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Research Article

Assessment of Haematological Complications and Prognostic Value of Oxidative Stress Markers in Viral Hepatitis B Patients

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Abstract

Background and Objective: Viral hepatitis infection could develop several complications include chronic infection, acute or sub-acute hepatic necrosis, cirrhosis, liver failure and hepatocellular carcinoma. The goal of this study was to estimate the effect of viral hepatitis B infection on some haematological and oxidative stress markers in men patients. **Materials and Methods:** Thirty voluntary men from the El Oued region divided equally into hepatitis B patients and control groups. Prediction tests of oxidative stress markers were estimated using ROC curve analysis. **Results:** Results showed that the leukocytes and platelet lines cells were significantly increased ($p < 0.01$) but the erythrocyte line was significantly decreased ($p < 0.05$) in the hepatitis group as compared to the control group. In this experimental study, the result showed a significant increase ($p < 0.001$) of serum MDA level, SOD and catalase activities, also a significant decrease ($p < 0.001$) of serum GSH in the hepatitis group compared to the control group. ROC analysis indicated that all of MDA and GSH levels, SOD and catalase activities were a high specificity and important AUC percentage with high correlation with risk factors of hepatitis B. **Conclusion:** Results indicated that, hematotoxicity and change in oxidative stress markers were the origins of the severity of hepatitis B infection. In addition, MDA, SOD, GSH and catalase were considered important diagnostic tools for follow up and predicting complications of hepatitis B.

Key words: Hepatitis B, infection, oxidative stress, hematotoxicity, ROC analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hepatitis is a liver inflammation caused by a range of factors such as medications, chemicals, heavy alcohol consumption, or autoimmune disease, with viral infection being the most common cause, which is called viral hepatitis¹. This last is mostly caused by infection from the hepatitis A and B virus or hepatitis C virus. Despite the abundance of effective vaccines and advanced antiviral therapies, viral hepatitis remains a significant public health problem in the United States². Approximately, 30% of the world's population has been infected with the Hepatitis B Virus (HBV), making it one of the most common viral infections in the world³. Nearly 350 M individuals are chronically infected with Hepatitis B Virus (HBV) Worldwide⁴ and it is responsible for 500,000-1.2 M deaths per year⁵. Hepatitis B is mostly asymptomatic at the beginning of infection but, in some patients, it could cause acute illness, yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain⁶. Generally, hepatitis patients are identified during blood donor testing, insurance testing, after maternal screening or after serological testing when they present some symptoms⁷. HBV infection could develop several complications include chronic infection with chronic active hepatitis, acute or subacute hepatic necrosis, cirrhosis, liver failure and hepatocellular carcinoma⁸. Ascertaining hepatitis B status enables identification of people who need vaccination, protection of those at risk of reactivation and intervention to treat chronic infection treatment to prevent liver failure and its serious complications⁹. Several studies have been demonstrated that the persistence of infection, the progression of liver damage and carcinogenesis are all steps in which oxidative stress is involved¹⁰. Oxidative stress is an abnormal condition caused by an excess production of oxidants compared to the antioxidant¹¹. Many scientific researchers have shown that oxidative stress inactivates the metabolic enzymes and damage important cellular components and in this way, it contributes to the progression of major health problems¹². When viruses employ the synthetic processes of the host cell to replicate, they cause an increase in ROS production and antioxidant depletion¹³. The ensuing redox imbalance and Oxidative Stress (OS) causes parenchymal damage ranging from subclinical anicteric hepatitis to necroinflammatory hepatitis, cirrhosis and carcinoma¹⁴. Faced with these problems, this study aimed to evaluate the hematotoxicity risk and evaluate the sensitivity and specificity of oxidative stress markers in viral hepatitis B patients.

MATERIALS AND METHODS

Study design: Randomly enrolled 50 volunteer man patients aged between 24-70 years old who visited the Hospital, El Oued from January, 10 until April 10, 2021. These men were reported to the clinical study service (NCT00000632). According to the official dates approved by the Hospital: (1st visit). Total of 30 men patients consented and were randomly assigned who had been hepatitis (group 1) (n = 15) or control peoples (group 2) (n = 15). Contact information was received for 50 men initially interested in participating in the study. After losing contact with each other (5 cases) and others not interested to participate (13 cases) or did not complete medical visits for some of them (2 cases), the number reached 30 men who were enrolled in the study shown in Fig. 1.

Subjects of study: Ethical approval was requested and approved by the Ethics Committee of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued. this study is carried out on 30 men volunteers from the El Oued region, aged between 24-70 years, who were divided into 2 groups, a group of 15 healthy control aged 37.47 ± 3.18 years and 15 has hepatitis B aged 38.60 ± 3.31 years.

Inclusion and exclusion criteria: Inclusion criteria for voluntary persons (patients and controls) live in the El Oued region, patient's clinical diagnosis shows hepatitis B disease confirmed by ELISA test and specialist doctor's patients have hepatitis B but no other type of acute or chronic disease. Regarding the controls are healthy people do not suffer from chronic or acute diseases. Exclusion criteria were to eliminate the factors which might affect oxidative stress parameters, we excluded all diabetics, arterial hypertension, anaemia or any evidence of endocrine disorders in their medical history from patient groups and healthy controls.

Sample collection: About blood sampling for both groups is done morning fasting. It is performed in the vein of the end of the elbow. Blood samples were collected in 3 tubes. Dry tubes are centrifuged at 2500 rpm for 5 min, then obtained the serum to achieve the dosage of oxidative stress (MDA, GSH, SOD and ORAC) parameters. The anticoagulant tube (EDTA) is mixed well and then assays the haematological parameters. The anticoagulant tube (Heparin) is centrifuged at 2000 rpm for 5 min, then recover the plasma to realize the dosage of the biochemistry parameter.

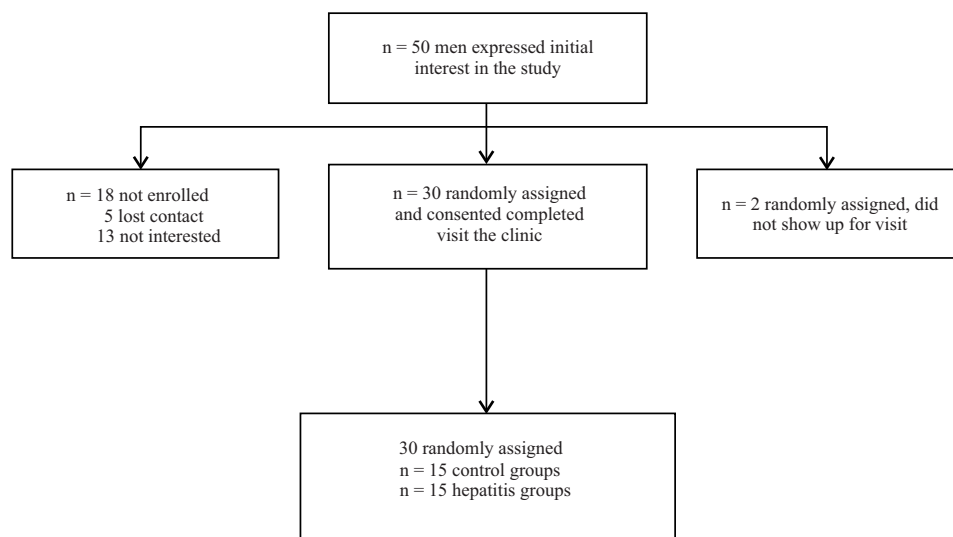


Fig. 1: Flowchart of subject recruitment, enrollment and retention from patients

Haematological, biochemical and oxidative stress

measurements: Hematological analysis (FNS) is performed by the haematology auto analyzer. Total bilirubin and uric acid were determined by auto analysis (BIOLIS24j) using commercial kits from Spinreact, (total bilirubin-20131, direct bilirubin 1001047, uric acid-1002011 aspartate aminotransferase-92025, alanine aminotransferase-92027, alkaline phosphatase-53123). Serum Malondialdehyde (MDA) and reduced glutathione (GSH) were measured according to the method described by Yagi¹⁵ and Weckberker and Cory¹⁶, respectively. Serum catalase enzyme activity was determined by Aebi method¹⁷. The activity of superoxide dismutase (SOD) was measured using the Beauchamp and Fridovich technique¹⁸.

Statistical analysis: The data were given as Mean $\pm$ Standard error of the Mean $\pm$ SEM. The independent samples were tested using the student's t-test. Minitab 14 is a statistics program. The coefficient of Pearson's correlation test was performed to examine the correlations between the various parameters. The diagnostic model of hepatitis B with several factors was based on OR risk factored regression analysis using SPSS statistics 25 software and Excel 2007 (Microsoft). The Area Under Curves (AUC) and Receiver Operating Characteristics (ROC) statistical parameters were utilized to demonstrate the potency of a biomarker in the detection of hepatitis B. The results of specificity, sensitivity, AUC and 95% Confidence Interval (CI) were calculated, $p < 0.05$ indicates a statistically significant difference.

RESULTS

Description of the study population: The general data of demographic characteristics of the 2 groups of subjects include age, body weight and blood group. The mean and standard deviation of age and bodyweight are 37.47 ± 3.18 years and 72.5 ± 3.81 kg in control and 38.60 ± 3.31 years and 77.2 ± 2.76 kg in the hepatitis group respectively. The blood type of hepatitis participants was mostly A+ (70%) or O+ (30%). Bilirubin and transaminases are among the main markers that indicate the safety of liver function. Current results indicate that total and direct bilirubin level and transaminase (AST and ALT) activities were significantly decreased ($p < 0.01$) in the hepatitis patients group as compared to the control group. This confirms that the liver suffers from dysfunction due to viral infection. On the other hand and as confirmation of liver dysfunction in patients, the results obtained show that the concentration of the uric acid decreased in patients compared to the control as it moved from 50.91 - $36.34 \mu\text{mol L}^{-1}$. As well as the activity of alkaline phosphatase increased in patients compared to control as it moved from 203.2 - 369.7 U L^{-1} anicteric as shown in Table 1.

Biochemical risk factor study: The odds ratio is frequently used to demonstrate the strength of an association between risk factors and clinical outcomes. Odds Ratio (OR) values of transaminases (1.73 and 1.91), ALP (16), bilirubin (12.96 and 3.95) and uric acid (16.01) levels show that the change of these biochemical markers was shown to be significant risk factors ($p < 0.05$) for the hepatitis disease in Table 2.

Table 1: Demographic, clinical and laboratory features between the study groups

Parameters	Control (n = 15)	Hepatitis (n = 15)	p-value
Age (year)	37.47±3.18	38.60±3.31	0.820
Body weight (kg)	72.5±3.81	77.2±2.76	0.168
Blood types			
A+ (%)	35	70	0.001
B+ (%)	30	0	0.001
AB+ (%)	15	0	0.001
O+ (%)	20	30	0.016
Serum AST (U L ⁻¹)	16.32±1.54	20.42±1.53	0.001
Serum ALT (U L ⁻¹)	15.12±0.83	19.09±1.88	0.005
Serum ALP (U L ⁻¹)	203.2±12.2	369.7±34.6	0.000
Serum uric acid (μmol L ⁻¹)	50.91±1.06	36.34±2.57	0.000
Total bilirubine (mg L ⁻¹)	4.36±0.24	6.31±0.65	0.000
Direct bilirubine (mg L ⁻¹)	1.37±0.14	2.81±0.64	0.003

Table 2: Comparison of the biochemical factors of control and hepatitis patients (N = 30)

Parameters	Control (%)		Hepatitis (%)		OR	CI 95%	p-value
	Positive	Negative	Positive	Negative			
Aspartate aminotransferase	46.15	53.84	33.33	66.66	1.73	0.975-3.068	0.041
Alanine aminotransferase	61.53	38.46	46.15	53.84	1.91	1.09-3.365	0.017
Alkaline phosphatase	63.63	36.36	10.00	90.00	16.00	7.405-34.571	0.000
Uric acid	57.14	35.71	9.09	90.90	16.01	7.179-35.699	0.000
Total bilirubine	53.33	46.66	7.69	92.30	12.96	5.699-29.511	0.000
Direct bilirubine	64.28	35.71	30.76	69.23	3.95	2.197-7.128	0.000

Table 3: Hematological parameters levels in control and hepatitis groups

Parameters	Control (n = 15)	Hepatitis (n = 15)	p-value
White blood cells (10 ³ μL ⁻¹)	6.542±0.397	8.382±0.636	0.000
Lymphocytes (10 ³ μL ⁻¹)	3.28±0.18	3.15±0.24	0.463
Monocytes (10 ³ L ⁻¹)	1.23±0.11	1.64±0.19	0.006
Granulocytes (10 ³ μL ⁻¹)	2.3±0.2	4.12±0.27	0.000
Red blood cells (10 ⁶ μL ⁻¹)	4.87±0.09	4.47±0.22	0.017
Hemoglobin (g dL ⁻¹)	14.70±0.25	13.57±0.75	0.040
Hematocrit (%)	42.16±0.69	39.32±1.97	0.048
Mean corpuscular volume (fl)	84.79±1.54	89.16±0.7	0.000
Platelet (10 ³ μL ⁻¹)	175.5±5.57	211.93±6.58	0.000
Platelet crit (%)	21.61±1.98	22.23±0.87	0.312
Mean platelet volume (fl)	10.12±0.09	10.8±0.43	0.032
Platelet distribution width (%)	11.12±0.22	11.5±0.27	0.059

Haematological markers: Blood parameters are among the important markers that indicate the extent of the effect of liver dysfunction due to viral infection on blood components, which are divided into 3 sections: Leucocytes line, erythrocytes line and platelets line. The results of the haematological analysis for the control and hepatitis group were illustrated in Table 3. The result of the leukocytes line shows that the WBC (6.542±0.397, 8.382±0.636), MONO (1.23±0.11, 1.64±0.19) and GRA (2.3±0.2, 4.12±0.27) were significantly increased (p<0.01) in the Hepatitis group as compared to the control group, LYM level was shown that no significant differences (p>0.05). In the erythrocyte line, the result shows that Hb (14.70±0.25, 13.57±0.75), RBC (4.87±0.09, 4.47±0.22) and HCT (42.16±0.69, 39.32±1.97) were

significantly decreased (p<0.05) while MCV (84.79±1.54, 89.16±0.7) was significantly increased (p<0.001) in patients group as compared to the control group. The Platelet line results show that MPV (10.12±0.09, 10.8±0.43) was significant increased (p<0.05) in the hepatitis group as compared to the control group. These results clearly illustrate the negative effect of viral hepatitis on blood components.

Oxidative stress markers: The analysis of blood oxidative stress parameters in control and hepatitis patient are shown in Table 4. Current result explained a significant increase (p<0.001) of serum MDA (3.509±0.472, 12.03±1.14 nM g⁻¹ Hb) level, SOD (1.67±0.08, 7.66±2.13 UI g⁻¹ Hb) and

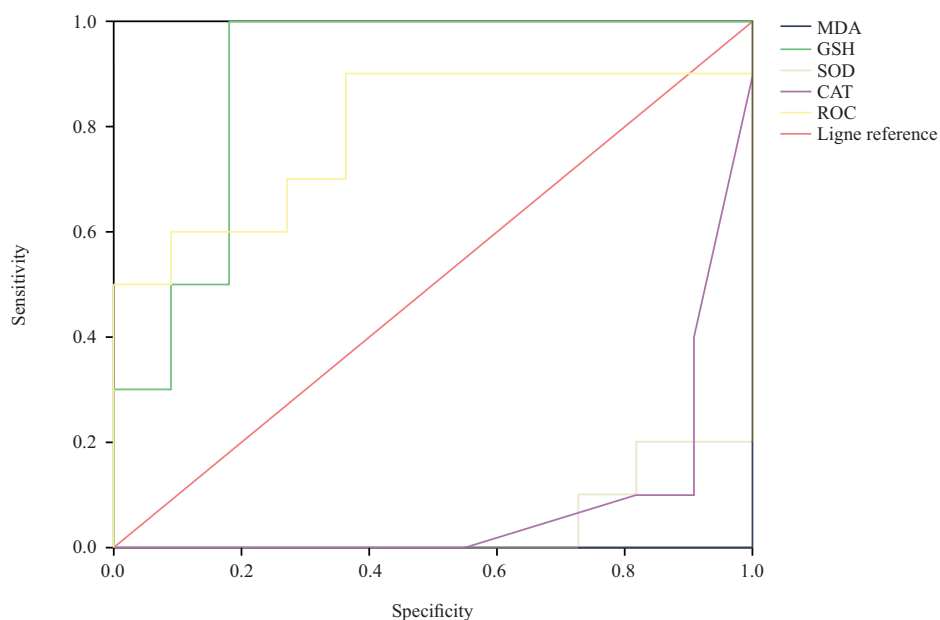


Fig. 2: Receiver Operating Characteristic (ROC) curve for oxidative stress markers

Table 4: Oxidative stress parameters in the blood of control and hepatitis groups

Parameters	Control (n = 15)	Hepatitis (n = 15)	p-value
Serum MDA (nM g ⁻¹ Hb)	3.509±0.472	12.03±1.14	0.000
Serum GSH (nM g ⁻¹ Hb)	2.595±0.269	1.012±0.231	0.000
Serum SOD (UI g ⁻¹ Hb)	1.67±0.08	7.66±2.13	0.000
Serum CAT (UI g ⁻¹ Hb)	0.01±0.0005	0.07±0.02	0.000
Serum ORAC (UI L ⁻¹)	0.5677±0.0890	0.960±0.132	0.000

Table 5: Sensitivity, specificity and AUC values of some oxidative stress markers

Variables	Sensitivity (%)	Specificity (%)	AUC	CI 95%	p-value
Malondialdehyde	63.6	100	0.00	0.000-0.000	0.000
Superoxide dismutase	54.5	100	0.058	0.000-0.148	0.000
Reduced glutathione	45.5	09.1	0.901	0.761-1.000	0.001
Catalase	81.8	100	0.074	0.000-0.192	0.001
ORAC	70.0	36.4	0.791	0.580-1.000	0.024

AUC: Area under the ROC curve, CI: Confidence interval and p: Significance level

catalase (0.01 ± 0.0005 , 0.07 ± 0.02), also a significant decrease ($p < 0.001$) of serum GSH (2.595 ± 0.269 , 1.012 ± 0.231 nM g⁻¹ Hb) in patient group compared to control group. These results prove the close link between viral inflammation and oxidative stress, which in turn may be the main cause of complications in this disease.

Receiver operating characteristic analysis of oxidative stress parameters: Results of ROC curve analysis obtained in Table 5 and Fig. 2. Current results explained that serum MDA, serum SOD, serum GSH and serum catalase were significant predictive factors ($p < 0.05$) with an important percentage of sensitivity (63.3, 54.5, 45.5, 81.8 and 70%), respectively, with AUC value (0.00, 0.058, 0.901, 0.074

and 0.791), respectively and a high percentage of specificity in serum MDA, SOD and CAT (100%) and a lower percentage of specificity in serum GSH (9.1%). The results obtained from this test show that the parameters of oxidative stress, especially MDA, catalase and SOD, have the property of predicting the complications of the disease before they occur, which contributes to reducing the risk of the disease.

Correlation between oxidative stress and risk factors: The correlation results between oxidative stress parameters and biochemical risk factors markers for healthy and hepatitis patients are shown in Table 6 and 7. Concerning control group, we found just 1 positive correlation between MDA

Table 6: Correlation between serum non-enzymatic oxidative stress markers and biochemical risk factors in healthy and hepatitis patients

Risk factors	MDA		GSH	
	Control	Hepatitis	Control	Hepatitis
Aspartate aminotransferase	R = 0.429 P = 0.188	R = -0.162 P = 0.614	R = -0.368 P = 0.295	R = -0.417 P = 0.085
Alanine aminotransferase	R = 0.228 P = 0.500	R = -0.139 P = 0.651	R = 0.052 P = 0.886	R = -0.487 P = 0.041
Alkaline phosphatase	R = 0.669 P = 0.049	R = 0.408 P = 0.242	R = 0.071 P = 0.855	R = -0.023 P = 0.937
Uric acid	R = -0.060 P = 0.860	R = -0.597 P = 0.050	R = -0.252 P = 0.482	R = 0.751 P = 0.001
Total bilirubine	R = 0.130 P = 0.686	R = -0.133 P = 0.649	R = 0.130 P = 0.704	R = -0.053 P = 0.825
Direct bilirubine	R = -0.201 P = 0.554	R = 0.649 P = 0.774	R = 0.239 P = 0.506	R = 0.261 P = 0.266

Table 7: Correlation between serum enzymatic oxidative stress markers and biochemical risk factors in healthy and hepatitis patients

Risk factors	SOD		CAT	
	Control	Hepatitis	Control	Hepatitis
Aspartate aminotransferase	R = 0.125 P = 0.698	R = 0.413 P = 0.182	R = -0.291 P = 0.385	R = 0.354 P = 0.125
Alanine aminotransferase	R = -0.007 P = 0.982	R = -0.273 P = 0.391	R = 0.018 P = 0.957	R = -0.337 P = 0.147
Alkaline phosphatase	R = 0.349 P = 0.323	R = -0.548 P = 0.127	R = 0.680 P = 0.063	R = -0.705 P = 0.005
Uric acid	R = 0.234 P = 0.586	R = -0.197 P = 0.441	R = -0.188 P = 0.580	R = -0.259 P = 0.417
Total bilirubine	R = 0.070 P = 0.813	R = -0.097 P = 0.752	R = -0.085 P = 0.793	R = -0.085 P = 0.708
Direct bilirubine	R = -0.171 P = 0.577	R = -0.040 P = 0.896	R = -0.182 P = 0.593	R = -0.003 P = 0.989

and ALP activity ($R = 0,669$, $p < 0.05$). But in hepatitis group, found, a negative correlation between GSH/ALT ($R = -0.487$, $p < 0.01$), GSH/Uric acid ($R = 0.751$, $p = 0.001$) and CAT/ALP activity ($R = -0.705$, $p = 0.005$). As well, a negative correlation was found also between MDA/Uric acid ($R = -0.597$, $p < 0.05$), GSH/ALT activity ($R = -0.487$, $p = 0.041$). There was no correlation ($p > 0.05$) between the rest of the correlation tests in patient groups. This strong correlation that exists between some biochemical parameters and oxidative stress markers confirms that oxidative stress causes an imbalance in the functions of organs and thus contributes to changes in biochemical parameters.

DISCUSSION

Results of the current study illustrated a significant decrease of erythrocyte line parameters in hepatitis patients when compared with healthy individuals which don't correspond with the results of the study of Ekiz *et al.*¹⁹, they found that hemoglobin and hematocrit levels were similar in both groups. These decreased values can be explained by a

secondary complication, aplastic anaemia, which is characterized by failure of bone marrow induced after an attack of acute B hepatitis²⁰. Moreover, the leukocyte line parameters were significantly increased in the hepatitis patients as compared to the control. A high WBC count is a marker of inflammatory activity in the body²¹ and might be an indicator of sepsis occurring in patients with hepatitis B virus-related acute-on-chronic liver failure²². In particular, Interacting with virions or virus proteins, monocytes/macrophages play an important function in the disease process²³. Besides, neutrophils enhance antiviral defences and they exhibit both pathologic and protective functions²⁴. In the experimental study, the result shows a significant increase in PLT and MPV with a low significant decrease of PDW in hepatitis patients as compared to control. According to a previous study, increased levels of PLT may be due to sequestration of platelets in the spleen without obvious splenomegaly and portal hypertension²⁵. On the other hand, MPV is a marker of platelet function and activity and a novel index of inflammation and its intensity²⁶. In the current study, there is an increase in MDA levels in patients compared to

controls. This result is in agreement with the studies of Alavian and Showraki²⁷ and Sfarti *et al.*²⁸. Particularly, the levels of MDA were elevated in studied HBV patients, this indicates that HBV stimulates the generation of ROS. It had been reported that ROS act as second messenger molecules and key elements to activate the nuclear factor kappa beta (NFκβ) in the early events of inflammation. NFκβ activates the inducible nitric oxide synthase (iNOS) which produces a high amount of NO²⁹. Also, it reacts with O₂, producing peroxynitrite (ONOO), a powerful oxidant, which damage the biological molecules³⁰. The generation of O₂, NO and other ROS and Reactive Nitrogen Species (RNS) accelerate peroxidation of native membrane lipids leading to loss of membrane integrity, membrane damage and subsequent release of the cytosolic contents³¹. In this experimental study, the result shows that a significant decrease of reduced glutathione (GSH) in HBV patients as compared with the controls group. Glutathione's antioxidant system is imbalanced in damage to hepatocytes, so it decreases with disease severity³². The increased peroxidation contribute to disease progression by reduction of antioxidant activity that favours potentiates carcinogenesis viral replication³³. In addition, the availability of cysteine, the unstable precursor of GS, is a critical determinant of cellular GSH levels³⁴. Vairetti *et al.*³⁵ suggest that due to the disturbed hepatic functions during HBV infection, GSH synthesis is decreased. In a current experimental study, the result shows that a significant increase in CAT activity compared to control groups. It was reported that catalase effectively reduces HBx protein levels by reducing its stability and accelerating its degradation³⁶. A previous study found that oxidative stress induces the activation of c-Abl and Arg which join the Abl family of mammalian non-receptor tyrosine kinases³⁷. It was approved that, by association, c-Abl and Arg activate catalase by phosphorylation at both Tyr231 and Tyr386³⁸, which explain the increase of catalase activity during hepatitis B infection as observed in the current experience that there was an increase in the level of SOD in patients compared with controls. These results are in agreement with the studies realized by Liu *et al.*³⁹ and Mesuta *et al.*⁴⁰. Administration of specific antioxidants, such as superoxide dismutase (SOD), is effective in attenuating the inflammation and tissue damage observed in experimental models of hepatitis⁴¹. The most likely sources of these oxidizing agents are phagocytic leukocytes infiltrating tissues. Activation of macrophages by interaction with some pro-inflammatory media or a specific bacterial product of specific receptors on the plasma membrane of leukocytes leads to the assembly of the flavoprotein NADPH oxidase, which catalyzes the production of large amounts of the superoxide fraction⁴².

CONCLUSION

Current results indicated oxidative stress associated with the HBV infection which contributes to the imbalance of antioxidant defence system and overexpression of free radicals and leads to cell membrane alteration and disease progression. Therefore, serum MDA, CAT, SOD and GSH activity are shown to be predictive and new reliable markers for hepatitis B suggested inserting these markers into the analytical diagnostic list for prediction of hepatitis B.

SIGNIFICANCE STATEMENT

This study explored the relationship between the complications of viral hepatitis and oxidative stress. This study also discovered that can predict the complications of viral hepatitis utilizing some biochemical parameters and from it can avoid these complications to preserve the lives of patients.

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