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A NON INVASIVE METHOD FOR DIAGNOSIS AND ASSESSMENT OF PORTAL HYPERTENSION IN EGYPTIAN CIRRHOTIC PATIENTS USING PLASMA MALONDIALDEHYDE LEVEL

By

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Abstract

Liver cirrhosis is a common disease affecting Egyptian patients. Complications of liver cirrhosis usually start once portal hypertension increases. To the authors' knowledge, there are no available non-invasive methods for assessment of the severity of portal hypertension. This study evaluated the value of plasma Malondialdehyde (MDA) - a lipid peroxide marker for oxidative stress, as a diagnostic biomarker, to assess severity of Portal Hypertension in Egyptian cirrhotic patients. It is a case-control study conducted on a total of 150 Egyptian patients divided into two groups. GI: 100 patients diagnosed as liver cirrhosis with esophageal varices proved by upper GIT endoscopy. GII: 50 normal controls. Serum MDA was measured by ELISA technique.

The results showed that MDA assay between the two groups revealed significant statistical difference between the two studied groups. Correlation between MDA and the clinical parameters in the cirrhotic group showed a significant positive correlation with ascites, Child Pugh score, varices grade, while there was no correlation with sex.

Keywords: Malondialdehyde; portal hypertension; liver cirrhosis.

Introduction

Portal hypertension is one of the most common complications of liver cirrhosis and one of the major causes of death in cirrhotic patients (Koga *et al,* 2019). Portal hypertension occurs as a result of the increase in the portal vein blood flow resistance caused by alteration in liver structure as well as alteration in blood structure (Parola and Robino, 2001).

Hepatic venous pressure gradient (HVPG) measurement is considered the gold standard method for the assessment of presence and severity of Portal Hypertension (PHT). However, it is an invasive tool and requires an experienced radiologist in its measurement (Groszmann *et al,* 2005).

Oxidative stress (OS) refers to a condition under which an organism or cell reactive oxygen species (ROS) are excessively produced and the antioxidant defense function is weakened, which causes big imbalance and damage to the organism cell (Catapano *et al*, 2000). Oxidative stress response accelerated liver fibrosis and also, initiates hepatic epithelioid malfunction, which is adjusted by the bioavailability of nitric oxide (NO) in the intrahepatic microcirculation (Rodriguez *et al*, 2007). End products of lipid peroxidetion have been used as biomarkers of the oxidative stress. These include malondialde hyde (MDA), isoprostanes and 2-propenal (acrolein). These compounds can be quantified in serum and urine and have the advantage of being relatively stable. Therefore, they can be used as an indirect measure of the oxidative stress (Raj *et al,* 2011)

Bajpai *et al.* (2017) described that the malonaldehyde (MDA) and the superoxide dismutase (SOD) were the most recognized predicative markers for the OS. MDA is the most representative indicator of OS in the body, while SOD is representative of the body's antioxidant system, and its function is to clear ROS. Also, Aziz *et al.* (2015) found that MDA levels significantly increased in

human schistosomiasis and correlated significantly with hepatic fibrosis. This may confirm the role of LPO byproducts in schistosomiasis pathogenicity, and proposing malondialdehyde as a biomarker for schistosomiasis morbidity.

This study aimed to evaluate the value of plasma Malondialdehyde (MDA); a lipid peroxide marker for oxidative stress, as a diagnostic biomarker to assess severity of portal hypertension in the Egyptian cirrhotic patients.

Materials and Methods

The study was approved by the Research Ethics Committee of Ain Shams University reference number: 000017585. Also, informed written consent was obtained from each participant before enrolment in the study.

This is a case-control study conducted on a total of 150 Egyptian patients selected from Internal Medicine and Hepatology outpatient clinics and inpatient wards at Ain Shams University's Hospitals and National Hepatology and Tropical Medicine Research Institute in the period from May 2017 to June 2018. All procedures followed were in accordance with the ethical standards of the responsible Committee on Human Experimenttation (Institutional and National) and with the Helsinki Declaration of 1975, as revised in 2000.

This was a case control study; conducted on 150 patients classified into 2 groups, GI: 100 patients diagnosed as the liver cirrhosis with esophageal varices proved by upper GIT endoscopy and GII: 50 normal controls. MDA serum was measured by ELISA.

The excluded criteria of patients***:*** Presence of bleeding esophageal varices, use of the vasoactive and antioxidant agents within a week, portal vein thrombosis, cardiac, renal or respiratory failure, hepatocellular carcinoma, abuse of alcohol and presence of active infection.

All patients were subjected to the full history taking, clinical examination, laboratory investigations including: fasting blood glucose, liver function tests (AST, ALT, prothrombin time, INR, serum albumin, total & direct bilirubin), renal function tests (creatinine, urea, Na, & K), complete blood count (CBC) and measurement of serum MDA using ELISA technique.

Child-Pugh score which assessed bilirubin, albumin, INR, presence and severity of ascites and encephalopathy, was used to classify patients in class A, B or C. (Cholongitas *et al*, 2005)

Radiological examination: Abdominal ultrasound with measurement of portal vein (PV) caliber and PV duplex. Equipment used: Hitachi, EUB-5500, 2-5 MHz convex probe, (China, Mainland); the patients were examined in supine position with emphasis on liver by an experienced sonographist, who was blind to all biochemical characteristics of the participants.

Endoscopy: All patients underwent an upper gastrointestinal endoscopy after premedication with intravenous midazolam (2.5-7.5mg), by video-endoscope after an overnight fast. During the upper gastrointestinal endoscopy, esophageal varices were graded as follow: 1- Small (Grade 1) minimally elevated veins above the esophageal mucosal surface, 2- Medium (Grade 2) tortuous veins occupying less than one-third of esophageal lumen and 3- Large (Grade 3): occupying more than one-third of esophageal lumen (De Franchis2005)

Serum MDA: Serum MDA was measured by Enzyme Linked Immunosorbent assay (ELISA) using Human MDA ELISA KIT by Glory Science Co., Ltd 2400 Veterans Blvd. Suite 16 - 101, Del Rio, TX 78840, USA.

Sample collection and preparation: Four mL of peripheral venous blood were collected under complete aseptic conditions into plain vacutainer tube and samples were clotted for 30 minutes before centrifu\gation for 15 minutes at 1,000xg. The freshly prepared serum was immediately assayed or stored in aliquots at -20°C until used for assaying serum MDA (=Myo Bio Source Human MDA ELISA Kit Cat. No. MBS728071).

Assay procedure: 1. Wells for diluted cali-

brators, blank and samples were determined. 50µL of calibrators, blank and samples were added into the appropriate wells, respectively followed by 50µL of detection reagent A. Plate was shaken gently by microplate shaker, covered with a sealer and incubated for 1hr at 37°C. 2. Solution in plate was aspirated and washed with 350µL of wash solution was added to each well using an auto washer. 3. A total of 100µL of detected reagent B working solution were added to each well, incubated for 30 minutes at 37°C after covered with plate sealer. 4. Aspiration wash process was repeated five times as in step 2. 5. A total of 90µL of substrate solution was added to each well and covered with a new plate sealer. After the incubation for 15-25 minutes at 37°C and protecting the plate from light, the liquid turned blue by addition of substrate solution. 6. A total of 50µL of stop solution was added to each well. Liquid turned yellow by adding stop solution. 7. Micro plate reader was run and measured at 450nm immediately. A standard curve was drawn on graph paper by plotting absorbance obtained from each standard on vertical (Y) axis against its concentration in ng/mL on the horizontal axis. Absorbance value of each sample determined the corresponding MDA concentration from the standard curve. Original concentration was calculated by multiplying the dilution factor. Assay range was 7ng/mL- 40ng/mL.

Statistical analysis: Data were coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 19.0.1 for windows; SPSS Inc, Chicago, IL). Analysis was conducted, using mean, standard deviation (±SD) for quantitative data. These tests were used: Two tailed Student's t test when comparing between two means and paired sample t-test of significance was used when comparing between related samples. P value <0.05 was considered significant. Sensitivity, specificity, PPV, NPV and accuracy were calculated. Analysis of variance (ANOVA) compared among different times in same group in quantitative data.

Results

Ages ranged between 40 & 67 years (53.4±5.813), and the age of controls ranged between 43 & 67 (53.25±6.935), without significant difference between them with P=0.927 (Tab. 1).

Table 1: Comparison between groups as regards ages

Age	Cases			Controls			T	P-value
Range	40	-	67	43	-	67	0.092	0.927
Mean ±SD	53.400	±	5.813	53.250	±	6.935		

Males were 98 (65.3%) & females were 52 (34.7%) without significant difference (P=0.098).

Table 2: comparison between the two groups as regards sex.

Sex	Cases		Controls		Total		Chi-Square	
	No.	%	No.	%	No.	%	X^2	P-value
Male	64	64.00	34	68.00	98	65.3	0.098	0.754

In cirrhotic patients 20 (20%) were child A, 38(38%) were child B & 42 (42%) were child C. Esophageal varices in cirrhotic 28 controls (28%) had small sized varices, 32 (32%) had medium sized and 40 (40%) had large sized varices (Tab. 3).

Table 3: Child`s Pugh score and esophageal varices in cirrhotic group.

Child's Pugh score	No.	%
Child A	20	20.00
Child B	38	38.00
Child C	42	42.00
Total	50	100.00
OES. V		
	No.	%
Small	28	28.00
Medium	32	32.00
Large	40	40.00
Total	100	100.00

MDA essay, in patients the MDA essay ranged between 300 and 1830 nm with mean level 934.860±481.574 nm, while in controls MDA essay ranged between 160 and 310 with mean level 238.250±45.330 nm, this revealed significant difference between the two groups with P <0.001 (Fig. 1**)**

Correlation between MDA and clinical parameters in cirrhotic group showed a significant positive correlation with ascites (p<0.001), child Pugh score (p<0.001), varices grade (p<0.001) and PHG grade (p<0.001), but without correlation with sex (Tab. 4).

Table 4: correlation between MDA and clinical parameters in cirrhotic group.

Variables		MDA				T-Test or ANOVA	
		No.	Mean	±	SD	T or F	P-value
Sex	Male	64	906.656	±	491.528	-0.548	0.586
	Female	36	985.000	±	473.004		
Ascites	Negative	28	634.143	±	262.850	4.127	<0.001*
	Mild	25	1069.643	±	474.076		
	Moderate	27	911.071	±	447.270		
	Marked	20	1266.875	±	601.551		
Child's	Child A	20	639.778	±	408.978	5.222	<0.001*
	Child B	38	831.000	±	384.385		
	Child C	42	1160.238	±	507.557		
OES. V	Small	28	471.643	±	197.798	31.770	<0.001*
	Medium	32	828.750	±	368.259		
	Large	40	1344.000	±	347.084		
PHG	Negative	20	438.300	±	194.522	15.248	<0.001*
	Mild	26	790.385	±	379.838		
	Severe	54	1188.333	±	430.568		

Correlating MDA with laboratory parameters showed significant statistical correlation with platelets (p<0.05), albumin (p<0.05) and INR (p<0.05), but without significant in age, TLC, HGB, Creatinine, ALT, AST and T. Bilirubin (Tab. 5).

Table 5: correlation between MDA and laboratory parameters.

Correlations	MDA	
	R	P-value
TLC	-0.118	0.415
HGB	-0.083	0.566
Platelet	-0.345	0.014*
Creatinine	0.158	0.272
ALT	-0.044	0.759
AST	0.137	0.342
Albumin	-0.337	0.017*
T.bilrubin	0.268	0.060
INR	0.378	0.007*

Assessing value of MDA in diagnosis of portal hypertension was found at best cut off value of MDA > 410. It was associated with 96% sensitivity & 98% specificity in diagnosis of portal hypertension. Positive predictive value was 97.5% and negative predictive value was 90.9%. The test accuracy was 97.3% (Tab. 6 & Fig. 3).

Table 6: MDA in diagnosing portal hypertension in Egyptian cirrhotic patients.

Sensitivity	Specificity	PPV	NPV
96	98	97.5	90.9

Discussion

Portal hypertension (PH) is considered one of the main complications of liver cirrhosis that leads to increase in incidence of ascites, variceal hemorrhage and decompensation. Thus, early PH diagnosis is the important

key in the managing patients with cirrhosis (Bosch *et al,* 2008)

The guidelines recommend the diagnosis of PH by the measurement of hepatic venous pressure gradient (HVPG) which was an invasive procedure and is only available in specialized centers. Noninvasive markers could be an appropriate alternative, but none of the markers which had been investigated, so far, have shown satisfactory specificity and sensitivity results (De Franchis, 2008). The oxidative stress (OS) has been shown to increase the progression of liver fibrosis during the chronic liver injury (Parola and Robino, 2001).

The OS also leads to the hepatic endothelial dysfunction through modulating NO bioavailability in the intrahepatic microcirculation. Consequently, the increased oxidative stress is important for the development of portal hypertension in cirrhosis (Catapano *et al,* 2000).

The Malondialdehyde (MDA) is the chemical product that results from lipid peroxidation of polyunsaturated fatty acids. The amount of MDA in tissues can give an idea about the degree of lipid peroxidation; moreover, it can be used as a marker for oxidative stress (Jain *et al,* 2002).

In this study, value of MDA was measured in 150 subjects divided into two groups, the first group contains 100 patients suffers from liver cirrhosis complicated with portal hypertension while second group contained 50 healthy subjects. There was a statistically significant difference between both groups regarding the MDA level with ($P < 0.001$) where the cases groups was higher than the control group. This result agreed with (Kuei-Chuan *et al,* 2009) who also found that the MDA level in cases group was higher than that of control group which suggested that more oxidants stress and weaker antioxidants protection exist in cirrhotic patients than in control subjects. Also, the present result agreed with Sheng-Lan *et al.* (2015) who found that the MDA level in patients was higher than that of control ones.

In the present study, there was a positive significant correlation between MDA value and presence of ascites in cirrhotic patients.

This result agreed with Kuei-Chuan *et al.* (2009) who mentioned that the MDA was slightly higher in the patients with ascites-regardless its degree- than in the patients without ascites. However, in the present study, there was positive significant correlation between the MDA level and degree of ascites.

In the present study, there was a positive significant correlation ($P<0.001$) between MDA value and Child Pugh score of the cirrhotic patients where MDA level in Child C Pugh score was higher than MDA level in Child B Pugh score was also higher than MDA level in Child A Pugh score. These results agreed with Sheng-Lan *et al.* (2015) regarding correlation between MDA and Child Pugh score ($P<0.001$).

Also, the present results agreed with Kuei-Chuan *et al.* (2009) who suggested that the plasma MDA levels might increase in the concordance with the progression of the severity of both the cirrhosis and fibrosis.

In this study, there was a positive significant correlation between the MDA level, the grade of esophageal varices and PHG with p values <0.001, which agreed with Sheng-Lan *et al.* (2015) regarding the correlation between MDA value and esophageal varices grade ($p<0.001$).

In the present study, a positive significant correlation between MDA level and portal vein diameter ($p<0.001$) was found. Sheng-Lan *et al.* (2015) reported that the variation of MDA level relate to the severity of portal hypertension as the vessel diameter width of venous system was positively related to the portal vein pressure in portal hypertension caused by cirrhosis, and the main vessel width represented the portal pressure to the some extent.

In the present study, there was positive significant correlation between MDA value and INR ($p<0.001$) that reflected the presence of positive correlation between MDA

value and the worsening of synthetic liver function due to the progression of liver cirrhosis, although the present study showed a negative correlation between MDA value and plasma albumin level (p =0.017).

In the present study, serum MDA best cut off value of > 410ng/ml was associated with 96% sensitivity and 98% specificity in the diagnosing of portal hypertension. The positive predictive value was 97.5% and the negative predictive value was 90.9%. The accuracy of the test was 97.3%. These results agreed with Kuei-Chuan *et al.* (2009) who reported that a slightly higher cut off value of MDA >426.5ng/ml was associated with sensitivity 78.6% and specificity 100%. Also, the present results agreed with Sheng-Lan *et al.* (2015) who stated that a slightly higher cut off value of MDA >426.5ng/ml was associated with sensitivity 78.2% and specificity 86.2%.

In the present study, the low cut value off can be explained by the difference of the etiology of cirrhosis which is mainly viral in the present study while alcoholic cirrhosis was predominant in the other two studies and also can be explained by the prevalence of the schistosomiasis infection among the Egyptian cirrhotic patients which markedly affected the severity of the portal hypertension.

In the present study, the limitations were small number of the participants. Also, prospective studies were required to compare between cirrhotic patients with portal hypertension, and cirrhotic patients with non-portal hypertension to elucidate whether the Malondialdehyde level was related either to the cirrhotic stage or the presence of portal hypertension.

Conclusion

Generally speaking, the portal hypertension is defined by the pathological increase in the pressure of the portal venous system, and cirrhosis is the commonest cause of the portal hypertension.

The outcome results showed that the MDA level was significantly higher in the cirrhotic patients with portal hypertension than in the healthy ones and directly correlated with the PVD, esophageal varices, PHG, Child Pugh score.

The MDA proved to be the marker for diagnosing the liver cirrhosis with the portal hypertension and also for assessing severity of portal hypertension in Egyptian patients.

Competing interests: Authors declared that they neither have conflict of interest nor received funds.

Authors' contributions: A.A.S., A.A.G., G. R.S., R. T.A. & H.M. conceived and planned the experiments. K.W.A.Y. contributed to sample preparation. N. H.M. performed the ultrasound to the patients.

All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors read and approved the manuscript.

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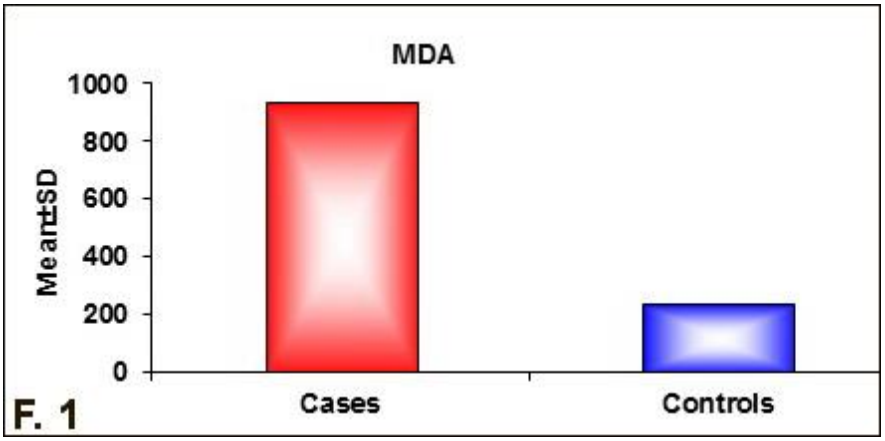
Explanation of figures

Fig.. 1: Comparison between two groups as regards MDA essay.
Fig.. 2: Correlation between MDA and portal vein diameter.
Fig. 3: Diagnostic performance of MDA in diagnosing portal hypertension in Egyptian cirrhotic patients.


MDA
1000
800
600
400
200
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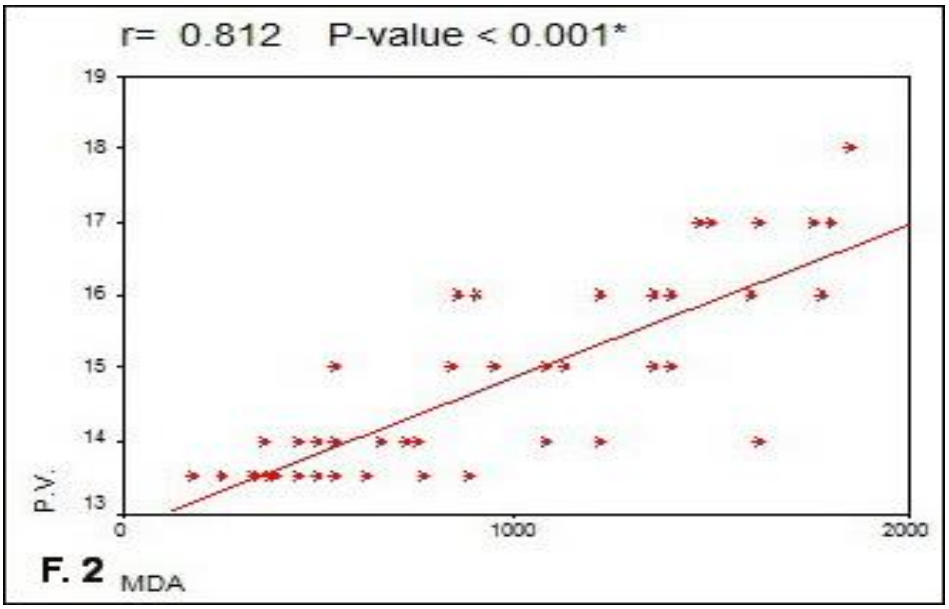
Mean±SD
Cases
Controls
F. 1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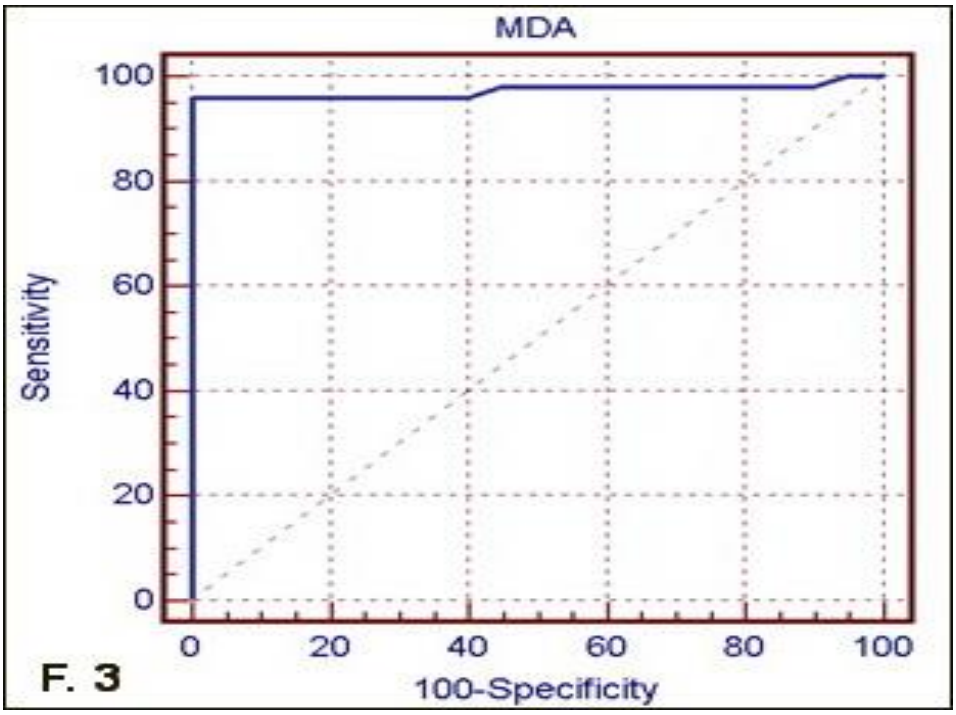
MDA
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