants and Oxidative damage products MDA

The antioxidants enzyme (SOD, CAT and GPx) and antioxidants non- enzyme (GSH, Vit E) uses the SOD, CAT, GPx, GSH, Vit E and MDA human ELIZA Kit in serum as demonstrated the manufacturer's instructions in (Sun long /China).

The biochemical parameters:

The reagents kits for serum Alanine amino transferase (ALT), aspartate amino transferase (AST), measurements were purchased from Dialab/Austria for serum total cholesterol (T. CHOL), high-density lipoprotein. Cholesterol (HDL), Low-density lipoprotein. Cholesterol (LDL), triglycerides, glucose, Albumin (ALB), alkaline phosphtase (ALP), direct and total bilirubin from Biolabo/France

Statistical analyses

Statistical analysis was performed by using a statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2019 for Windows. Data were expressed as Mean, standard deviations (SD) and Median. Normally distributed continuous variables were compared using independent t-test (two-tailed), whereas Kruskal-Wallis test used for those variables that were not normally distributed, also used spermans correlation coefficients when calculated the Correlations. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

In table (3.1) showed significant increase of BMI in NAFLD groups compared to control group (P<0.001), this study was agreed with previous studies [4, 14], BMI which is considered to be strictly associated with the entity of liver steatosis and fibrosis in obese and liver was higher in respect to normal values in a large number of our cases, while it was within the normal range in 11%. Hence, the increase of body weight is frequently associated with the appearance of NAFLD, even if it is not a necessary factor [15].

The liver function test (ALT, AST, ALP & ALB) shown significant increase except ALB significant decrease p < 0.001. NAFLD is the most common cause of abnormal liver function and is characterized by hepatic steatosis in individuals with little or no alcohol consumption [3]. In other words, these biochemical markers are often more pronounced in the presence of a metabolic disorder caused by liver disease than just by obesity, since these are factors closely related to the pathophysiology of NAFLD [16]. This study was match with previous studies [1, 16]. Moreover, in these studies, elevation of liver enzymes, in obese childern (Table 3.4), was positively correlated with NAFLD in which the changes in these enzymes could reflect the changes in fatty liver content found in obese children with NAFLD. Given the increased prevalence of obesity and other metabolic disorders among children, it has become very urgent to get children screened for NAFLD. North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) has recommended that the crucial step for NAFLD screening among children is to check over for elevation of liver enzymes (ALT, AST, and ALP) above twice the upper limit of normal values [17]. However, Our finding showed significant increase Glucose in NAFLD groups compared to control group 1 (p=0.003), consistent with previous studies [14, 18, 19] mention Nonalcoholic fatty liver disease was closely associated with insulin resistance,

independent of body mass index and fat distribution. This suggests that in nonalcoholic fatty liver disease, insulin resistance may be a primary phenomenon. In addition to obesity-associated insulin resistance, whereas in normal subjects' sensitivity to insulin may also depend primarily on obesity. While, table (3.4) the average glucose concentration, there is no significant difference in the group of Non-alcoholic liver diseases in children when compared with the control children, despite the strong relationship between body mass index, glucose and insulin resistance in Non-alcoholic liver diseases, the reason of this difference may be young age of patient or the result of that disease is in the first stage and not in an advanced stage, as in the first group, consistent with [20-22].

This dyslipidemia is characterized by significant increased plasma cholesterol and LDL (p<0.001) (Table 3.2), significant decrease of HDL whereas triglyceride and VLDL levels increase. Earlier observations in NAFLD patients showed that dyslipidemia was present in (20-92)% of the patients [16]. This study was match with another studies [1, 3, 14]. Dyslipidemia is often observed years before type 2 diabetes is diagnosed, indicating that lipid disturbances might be a primary event of type 2 diabetes. Emerging results indicate that the underlying defect is hepatic overproduction of large very low density lipoprotein (VLDL) particles. Thus, it is important to elucidate the mechanisms behind the overproduction of large VLDL particles in diabetic dyslipidemia, insulin resistance, and NAFLD [3]. Whereas, Table (3.5) shown statistically significant increase difference (p<0.001) between Obese kids group patient and the control group for all total cholesterol, triglyceride, VLDL and LDL except HDL significantly decrease. In the present study, the NAFLD children showed higher cardiovascular risk and more metabolic syndromes than non-NAFLD children with obesity. The lowering HDL among obese kids in the current study was similar to the findings found in obese children with NAFLD in Germany [23] and China. The greater levels of TG seen among obese kids with NAFLD were supported by recent findings on Malaysian obese children with NAFLD [22, 24]. It has been suggested that children with NAFLD are vulnerable to develop serious changes in their lipid profile compared to those children without NAFLD. The results here strongly indicated that the obese children with NAFLD could develop serious metabolic and cardiovascular diseases later on.

Table (3.1): Statistical analysis for Age, sex, BMI, Glucose and liver function in total Non-alcoholic fatty liver disease group (NAFLD) compared to the total control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Total Control (N=60)			Total NAFLD (N=60)			P value
	mean	SD	Median	mean	SD	Median	
Age (year)	35.5	22.2	39.5	33.6	20.3	40.5	0.635
Sex male/ female	38/22			29/31			0.141
BMI (Kg/m^2)	23.0	2.65	23.5	34.0	6.47	33.4	<0.001
Glucose mg/dl	93.6	16.4	90.0	155	79.9	118	<0.001
ALT U/L	22.6	6.82	22.0	54.8	21.9	60.0	<0.001
ALP U/L	111	18.1	110	256	152	196	<0.001
AST U/L	28.8	24.0	27.0	53.1	17.6	58.0	<0.001
ALB g/dl	4.38	0.601	4.40	3.39	0.650	3.40	<0.001

Table (3.2): Statistical analysis for lipid profile in total Non-alcoholic fatty liver disease group (NAFLD) compared to the total control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Total Control (N=60)			Total NAFLD (N=60)			P value
	mean	SD	Median	mean	SD	Median	
T. CHOL mg/dl	143	17.8	140	214	36.2	210	<0.001
TG mg/dl	82.1	49.6	70.0	149	113	128	<0.001
VLDL mg/dl	16.4	9.91	14.0	29.8	22.6	25.5	<0.001
HDL mg/dl	55.3	11.2	52.5	26.9	10.5	25.0	<0.001
LDL mg/dl	70.9	20.8	70.0	157	39.9	152	<0.001

Table (3.3): Statistical analysis for MDA, antioxidant enzymes and antioxidant non-enzymes concentration in total Non-alcoholic fatty liver disease group (NAFLD) compared to the total control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Total Control (N=60)			Total NAFLD (N=60)			P value
	mean	SD	Median	mean	SD	Median	
CAT KU/L	0.399	0.712	0.225	41.0	13.6	41.0	<0.001
SOD pg/ml	1.78	0.455	1.84	9.93	3.11	9.21	<0.001
Gpx u/l	11.8	5.80	10.2	16.3	7.09	16.2	<0.001
GSH µg/ml	1.90	0.574	1.80	2.04	0.953	1.76	0.752
VIT.E pg/ml	10.5	5.01	11.6	1.94	0.704	1.76	<0.001
BIL.T mg/dl	0.629	0.499	0.430	0.516	0.748	0.430	.351
BIL.D mg/dl	0.296	0.238	0.210	0.209	0.152	0.150	.070
MDA pg/ml	32.9	7.67	34.0	39.9	7.00	39.5	<0.001

Parameters related to oxidative stress in the liver of patients with NAFLD are shown in table (3-3). catalase, superoxide dismutase, Glutathione peroxidase, Malondialdehyde assessed by the content of hepatic protein carbonyls, were significantly increased (P<0.05) in the liver of patients with NAFLD when compared to control group this study was agree with Świderska et al. [4], Monserrat-Mesquida et al. [18] whereas vitamin E significant decrease (P<0.05), Glutathione, Bilirubin total and Bilirubin direct were non-significant (P>0.05). In contrast, statistically significant increase difference (p<0.001) between Obese kids group patient and the control group for CAT, Gpx, SOD BIL.T, and MDA, whereas BIL.D and VIT.E significantly decrease. Also, shown none. Significant difference (p>0.05) for GSH, as show in Table (3.6).

Superoxide dismutase and catalase were increase levels in NAFLD when compared to control groups this study was match with another previous studies [18]. Alternations of the balance between pro-oxidant and antioxidant factors lead to oxidative stress. As it was shown recently, in NAFLD pathogenesis higher production of ROS causes cell damage, induces cytokine production (e.g. IL-1, -6, -18) and oxidative damage markers (MDA) also increase [18], and activates inflammatory cells, which further stimulates the formation of ROS [25, 26]. Therefore, the enhancement of antioxidants observed in our study (↑SOD, ↑CAT, ↑GR) may indicate an adaptive reaction to

the increased production of ROS in NAFLD group. Interestingly, we observed not only changes in the activity of enzymatic antioxidants but also increased concentrations of reduced glutathione (GSH). It is well known that OS creates an oxidized form of glutathione (GSSG) that may be regenerated to GSH by glutathione reductase (GR). Considering that GR uses the NADPH reduction potential, as well as that NADPH is needed to a production of the active form of catalase (CAT), decreased CAT activity observed in our study may result from the reduced synthesis of this enzyme [27].

Indeed, in our study, we proved significantly higher oxidation to lipids (↑MDA) in early NAFLD and NASH group. Worth mentioning is increase in malonylodialdehyde (MDA) levels. This compound is characterized by high mutagenic and carcinogenic potentiall [19, 26]. This condition results in increased expression of proinflammatory cytokines and may predispose to ischemia/ fibrosis of the liver parenchyma. MDA also creates adducts with proteins and lipids, which further causes oxidative damage and disrupts many metabolic processes (e.g., protein synthesis or oxidative phosphorylation in mitochondria). Interestingly observed higher concentrations of MDA in the liver and serum of advanced NAFLD patients compared to early NAFLD [4, 19, 26], also consist with another previous studies [28].

The main reason that leads to a decrease in the level of vitamin E concentration in non-alcoholic liver disease compared to control is what we saw in the increase in antioxidants. Oxidative stress plays a central role in the transition from simple steatosis to nonalcoholic steatohepatitis (NASH) [29]. The oxidative stress has been reported to be involved in the pathogenesis of various diseases such as hyperlipidemia, diabetes, and hypertension, which are all also associated with the obesity. Non-alcoholic fatty liver disease is associated with the obesity in the children, however, very little is known about the involvement of oxidative damage in the pediatric obesity-related NAFLD. As impaired free oxygen radical scavenging has been pro. posed as a mechanism in the progression of hepatosteatosis, all antioxidant enzymes increase levels Catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (Gpx) consistent with previous studies [20, 30].

Although there have been very few studies measuring the markers of oxidative stress among obese children with NAFLD, the significant reduction observed in the makers of both enzymatic and non –enzymatic antioxidants (GPx and GSH) were consistent with previous finding [30], obese children with NAFLD had significantly more oxidative lipid damage (MDA) than in the control group. Higher oxidative damage was also found among obese children with NAFLD in the previous studies. Torun [30] found that total oxidant measured in the serum of obese children with NAFLD was significantly greater compared to the control group.

Table (3.4): Statistical analysis for Age, sex, BMI, Glucose and liver function in Obese kids compared to the control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Control group (N=20)			Obese kids (N=20)			P value
	mean	SD	Median	mean	SD	Median	
Age (year)	9.45	3.30	9.00	10.2	3.44	11.0	0.547
Sex male/ female	10/10			13/7			0.337
BMI Kg/m^2	20.5	2.75	20.9	34.3	7.73	32.5	<0.001
Glucose mg/dl	94.9	16.2	90.0	107	21.0	104	0.055
ALT U/L	18.3	6.52	18.5	28.4	12.7	29.0	0.003
ALP U/L	108	13.8	105	383	200	366	<0.001
AST U/L	30.1	41.0	18.0	36.4	21.1	32.0	0.003
ALB g/dl	4.65	0.639	4.70	3.73	0.397	3.85	<0.001

Table (3.5): Statistical analysis for lipid profile in Obese kids compared to the control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Control group (N=20)			Obese kids (N=20)			P value
	Mean	SD	Median	mean	SD	Median	
T. CHOL mg/dl	127	8.82	126	192	16.7	189	<0.001
TG mg/dl	61.0	24.6	62.5	145	53.5	140	<0.001
VLDL mg/dl	12.2	4.92	12.5	29.0	10.7	28.0	<0.001
HDL mg/dl	50.3	6.64	50.0	36.5	8.23	37.5	<0.001
LDL mg/dl	64.1	6.97	65.5	127	18.2	125	<0.001

Table (3.6): Statistical analysis for MDA, antioxidant enzymes and antioxidant non-enzymes concentration in Obese kids compared to the control group, using independent t-test (two tailed) and Mann-Whitney U test.

Parameter	Control group (N=20)			Obese kids (N=20)			P value
	mean	SD	Median	mean	SD	Median	
CAT KU/L	0.625	1.21	0.250	36.2	13.1	35.5	<0.001
SOD pg/ml	1.55	0.323	1.55	9.99	3.83	9.13	<0.001
Gpx u/l	8.18	2.25	8.15	16.9	7.55	17.0	<0.001
GSH µg/ml	2.01	0.899	2.00	2.05	0.912	1.80	0.888
VIT.E pg/ml	5.08	3.66	3.85	1.62	0.286	1.60	<0.001
BIL.T mg/dl	0.308	0.288	0.210	0.742	1.25	0.470	<0.001
BIL.D mg/dl	0.435	0.278	0.310	0.161	0.0924	0.165	<0.001
MDA pg/ml	25.0	5.69	25.5	38.2	5.04	39.0	<0.001

4. Conclusions

To conclude, our study demonstrates an association of alter the levels of anti-oxidant enzymatic and anti-oxidant non-enzymatic in total NAFLD and obese kid patient, NAFLD patients exhibiting high alterations in liver function tests, also dyslipidemia appear in total NAFLD and obese kids' patient groups, also total NAFLD and obese kid patient showed highly decrease vitamin E compared to the control groups.

Conflict of interest

The authors have no conflict of interest.

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