

## Original paper

# Oxidative Stress and Antioxidant Defense in Patients With Jaundice

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## Abstract

**Background:** The objective of our study is to measure serum Malondialdehyde level (MDA), an index of oxidative stress, and Vitamin E level, a protective agent against oxidative stress, in Jaundice patients and study the possible correlation between them .

**Materials and Methods:** 80 patients with jaundice ( 38 female and 42 male) and 50 healthy control (23 female and 27 male) were enrolled in this study. About 10 ml of venous blood were obtained from the cubital vein using disposable needles and syringes. The thiobarbituric acid method was used to measure the Malondialdehyde (MDA) which reacted with thiobarbituric acid(TBA) to give pink color that was read at (535 nm). The concentration of vitamin E in serum was determined according to a modified Hashim and Schuttringer method.

**Results:** A significant increase ( $P<0.0001$ ) in serum Malondialdehyde (MDA) in jaundice patients as compared to that of normal healthy controls was observed. A significant decrease ( $P<0.0001$ ) in serum vitamin E in jaundice patients as compared to that of normal healthy controls has been noticed. No significant correlation between serum Malondialdehyde (MDA) and serum Vitamin E in jaundice patients was observed.

**Conclusion:** our data shows that antioxidant capacity decreases with an increase in oxidative stress in Jaundice patients but there is no significant correlation between them .

**Abbreviations:** The abbreviations used are: dL, deciliter; MDA, Malondialdehyde;  $\mu$ l, microliter; r, Correlation factor ;rpm , Round per minute; TCA, Trichloroaceticacid; TBA, thiobarbituric acid ; ROS, Reactive oxygen species.

## Introduction

Jaundice is a physical sign characterized by a yellow appearance of the patient. It results from deposition of bile pigment (Bilirubin) in the skin, mucous membranes, and sclera. It is the most characteristic clinical manifestation of hepatic disease. <sup>(1)</sup> The degree of serum bilirubin elevation can be estimated by physical examination. A clinical examination cannot detect jaundice until the serum bilirubin is greater than 2 mg /dL (34 $\mu$ mole/litter), twice the normal upper limit .<sup>(2)</sup> Jaundice is not specific to liver disease and may indicate other disorders, including hemolysis and disorders of bilirubin metabolism.<sup>(1)</sup> Oxygen free radicals have an important role in the

pathogenesis of acute and chronic liver disease.<sup>(3)</sup> The free radical can be defined as a chemical species, an atom or molecule that has one or more unpaired electrons in valance shell and is capable of existing independently. A free radical contains an odd number of electrons, which make it unstable, short lived and highly reactive.<sup>(4)</sup> It becomes stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease.<sup>(5,6)</sup> Prime targets of reactive oxygen species are the polyunsaturated fatty acid in cell membranes causing lipid peroxidation, which may lead to the damage of the cell structure and function. Lipid peroxidation is an auto catalytic process whereby polyunsaturated fatty acids and

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phospholipids undergo degradation by a chain reaction to form lipid hydroperoxides in cell membranes, body fluids, etc.<sup>(7, 8)</sup> The increase in lipid peroxidation has been previously reported in various liver diseases<sup>(9)</sup> The process of lipid peroxidation can be followed by measurement of numerous break down products such as Malondialdehyde (MDA) as an indicator of lipid peroxidation.<sup>(10)</sup> Oxidative stress results from any significant change of the ratio between the formation of free radicals and endogenous antioxidant defense mechanisms.<sup>(4)</sup> Under normal physiological conditions, the generation of free radicals was halted or their effects were abolished by several methods of defence mechanisms grouped collectively under the term of antioxidants or scavenger System. So an antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate.<sup>(11)</sup> The term "oxidizable substrate" includes almost everything found in living cells, including proteins, lipids, carbohydrates and DNA. Antioxidant such as vitamin E acts to protect cells against effects of free radicals, which may contribute to the

development of chronic diseases such as chronic liver disease.<sup>(12, 3)</sup> The objective of our study is to measure serum malondialdehyde level, an index of oxidative stress, and vitamin E level, a protective agent against oxidative stress, in jaundice patients.

## Material & Methods Patients

Eighty patients with jaundice and fifty healthy control were enrolled in the study. They represent a selected sample of patients who attend the AL-Yarmouk Teaching Hospital and AL-Sadder Hospital. About 10 ml of venous blood were obtained from the cubital vein using disposable needles and syringes. The blood was allowed to coagulate at room temperature and centrifuged at 3000 rpm for 20 min. The resulting sera were separated and stored at -20 C° when it was not used immediately The assays were obtained by running duplicates for the test, control, and the standard. The results were expressed as mean  $\pm$  SD. The following table shows the host information which are used in this study.

Table1. Age and Sex distribution of the study population

	Sex		Age(mean $\pm$ SD)		Total (N)
	Male (N)	Female(N)	Male	Female	
Jaundice patients	42	38	32.6 $\pm$ 13.6	28.6 $\pm$ 12.6	80
Control	27	23	27.7 $\pm$ 10.03	35 $\pm$ 12.08	50

**Chemicals:** All common laboratory chemicals and reagents used in the study are of analytical grade, and they were obtained from the following companies : HCL from Hopkins and William, Thiobarbituric acid, Ethanol(99.9%), Ferric chloride FeCl<sub>3</sub>.6H<sub>2</sub>O,  $\alpha$ ,  $\alpha$ -Di pyridyl, and  $\alpha$ -tocopherol acetate from BDH-England. Trichloroacetic acid (TCA) from Randox Laboratory ,Ltd.England.

**Methods Determination of Malondialdehyde (MDA)**

Malondialdehyde (MDA) was formed from the breakdown of poly unsaturated fatty acid, served as a convenient index of peroxidation reaction. The thiobarbituric acid method<sup>(13)</sup> was used to measure the Malondialdehyde (MDA) which reacted with thiobarbituric acid (TBA) to give pink color that was read at (535 nm). Malondialdehyde concentration was calculated using the molar extinction coefficient of  $1.56 \times 10^5$ <sup>(14)</sup>.

**Reagent:** Dissolve 0.188g TBA and 7.5 gm TCA in a suitable volume of 0.25 N HCl. The mixture was shaken and heated

at 70C° until the solubility is completed then the volume was made up to a final volume 100 ml. **Procedure:** A volume of 1 ml of the reagent was added to 0.5 ml of serum sample. The tube was well mixed by vortex mixer and heated at 70c° for 20 min. After cooling the mixture was centrifuged for 15 min at (1000rpm) the clear supernatant was read at 535 nm against the blank which contained 1 ml of distilled water and 2 ml of reagent.

#### Determination of vitamin E

The concentration of vitamin E in serum was determined according to a modified of Hashim and Schuttringer(15) **Principle:** After the proteins in serum are precipitated by an equal volume of absolute ethanol, the obtained mixture is subjected to extraction by an equal volume of n-heptane (16). The  $\alpha, \alpha$  - dipyridyl is added to an aliquot of the upper layer to estimate the principal interfering substance,  $\beta$  carotene, at 460 nm. At this time an antioxidant- reduction reaction is carried out according to Emmerie-Engle procedure in which tocopherol is oxidized to tocopherol quinone by the addition of ferric chloride reagent, and the (Fe+2 ) in the resultant FeCl<sub>2</sub> is complex with  $\alpha, \alpha$  - dipyridyl to produce a red color which absorbs at 510 nm(15) (17) (18).

**Procedure:** A volume 0.5 ml of serum sample transferred into a screw capped tube, then 0.5 ml of Ethanol (99.9%) and 1.5 ml of heptane were added. The tubes were capped and shaken for 10 min and

centrifuged for 10 min at (1000 rpm). A volume of 0.7 ml of the upper heptane layer which , sharply separated, was pipette into a test tube, and 0.5 ml of  $\alpha, \alpha$  - dipyridyl reagent was added. While a voiding a direct light , 0.17 ml of ferric chloride reagent was added .

The spectrophotometer was set to zero absorbance at 510 nm with blank consisting of 1.4 ml heptane and 1 ml of  $\alpha, \alpha$  - dipyridyl reagent and 0.34 ml of ferric chloride reagent . The absorbance of the sample was read at 510 nm, exactly after 4 min of ferric chloride reagent

**Vitamin E Standard:** The standard of vitamin E was prepared as follows: A stock solution of 100 mg / dL was prepared dissolving 100 mg of  $\alpha$ -tocopherol acetate into a final volume of Ethanol (99.9%). One ml of this solution was then diluted to 10 ml with Ethanol 99.9% to form intermediate standard solution of 10 mg /dL .From the intermediate standard solution the following concentration were prepared by serial dilutions with Ethanol (99.9%): (0.1 , 0.3 , 0.5 , 0.7 , 0.9 , 1.1 , 1.3 , 1.5 , 1.7 , 1.9 , 2.1) mg /dL

#### Calculation

The standard curve (Figure 1) was obtained by plotting the absorbance against the corresponding concentrations of standard vitamin E, and it was used to determine the unknown vitamin E concentration of the serum samples.

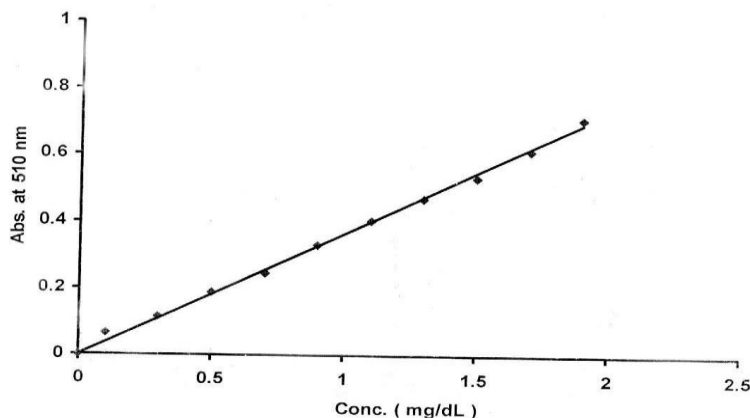


Figure 1. Standard curve for determination of vitamin E

## Results

Table 2 and figure 2 show the mean  $\pm$ SD of MDA level expressed as nmol/ml in sera of normal healthy controls, and jaundice patients. The results indicate a significant increase in the mean value of MDA in the sera of jaundice patients ( $0.12 \pm 0.03$  nmol/ml) when compared to

control group ( $0.05 \pm 0.004$  nmol/ml), whereas in Jaundice patients there is no significant difference between males & females as shown in Table 3. Concerning MDA level in serum of jaundice patients there was no significant correlation with age. ( Figure 4).

Table 2. Biostatistical calculation and students t.test for MDA level nmol/ml in sera of normal healthy controls and Jaundice patient

Serum MDA nmol/ml	Jaundice patients	Normal healthy control
Sample size	80	50
Mean $\pm$ SD	$0.12 \pm 0.03$	$0.05 \pm 0.004$
Range	0.06-0.17	0.04-0.08
Standard error of mean	0.003	0.0005
Confidence Interval of mean	0.12-0.12	0.049 – 0.051
t. test	17.22	
P value	0.0001*	

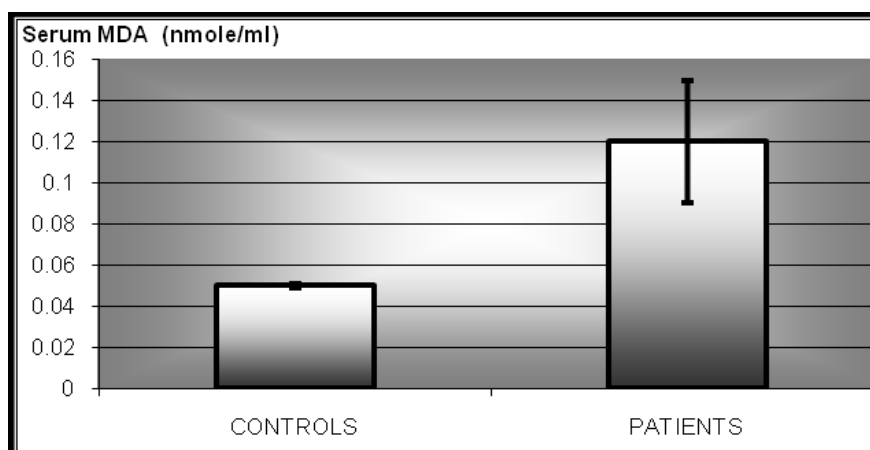


Figure 2. Serum MDA level in normal healthy controls, and Jaundice patients

Table 3. Biostatistical calculation and student t.test for MDA level nmol/ml in serum of Jaundice patients according to their sex.

MDA( nmol/ml)	Patient	
	M	F
Sex		
Sample size	42	38
Mean $\pm$ SD	$0.12 \pm 0.03$	$0.13 \pm 0.03$
Range	0.07-0.16	0.06-0.17
Standard error of mean	0.005	0.005
Confidence interval of mean	0.11-0.13	0.12-0.14
t. test	1.061	
P value	0.292	

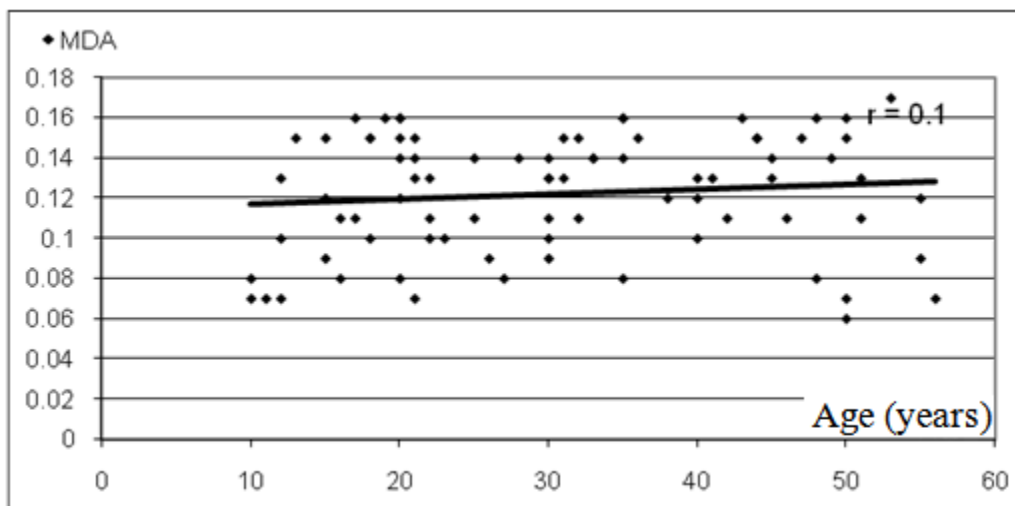


Figure 3. Scatter diagram of serum MDA and Age in jaundice patients

Table 4. Correlation between MDA, Vitamin E, and age in Jaundice patients

	r	Age(year)
	P value	
MDA(n mole/MI)	r	0.112
	p	0.112
Vitamin E (mg/Dl)	r	0.061
	p	0.589

Table 5 and figure4 show the mean  $\pm$ SD of vitamin E level expressed as mg/dl in sera of normal healthy controls, and jaundice patients. The results indicate a significant decrease in the mean value of vitamin E in the sera of jaundice patients ( $0.37 \pm 0.13$  mg/dl) when compared to control group ( $1.027 \pm 0.28$  mg/dl), whereas in jaundice

patients there is no significant difference between males & females as shown in table6. No significant correlation has been found between serum vitamin E level and age as in table4. Also there is no significant correlation between serum vitamin E levels and MDA in jaundice patients as figure 5.

Table 5. Biostatistical calculation and students t.test for Vitamin E level mg/dl in sera of normal healthy controls and Jaundice patients

Serum Vitamin E mg /dl	Jaundice patients	Normal healthy control
Sample size	80	50
Mean $\pm$ SD	$0.37 \pm 0.13$	$1.027 \pm 0.28$
Range	0.20-0.72	0.61-1.58
Standard error of mean	0.01	0.0395
Confidence Interval of mean	0.35-0.39	0.95 – 1.11
t. test	18.19	
P value	0.0001*	

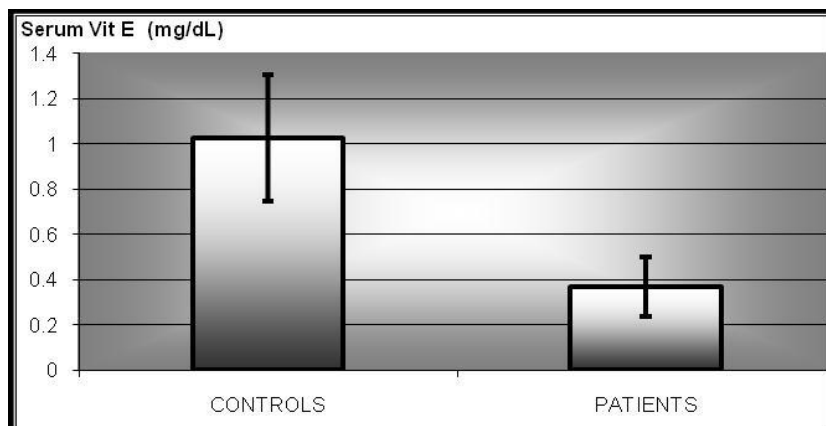


Figure 4. Serum vitamin E level in normal healthy controls, and Jaundice patients (mean± SD)

Table 6. Biostatistical calculation and student t.test for Vitamin E level mg/dl in serum of Jaundice patients according to their sex.

Vitamin E(mg/dl)	Patient	
	M	F
Sex		
Sample size	42	38
Mean ±SD	0.36±0.13	0.38±0.12
Range	0.20-0.72	0.20-0.70
Standard error of mean	0.02	0.02
Confidence interval of mean	0.32-0.40	0.34-0.42
t. test	0.801	
P value	0.426	

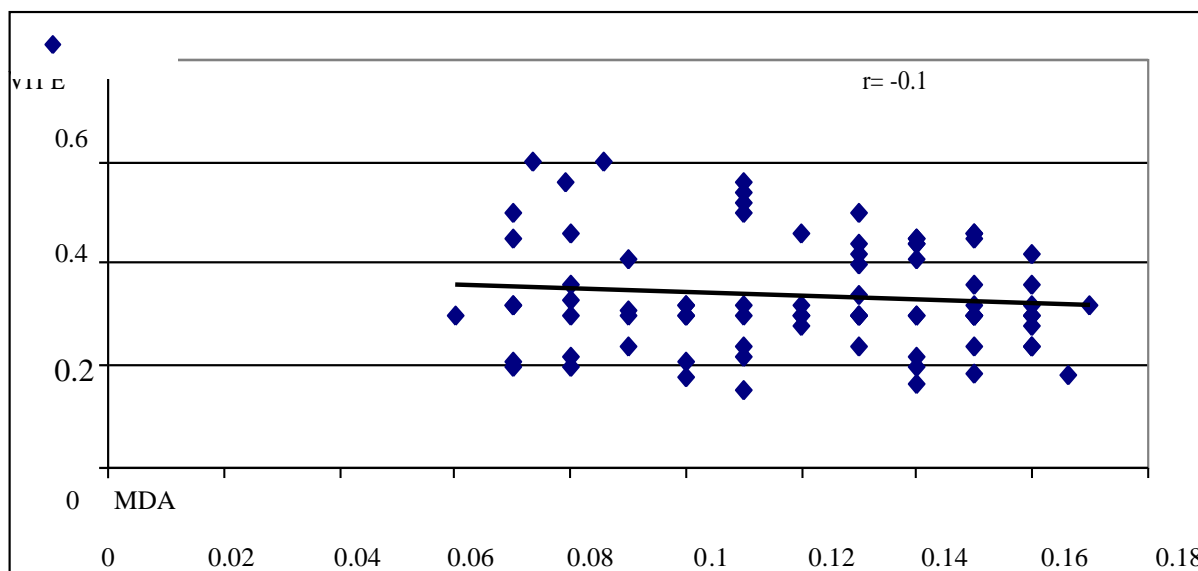


Figure 5. Scatter diagram of serum MDA and serum Vitamin E in jaundice patients

### Discussion

Measurements of parameters for oxidative stress are a well-accepted technique to express the extent of cell damage. (19) Previous studies have demonstrated that

MDA levels increase and antioxidant capacity decreases in acute and chronic hepatitis. (3) The result in this study indicate a highly significant increase in serum MDA levels in jaundice patients compared to normal healthy control

( $p < 0.0001$ ) as shown in table 2. These findings were in agreement with findings reported by Dikici et.al (2005)<sup>(20)</sup>; Bianchi et.al (1997)<sup>(21)</sup>; wan -shengko et.al (2005)<sup>(22)</sup>; Pratibha et.al (2004)<sup>(23)</sup>; Aksoy et.al (2003)<sup>(9)</sup>; Levent et.al (2006)<sup>(24)</sup> Increased levels or accelerated generation of ROS have been reported in the plasma of patients with liver disease and animal models of liver disease.<sup>(25, 26)</sup> The production of ROS in the liver may be linked to inflammation which has emerged as a primary mechanism of liver injury after pathophysiological insults. Activated kupffer cells and neutrophils release ROS in response to inflammatory cytokines in the liver<sup>(27)</sup>. ROS in excess abstracts hydrogen atoms from lipoproteins causing lipid peroxidation, of which MDA is the main product<sup>(28)</sup>. Within jaundice patients, the present study revealed that there was no significant difference in serum MDA level between males and females. No significant correlation is found between the serum MDA levels and age in jaundice patients as in Table 4. In the recent years increasing experimental and clinical data have provided compelling evidence for the involvement of free radicals/ROS in a large number of pathophysiological states, but still evidences are not enough to ascertain whether free radicals are the cause or consequence of pathology. This has led to increasing curiosity and interest among the scientists and researchers globally to evaluate potential benefits from the antioxidant.<sup>(4)</sup> Vitamin E is a term that encompasses a group of potent, lipid-soluble, chain-breaking antioxidants.<sup>(29)</sup> Vitamin E's function as an antioxidant is dependent upon its ability to break radical-propagated chain reactions. As a result, the formation of the tocopheroxyl radical, the odd-electron derivative of vitamin E, is an inherent part of any vitamin E based, antioxidative reaction<sup>(12)</sup>. Both in vitro and in vivo studies have demonstrated that  $\alpha$ -tocopherol inhibits LDL oxidation and decreases the release of reactive oxygen species.<sup>(12)</sup> Furthermore, it also has been

shown to reduce the release of pro-inflammatory cytokines, and inhibit monocyte-endothelial cell adhesion.<sup>(30)</sup> The results in this study indicate a highly significant decrease in serum vitamin E level in jaundice patients compared to normal healthy control ( $p < 0.0001$ ) as shown in table 5. These findings were in agreement with findings reported by Espat et.al (2000)<sup>(31)</sup>; sokol et.al (1985)<sup>(32)</sup>; Von Herbay et.al (1996)<sup>(33)</sup>.

There was no significant difference in serum vitamin E level within jaundice patients according to the sex. Vitamin E is a fat soluble vitamin which is absorbed from the human small intestine (enterocyte) after solubilization in bile salt micelles.<sup>(12)</sup> Very low serum concentrations of vitamin E have been consistently reported in patients with both chronic liver disease and cholestasis.<sup>(34)</sup> This decrease in vitamin E can be explained by the fact that in these patients, deficiency of bile salts in the intestinal lumen leads to impaired micelle formation and malabsorption of fat. Therefore the solubilization of vitamin E and its absorption by enterocytes is insufficient.<sup>(34)</sup> No significant correlation was found between serum vitamin E level and age. There was no significant correlation between serum vitamin E levels and serum MDA levels ( $r = -0.1$ ), (fig.5). The decrease in vitamin E level confirms the fact that vitamin E concentration decreases with an increase in oxidative stress as stated by each of Wisdom et.al (1991)<sup>(35)</sup>; wang et.al (1991)<sup>(36)</sup>; Davidge et.al (1992)<sup>(37)</sup>; and Mikhail et.al (1994)<sup>(38)</sup>.

This decrease has probably been contributed to the increased consumption of  $\alpha$ -tocopherol for free radical neutralization and its conversion to  $\alpha$ -tocopheroxyl radical.<sup>(39)</sup> In conclusion, our data shows that antioxidant capacity decreases with an increase in oxidative stress in jaundice patients but there is no significant correlation between them.

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