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The Relation of Biomarkers in Serum Non-Alcohol Fatty Liver Disease with Diabetes Mellitus Type 2 and NAFLD Obese among Adults in Basrah Governorate

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the term for a range of conditions caused by a build-up of fat in the liver. The goal of the study was to determine the link between lipid damage (MDA), enzymatic and non-enzymatic antioxidants, and various biochemical indicators in patients with NAFLD who had diabetes and obese adults. This study included comparison 100 patients and healthy control group, aged 18 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); lipid damage (malondialdehyde (MDA)), and biochemical markers (liver enzyme (ALT, AST & ALP), glucose, Albumin and lipid profile in the serum samples were measured. The NAFLD with DM and obese adult showed increasing of glucose, BMI, ALT, AST, ALP, T. CHOL, LDL, TG, VLDL, SOD and CAT levels excepted HDL. It showed a decreasing. Whereas decrease of Vitamin E and ALB levels compared to control group. Our findings show that the serum enzymatic anti-oxidant, non-enzymatic anti-oxidant, dyslipidemia and liver disfunction and vitamin E decrease are tightly and independently related to NAFLD with diabetes and obese adult status.

1. Introduction

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 (Adiels, Taskinen, & Borén 2008; Goldberg & Ginsberg 2006). The patients with DM and NAFLD have more aggressive disease with respect to cirrhosis and mortality than NAFLD patients without DM. The increased risk remained significant even after adjusting for potentially important confounders that can affect survival. The 18.2% liver-related mortality rate reported here is much greater than that of patients without DM with NAFLD and those reported for the general population (Falck-Ytter et al., 2001; Younossi et al., 2004). The alterations in glucose metabolism could be significant risk factor for NAFLD progression combined with obesity and diabetes mellitus (Leghi, Domenici, & Vannucchi 2015; Marchesini et al. 2003) reported alterations in the glucose metabolism, including T2DM, glucose intolerance and hyperglycemia, which were associated to NAFLD. Previous research strongly indicated that the NAFLD is highly

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associated with insulin resistance (Klisic et al. 2018; Macut et al. 2016). Therefore, the incidence of this disease was highly observed in the people with type 2 diabetes mellitus (DM2). More than 75% of people with (DM2) are affected by NAFLD (Giorda et al. 2017; Hazlehurst et al. 2016). In addition, there were strong evidence of association between NAFLD and the complications of diabetes mellitus type 2 like diabetic retinopathy and chronic kidney disease (Targher et al. 2010). The incidence of obesity is increasing dramatically and, at present, obesity is considered as a public health problem. The most important pathological consequences are nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus and cardiovascular disease. NAFLD includes a spectrum of hepatic abnormalities ranging from hepatic steatosis to more severe pathologies like Non-Alcoholic Steatohepatitis (NASH) and cirrhosis. NAFLD prevalence increases with adiposity up to 85% in morbid obesity, defined as having a body mass index (BMI) of greater than 40 kg/m2 (Carazo et al. 2011; Fabbrini, Sullivan, & Klein 2010).

Nonalcoholic fatty liver disease (NAFLD) is a constellation of progressive liver disorders that are closely related to obesity. NAFLD prevalence has increased with the change in eating habits, thus identifying an effective treatment for NAFLD is a significant public health objective. Lifestyle-related factors such as poor diet, obesity, excessive alcohol intake, diabetes, and hyperlipidemia have all been proposed to contribute to NAFLD. In addition to the development of a fatty liver, NAFLD patients may also exhibit inflammation, necrosis and fibrosis of the liver, which are known as nonalcoholic steatohepatitis (NASH) (Pezeshki et al. 2015).

This study aims to assess the levels of enzymatic and non-enzymatic antioxidants, oxidative damage to lipids, and biochemical indicators (glucose, liver function tests, and lipid profile contents) in the serum of NAFLD patients with diabetes mellitus type 2 and in the NAFLD obese adult patients compared with control group**.**

2. Materials and Methods

2.1. Patient Characteristics

This study included 100 patients and healthy control group (male= 57 and female= 43) NAFLD with 20 Diabetes groups (male=14 and female= 6) and 30 NAFLD obese adult group (male= 15 and female= 15) 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 50 adult generally healthy volunteers (28 men and 22 women).

The registered subject's patients 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 (18-75) years old. All subjects included in the study (both patients 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).

2.2. Blood Samples

Peripheral 5 ml venous blood samples were taken into clot tube to obtain serum. The blood was centrifuged at 4000 RPM for 10 min. The resulting serum was collected and stored at 20^{-} C until were analysis.

2.3. 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).

2.4. 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

2.5. 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

Tables 1 and 4 show statistically significant increase difference ($p<0.05$) between NAFLD with Diabetes and obese groups patients and the control group for BMI, Glucose, ALT, ALP and AST, while ALB was significant decrease. These results have suggested that the alterations in glucose metabolism could be significant risk factor for NAFLD progression combined with obesity and diabetes mellitus (Leghi et al. 2015), more than 75% of people with (DM2) are affected by NAFLD (Giorda et al. 2017; Hazlehurst et al. 2016). In addition, diabetic patients with NAFLD have more risk for the development of cardiovascular disease (Klisic et al. 2018) and also more mortality rate due to the chronic liver disease (Zoppini et al. 2014) comparing to those diabetic people who have no NAFLD, also BMI considered risk factor for NAFLD progression combined with obesity and diabetes mellitus. Additionally, Obesity in turn increases the prevalence of diabetes, dyslipidemia, hypertension, and ultimately of the metabolic syndrome (Marchesini et al. 2003) this study match with (Klisic et al. 2018; Macut et al. 2016).

The results of albumin among studied groups indicated that the levels of albumin were significantly lower among patients NAFLD with DM, due to influence of the liver and thus the efficiency of the liver in making albumin decreases, this study was consistent with previous studies (Hazlehurst et al. 2016; Klisic et al. 2018). These liver 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. It is

well documented that these liver enzymes are markers of liver injury and may be useful surrogate measures of NAFLD (Sanyal et al. 2015). In fact, NAFLD has been reported to be most common causes of chronically elevated liver enzymes and is often the tipping point for further diagnostic evaluation (Vernon, Baranova, and Younossi 2011). Previous research indicated that ALT and AST could be good markers being measured to assess many of cardiovascular diseases complications among patients with NAFLD (Sanyal et al. 2015). The World Gastroenterology Organization (WGO) has updated its guidelines by mentioning that in morbid obesity, diabetes and ALT and AST could be associated with high risk of progression to NAFLD (Klisic et al. 2018). Therefore, it was recently suggested that people who have greater levels of liver function tests would be qualified for ultrasonography for detection of NAFLD avoiding the potential cost of treatment that would be higher in the developing country (Monserrat-Mesquida et al. 2020).

Tables 2 and 5 show statistically significant increase difference ($p<0.05$) between Diabetes type 2 and obese groups patients compare the control group for all parameter total cholesterol, triglyceride, VLDL and LDL, except HDL showed significantly decrease. Dyslipidemia (hypertriglyceridemia, hypercholesterolemia, or both) has been reported in (20–92) % of patients with NAFLD. Most of these patients had other components of the metabolic syndrome, highlighting the importance of diagnosing the metabolic syndrome and its effect on NASH and NAFLD (Grajower 2020). This study was consist with previous study (Klisic et al. 2018).

Tables 3 and 6 show statistically significant increase difference ($p<0.05$) between Diabetes type 2 and obese groups patients and the control group for CAT, Gpx and SOD, whereas vitamin E show significant decrease, also, shown non- significant difference *($p>0.05$)* for, GSH, BIL.T, BIL.D and MDA.

There are very few studies that have examined the rate of antioxidants in the Non-alcoholic liver disease group with diabetes mellitus. In these studies, patients with diabetic and NAFLD have had significant changes in their antioxidants levels and oxidative damage targeting different biological molecules (Klisic et al. 2018; Narasimhan et al. 2010). The lack of significant changes in non-enzymatic antioxidant (both total and direct bilirubin) and the significant reduction of non-enzymatic antioxidant (Vit.E) measured in the serum of diabetic patients with NAFLD presented here was consistent with the findings among diabetic with NAFLD (Klisic et al. 2018; Narasimhan et al. 2010; Pacana & Sanyal 2012).

Glutathione peroxidase 1 (GPx1) is a seleno-protein that reduces hydroperoxides by means of glutathione. Its role is mainly that of an antioxidant. GPx1 knockout mice tolerate moderate oxidative stress, but are highly susceptible to severe oxidative damage. Moreover, GPx1 knockout mice increase insulin sensitivity. In contrast, GPx1 overexpressing mice are more resistant to acute oxidative stress and develop insulin resistance and obesity. Although an excess in oxidative damage is associated with the pathology of many human diseases, a growing body of evidence shows that low levels of reactive oxygen and nitrogen species are required for normal cellular functioning and intracellular signalling (Carazo et al. 2011). Another study reported that muscle-specific disruption of ADIPOR1 decreased muscle oxidative stress-detoxifying enzymes and mitochondrial content (Iwabu et al. 2010). In addition, ADIPOR2 overexpression in a model of diabetic and obese mice increased the expression of hepatic antioxidant enzymes (Yamauchi et al. 2007).

Although the significant changes observed in both enzymatic and non-enzymatic antioxidants among diabetic patients with NAFLD, this was not reflected in the levels of oxidative damage measured by MDA in which MDA levels were not significantly changed among patients compared to the control group. This result was inconsistent with the previous studies (Klisic et al. 2018). This difference could be due to the differences in the sample size between studies as the sample size in the current study was smaller than in the other studies which in turn it could have hidden the significant changes of the lipid damage. In addition, these equivocal results among studies have shed the light of the importance of doing more research measuring different markers of antioxidants and oxidative damage in patients with diabetic and NAFLD.

Although the exact mechanism driving the link between diabetic and NAFLD is not well known, oxidative stress could be an option controlling this link. Diabetic patients have more visceral adipose tissue which could be the main source of reactive oxygen species (ROS), higher pro-inflammatory adipokines and cytokines (Klisic et al., 2014). This increment with ROS could exceed the levels of both antioxidant enzymatic and non-enzymatic defense which altogether make the milieu of increased inflammation and oxidative stress (Klisic et al. 2021; Savini et al. 2013). In addition, ROS could influence on insulin signaling pathways, thus leading to consequent insulin resistant state, increased free fatty acids hepatic influx, increased lipogenesis, as well as triglyceride storage, inducing hepatocytes dysfunction or death (Klisic et al., 2021).

The data of this study indicate that patients with NAFLD and DM experience greater rates of cirrhosis and mortality. This has important clinical and prognostic implications for patients with NAFLD. Patients with clinical evidence of NAFLD and DM may have more progressive liver disease. Such patients should be the target of future investigations into the pathogenesis of NAFLD and NASH and clinical trials designed for the treatment of NASH (Falck-Ytter et al., 2001; Younossi et al., 2002).

Table 1
Statistical analysis for Age, sex, BMI, Glucose and liver function in NAFLD with Diabetes compared to the control group, using independent t-test (two tailed) and Mann-Whitney *U* test

Parameter	Control group (N=20)			NAFLD with Diabetes (N=20)			P value
	mean	SD	Median	mean	SD	Median	
Age (year)	46.1	17.9	51.0	46.2	14.3	47.5	0.977
Sex male/ female		7/13			14/6		0.027
BMI Kg/m2	23.9	1.28	24.1	33.8	5.91	33.2	<0.001
Glucose mg/dl	92.2	20.0	90.0	252	66.7	245	<0.001
ALT U/L	24.5	6.76	24.0	66.7	11.6	63.0	<0.001
ALP U/L	119	21.1	119	204	56.4	202	<0.001
AST U/L	29.0	6.57	30.0	61.0	6.37	60.5	<0.001
ALB g/dl	4.24	0.584	4.35	3.17	0.663	3.10	<0.001

Table 2
Statistical analysis for lipid profile in NAFLD with Diabetes compared to the control group 2, using independent t-test (two tailed) and Mann-Whitney *U* test.

Parameter	Control group (N=20)			NAFLD with Diabetes (N=20)			P value
	Mean	SD	Median	mean	SD	Median	
T. CHOL mg/dl	143	13.7	143	215	43.8	208	<0.001
TG mg/dl	94.0	59.6	80.0	185	175	140	0.017
VLDL mg/dl	18.8	11.9	16.0	37.0	34.9	28.0	0.017
HDL mg/dl	63.8	11.9	64.5	19.7	5.09	19.0	<0.001
LDL mg/dl	60.8	24.7	58.5	158	44.4	156	<0.001

Table 3
Statistical analysis for MDA, antioxidant enzymes and antioxidant non-enzymes concentration in NAFLD with Diabetes compared to the control group 2, using independent t-test (two tailed) and Mann-Whitney *U* test.

Parameter	Control group (N=20)			NAFLD with Diabetes (N=20)			P value
	mean	SD	Median	mean	SD	Median	
CAT KU/L	0.286	0.130	0.235	46.7	11.4	47.3	<0.001
SOD pg/ml	1.97	0.429	2.05	9.82	0.792	9.85	<0.001
Gpx u/l	11.1	7.85	7.10	14.8	7.42	12.0	0.034
GSH µg/ml	1.76	0.303	1.75	2.15	0.939	1.91	0.228
VIT.E pg/ml	14.6	2.59	15.0	1.99	0.556	1.80	<0.001
BIL.T mg/dl	0.410	0.238	0.325	0.450	0.215	0.420	0.580
BIL.D mg/dl	0.173	0.161	0.125	0.151	0.088	0.135	0.794
MDA pg/ml	38.6	5.68	39.5	40.6	6.75	41.0	0.578

Table 4
Statistical analysis for Age, sex, BMI, Glucose and liver function in NAFLD Obese Adult compared to the control group, using independent t-test (two tailed) and Mann-Whitney *U* test

Parameter	Control group (N=30)			NAFLD Obese Adult (N=30)			P value
	mean	SD	Median	mean	SD	Median	
Age (year)	49.7	51.0	14.8	45.5	13.5	46.0	0.255
Sex male/ female		21/9			15/15		0.1382
BMI Kg/m2	24.4	1.62	24.3	36.3	4.38	36.3	<0.001
Glucose mg/dl	91.5	18.1	90.0	180	89.4	141	<0.001
ALT U/L	24.6	5.72	24.5	68.6	10.3	66.0	<0.001
ALP U/L	114	20.8	116	196	58.9	188	<0.001
AST U/L	28.3	6.74	29.5	62.1	6.46	62.0	<0.001
ALB g/dl	4.20	0.566	4.35	3.19	0.699	3.10	<0.001

Table 5
Statistical analysis for lipid profile in NAFLD Obese Adult compared to the control group, using independent t-test (two tailed) and Mann-Whitney *U* test.

Parameter	Control group (N=30)			NAFLD Obese Adult (N=30)			P value
	mean	SD	Median	mean	SD	Median	
T. CHOL mg/dl	152	16.5	155	221	40.1	230	<0.001
TG mg/dl	95.5	57.3	90.0	157	149	118	0.041
VLDL mg/dl	19.1	11.5	18.0	31.3	29.9	23.5	0.041
HDL mg/dl	57.9	11.9	58.0	21.8	8.42	20.5	<0.001
LDL mg/dl	75.0	22.3	77.5	168	40.9	172	<0.001

Table 6
Statistical analysis for MDA, antioxidant enzymes and antioxidant non-enzymes concentration in NAFLD Obese Adult compared to the control group, using independent t-test (two tailed) and Mann-Whitney *U* test.

Parameter	Control group (N=30)			NAFLD Obese Adult (N=30)			P value
	mean	SD	Median	mean	SD	Median	
CAT KU/L	0.269	0.134	0.220	41.7	12.3	41.0	<0.001
SOD pg/ml	1.86	0.462	1.94	10.1	3.12	9.65	<0.001
Gpx u/l	14.1	6.33	15.0	16.1	6.92	16.2	0.243
GSH µg/ml	1.27	0.450	1.00	1.43	1.10	1.00	0.447
VIT.E pg/ml	13.2	3.44	14.0	1.53	0.900	1.00	<0.001
BIL.T mg/dl	0.783	0.513	0.720	0.374	0.229	0.335	<0.001
BIL.D mg/dl	0.213	0.158	0.180	0.216	0.176	0.150	0.945
MDA pg/ml	36.7	5.45	37.5	40.0	6.46	40.0	0.037

4. Conclusions

This work demonstrates that NAFLD with diabetes are reciprocal risk factors. When they occur together, an increasing body of data was observed. The diabetes is more difficult to manage and while the NAFLD is more likely to progress. The parameters is also related to oxidative stress in the liver of patients with the NAFLD (catalase, superoxide dismutase, Glutathione peroxidase and Malondialdehyde) assessed by the content of hepatic protein carbonyls. They were significantly increased with the NAFLD when compared to control group, whereas vitamin E significant decreases. In patients of the NAFLD obese adult increase levels of glucose, BMI, ALT, AST, ALP, T. CHOL, LDL, TG, VLDL, SOD, CAT and MDA, also decrease level of HDL, ALB, BIL.T and Vitamin E. Whereas no significant difference for Gpx, BIL.D and GSH compared to healthy obese adult group.

Competing Interests

The authors have declared that no competing interests exist.

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