



EVALUATION OF SERUM ANTIOXIDANT CAPACITY AND LIVER FUNCTIONS IN HEPATITIS B AND C VIRAL INFECTIONS

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ABSTRACT

OBJECTIVE: To investigate the serum antioxidant capacity and status of liver function parameters in Hepatitis B virus (HBV) and Hepatitis C Virus (HCV) infected patients.

METHODS: In this case-control study, fifty patients with HBV (28 male & 22 females), fifty HCV patients (28 male & 22 females) and age and gender matched fifty healthy controls were enrolled from May to August 2016. The antioxidant status was determined by measuring trolox equivalent oxidant capacity (TEAC) through the Ferric Reducing Ability of Plasma assay. Serum levels of alkaline phosphates (ALP), Alanine amino transferase (ALT) and Albumin were estimated.

RESULTS: Serum ALT levels (U/L) were 119.69 ± 5.03 & 24.3 ± 3.01 in male HBV patients and controls; 176.34 ± 75.48 and 25 ± 3.01 in female HBV patients & Controls; 114.71 ± 5.03 and 22.61 ± 2.33 in male HCV patients and controls and 165 ± 5.03 & 25 ± 3.01 in female HCV patients & Controls respectively. Serum ALP levels (U/L) were 317.69 ± 5.13 , 240.17 ± 23.9 in male HBV & controls, 159.34 ± 5.03 & 138.4 ± 3 in female HBV & Controls; 129.28 ± 4.57 , 230.47 ± 33.5 in male HCV & controls and 169 ± 3.65 and 165.10 ± 5.70 in female HCV & Controls respectively ($p < 0.01$). Serum TEAC ($\mu\text{g}/\text{mL}$) were 266.1 ± 13.71 , 413 ± 18.21 , 214.42 ± 11.1 , 395.71 ± 16.33 in male HBV & control and female HBV & control respectively as compared to 264.6 ± 14.55 , 409.8 ± 17.54 , 273.4 ± 13.84 and 395.5 ± 14.23 in male HCV & control and female HCV & control respectively ($p < 0.01$).

CONCLUSION: A significantly high HBV & HCV induced oxidative stress and deranged liver parameters were observed in male and female patients, suggesting a possible association between viral pathogenesis and level of oxidative stress.

KEYWORDS: Hepatitis B (MeSH); Hepatitis B virus (MeSH); Oxidative Stress (MeSH); Hepatitis C (MeSH); Hepacivirus (MeSH); Hepatitis B Surface Antigens (MeSH); Antioxidant Capacity (Non-MeSH); Trolox equivalent oxidant capacity (TEAC) (Non-MeSH); Ferric Reducing Ability of Plasma (Non-MeSH); Alkaline phosphates (Non-MeSH); Alanine amino transferase (Non-MeSH); Albumins (MeSH).

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INTRODUCTION

The hepatitis B & C viruses are significant pathogens which can cause fibrosis, cirrhosis, hepatocellular cancer through liver cells damages. It is reported that 15-40% of hepatitis B virus (HBV) & hepatitis C virus (HCV) infections lead to cirrhosis and hepatocellular carcinoma.¹ HCV infections lead to metabolic dysfunctions and generate reactive oxygen species (ROS) in the body to produce high oxidative stress that cause

pathogenesis.² Hepatitis viruses are primary in ROS production that may lead to oxidative damages of biomolecules such as lipids, proteins, and deoxyribonucleic acid (DNA).³ The deficiency of antioxidants leads increase in reactive oxygen species concentration which causes significant destruction in DNA, ribonucleic acid (RNA) and other biomolecules to produce disorders in the body.⁴ The synthesis of ROS potentiated by hepatitis viruses particularly HCV,

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induce oxidative stress and promotes hepatic and extrahepatic complications.⁵ The improved cellular redox status affects the viral activity. The redox balance disturbance lead to the clinically latent parasitic virus activation, so a wide variation is found in the incubation periods of HBV and HCV infection. In severe oxidative stress condition, the excessive generation of ROS damages to cell. Oxidative stress is a basic outcome of various conditions like autoimmune, viral and alcoholic hepatitis.⁶ The cellular antioxidant defense mechanism become poor as reactive oxygen and nitrogen species production enhanced and promotes the complication associated with HBV and HCV.⁷ The blood-to-blood contact is considered as a primarily source of spreading HCV. Several studies have reported that ROS levels are significantly elevated in HCV replicating cells, such as liver tissue and in lymphocytes. Liver cells containing antioxidant, plays a vital function in phase II metabolism and protect from viruses induced damages. In liver diseases, ROS and toxic degradation products generated in the body either by biological agent (HBV, HCV) or chemical agent causes significant Glutathione depletion.⁸ The elevation of oxidative stress is also responsible for irreversible alteration in proteins, lipids and DNA contents. ROS induce causes alteration in many pathways that regulates genetic transcription, translation, apoptosis, and stellate cell activation.⁹

During oxidative stress, the processes of transcription and translation increases in mitochondria and electron transport chain is blocked which causes the release of Tumor Necrosis Factor- α (TNF- α) by liver parenchymal cells that destroy the mitochondrial cytochrome oxidase. ROS consume antioxidant enzymes and increases the deposition of oxidative lipids to enhance the lipid peroxidation and create vicious circle.¹⁰ Previously, in-vivo investigative studies suggested that exact quantification of the presence of free radicals in the body is not possible because they have very short life time. Therefore, they recommended indirect estimation of ROS by evaluating the serum antioxidant capacity by specific assays.¹¹

HBV or HCV infection increased oxidative stress by lowering superoxide dismutase and catalase activity; however, only children were involved in the study, and did not examine the impact of HCV or HBV upon the variation of other non-enzymatic antioxidants. Total antioxidant capacity was not assessed by FRAP which is given as a novel tool for testing "antioxidant power."^{17,12}

Virally induced liver cancer has been associated with the generation of oxidative microenvironment that eventually leads to oncogenic mutations in cell cellular signaling. In the liver, neoplastic transformation may also induce by oxidative stress generated by hepatitis viruses, therefore in the current study; we intended to evaluate the HBV & HCV-induced oxidative stress in patients and its effects on serum liver parameters including serum Alanine amino transferase (ALT), alkaline phosphatase (ALP), Albumin & bilirubin.

METHODS

This prospective study was conducted on 100 diagnosed patients of HBV (n=50; 28 males and 22 females) and HCV (n=50; 28; males and 22 females) infection. Patients were enrolled in the study after taking inform consent from each patient. The study was performed in the Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan, from February 2016 to October 2016. Ethical approval

was obtained from intuitional ethical review board of the Gomal University, Dera Ismail Khan.

Diagnosis of hepatitis B virus was based a positive hepatitis B surface antigen (HBsAg) test using immune-chromatographic screening method (Rapid test strip) and anti-HCV antibodies (ELISA second generation Ortho Diagnostic Systems, Raritan, NJ) was performed to confirm HCV infection. The patients with other chronic or autoimmune liver diseases were excluded from the study. The specific codes were used to label the samples to hide the identity of subjects. The control group comprised of 50 healthy individuals (28 males and 22 females) and they were selected on the basis of general physical examination. They showed no abnormal laboratory findings including liver function tests i.e., serum ALP, serum ALT, albumin and bilirubin. They were seronegative for HCV, HBV, human immunodeficiency virus (HIV), HBsAg, anti-HBc total, and anti-HCV tests. No previous history of hepatitis and/or chronic alcoholism was evident.

Exclusion criteria included patients with the history of coronary artery disease, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, systemic or local infection, corticosteroid usage, smoking, alcohol intake, rheumatoid arthritis, cancer and pregnancy.

Liver functions tests of hepatitis B & C patients and control group was determined by measuring serum level of alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Albumin (ALB) through spectrophotometric method at λ_{max} 400 nm, 365 nm and 600 nm. The serum level of bilirubin was determined by calorimetric method. λ_{max} 400nm, 365 nm and 600 nm. The serum level of bilirubin was determined by calorimetric method.¹³

The total antioxidant capacity was determined by using Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1999).⁷ This method was based on the reduction of ferric iron (Fe³⁺) and 2, 3, 5-triphenyl-1, 3, 4-triazole-2-azoniacyclopenta-1,4-diene chloride to ferrous (Fe²⁺) ion at low pH,

which leads to the formation of blue colored ferrous tripyridyltriazine (Fe²⁺ - TPTZ) complex, that is monitored by change in absorbs at 593 nm through spectrophotometer. FRAP reagent was prepared by mixing 300mM acetate buffer (pH 3.6), TPTZ 10mM in 40mM HCl and 20mM FeCl₃.6H₂O in ratio 10:1:1. The FRAP reagent (3ml) was mixed with serum sample (100 μ l) and absorbance was measured at 0 minute at 593nm. After vortexing, the samples were placed at water bath (37°C) for 4 minutes and absorbance was again measured. The change in absorbance selected for calculation of FRAP values by using equation. The trolox calibration curve was prepared to obtain FRAP values, and expressed as micromole of trolox equivalent per ml of sample.⁷

Results were forced under statistical parameters to further strengthen the validity and expressed as means and standard deviations (SD). The descriptive and inferential significance is observed through student t-statistics (t-Test for two sided comparison). For statistical analysis, SPSS version 20 (IBM, USA) was used. P value less than 0.05 was considered significant.

RESULTS

In this study, there was no statistically significant difference in mean ages and gender distribution of HBV patients, HCV patients and their control group was observed [Table I&II].

The study results revealed the serum ALP levels in HBV male patients (317.69 \pm 5.13 U/L) were significantly elevated as compare to control groups (240.17 \pm 23.9 U/L), whereas, serum ALP levels in HBV female patients (159.34 \pm 503 U/L) were found to be significant high as compared to their healthy female control (138.4 \pm 3 U/L) group [Table I].

Similarly, serum levels of ALT in HBV infected male (119.69 \pm 5.03 U/L) and female (176.34 \pm 75.48 U/L) patients were elevated significantly in comparison to their control groups (24.3 \pm 3.01 U/L & 25 \pm 3.01 U/L) respectively [Table I].

The study results also showed the significant reduction in serum ALB level

TABLE I: COMPARISON OF ANTIOXIDANT AND OTHER PARAMETERS CALCULATED FOR HEPATITIS B POSITIVE INDIVIDUALS AND CONTROLS

Variables	Males		P Value	Females		P Value
	HBV +ve (Patients)	HBV -ve (Controls)		HBV +ve (Patients)	HBV -ve (Controls)	
Age (years)	48.2±6.57	48.8±6.07	NS	45.63±8.05	45.43±8.04	NS
Serum Bilirubin (mg/dl)	1.07±0.76	0.5±0.30	*	1.06±0.74	0.45±0.14	*
Alanine Amino Transferase (U/L)	119.69±5.03	24.3±3.01	**	176.34±75.48	25±3.01	**
Alkaline Phosphatase (U/L)	317.69±5.13	240.17±23.9	**	159.34±5.03	138.4±3	*
Albumen (mg/dl)	3.5±0.72	4.1±0.62	**	3.23±0.69	4.12±0.75	**
Trolox Equivalent Antioxidant Capacity (µg/mL)	266.1±13.71	413±18.21	**	214.42±11.1	395.71±16.33	**

NS non-significant; * p-value <0.05, **p-value <0.001, Values expressed as Mean±Standard Deviation

of HBV infected male and female in comparison to their respective control male and female groups. The serum bilirubin levels in HBV infected male and female were found to be high as compare to their respective control male and female group [Table I].

Serum albumin in HCV infected patients were decline, significantly in comparison to their respective gender matched control group [Table II].

Serum ALT levels in HCV infected male (114.71±5.03 U/L) and female (165±5.03 U/L) were elevated significantly in comparison to their health matched control male (22.61±2.33 U/L) and female (25±3.01U/L) patients respectively [Table II].

Whereas, serum bilirubin levels in HCV infected male and female patients were raised significantly as compared to healthy control males and female [Table II].

The total antioxidant capacity of HBV, HCV infected male and female patients and their control group were determined by using trolox equivalent antioxidant capacity (TEAC) by FRAP assay and results revealed significantly reduction in the (TEAC)of HBV male& female patients as compared to their control male and female group [Table I].

The TEAC in HCV male and HCV female patients was significantly low as compared to their healthy controls [Table II].

Comparison between LFTs in Hepatitis B & C infection: During current study, LFT results of Hepatitis B & C patient has revealed significantly high serum ALP and ALT level found in HBV male patients in comparison to HCV male patients. HBV male patients showed high serum albumin level as compared to HCV male patients, while HBV and HCV female patients showed no significant difference in serum albumin level. The almost similar serum bilirubin level showed by both Hepatitis B & C infected patients [Table III].

In our study, LFT results of Hepatitis B & C patient's varied among male and female patients. Serum ALT levels were higher in female HBV (176.34±75.48 U/L) & HCV (165±5.03 U/L) patients as

TABLE II: COMPARISON OF ANTIOXIDANT AND OTHER PARAMETERS CALCULATED FOR HEPATITIS C POSITIVE INDIVIDUALS AND CONTROLS

Variables	Males		P Value	Females		P Value
	HCV +ve (Patients)	HCV -ve (Controls)		HCV +ve (Patients)	HCV -ve (Controls)	
Age (years)	46.71±7.23	46.57±7.08	NS	42.6±5.93	42.42±5.71	NS
Serum Bilirubin (mg/dl)	1.07±0.76	0.519±0.15	**	1.08±0.67	0.45±0.14	**
Alanine Amino Transferase (U/L)	114.71±5.03	22.61±2.33	**	165±5.03	25±3.01	**
Alkaline Phosphatase (U/L)	129.28±4.57	230.47±33.51	**	169±3.65	165.10±5.70	*
Albumen (mg/dl)	3.1±0.84	4.08±0.66	*	3.26±0.62	5.3±0.83	*
Trolox Equivalent Antioxidant Capacity (µg/mL)	264.6±14.55	409.8±17.54	**	273.4±13.84	395.5±14.23	**

NS non-significant; * p-value <0.05, **p-value <0.001, Values expressed as Mean±Standard Deviation

TABLE III: COMPARISON OF ANTIOXIDANT AND OTHER PARAMETERS AMONG MALE AND FEMALE HEPATITIS B & C POSITIVE PATIENTS

Variables	Males		Females		P Value
	HBV +ve	HCV +ve	HBV +ve	HCV +ve	
Age (years)	48.2±6.57	46.71±7.23	45.63±8.05	42.6±5.93	NS
Serum Bilirubin (mg/dl)	1.07±0.76	1.07±0.76	1.06±0.74	1.08±0.67	NS
Alanine Amino Transferase (U/L)	119.69±5.03	114.71±5.03	176.34±75.48	165±5.03	**
Alkaline Phosphatase (U/L)	317.69±5.13	129.28±4.57	159.34±5.03	169±3.65	NS
Albumen (mg/dl)	3.5±0.72	3.1±0.84	3.23±0.69	3.26±0.62	NS
Trolox Equivalent Antioxidant Capacity (µg/mL)	266.1±13.71	264.6±14.55	214.42±11.1	273.4±13.84	**

NS non-significant; * p-value <0.05, **p-value <0.001, Values expressed as Mean±Standard Deviation

compared to male HBV (119.69±5.03U/L) & HCV (114.71±5.03U/L) patients (p <0.0001). TEAC levels in male patients of HBV (266.1±13.71 U/L) & HCV (264.6±14.55 U/L) were almost similar, however, in female patients, levels were high (273.4±13.84 U/L) in HCV patients as compared to HBV patients (214.42±11.41 U/L). [Table III].

DISCUSSION

Normally, there are various causes of liver diseases, including hepatitis B and C viral infections. These infections are the most prevalent healthcare problem especially in under developing countries. According to the WHO (World Health Organization), more than 2 billion people are infecting from HBV and 58 million people infecting from HCV infections annually.¹⁴ Oxidative stress normally generated under normal metabolism in the living organisms, but, excessive production of oxidative stress is characterized increase in generation of ROS and a loss of equilibrium between enzymatic and non-enzymatic antioxidants present in the body. The hepatitis C virus stimulates Reactive oxygen species production in infected cells leads to generation of oxidative stress. Oxidative stress results in disturbance between the ROS production and antioxidant in cell.¹⁵

The present study evaluated both, serum levels of LFT and oxidative status in individuals affected from HBV and HCV infection and their healthy

control group. We found significant increased liver enzymes and oxidative stress levels, which indicating the decline in enzymatic and non-enzymatic antioxidant status in the body. Similarly, the previous studies on hepatitis viral infections have reported the elevation in oxidative stress, by observing the markedly changed in serum catalase, lipid peroxidation and GSH in hepatitis virus infected patients.¹⁶ The hepatic intracellular injury causes the markers like serum amino transferases to release into the systemic circulation.¹⁷

In this study, no remarkable difference was observed in levels of liver enzymes and oxidative stress, there was a linear correlation ship between increases in LFT parameters and oxidative stress in HBV and HCV infected patients, but we found in HCV patients, serum ALT levels were elevated and TEAC of were more decline than HBV patients and control group, which indicating the depletion of endogenous antioxidant components such as catalases, glutathione and uric acid. The notable increase in serum ALT, bilirubin level and decline in albumin level in Hepatitis C viral infected individuals has been reported¹⁸ suggesting that serum albumin and uric acid possess more prominent free radical scavenging activity than other antioxidants present in the body.

They also reported that reduced serum albumin levels in liver cirrhosis leads to increase in the severity of liver disease such as hepatitis viral

infections.¹⁹ In our study data, serum albumin levels were found to be decline in hepatitis viral infections particularly in HCV infection. The significant decline in TEAC in HCV patients may be related with decline in albumin level. Similarly, previous studies findings have also confirmed the reduced antioxidant status in hepatitis B & C virus infected patient.²⁰ The previous studies finding further reported that oxidative stress play an important role in the diseases and induction of HCV-induced inflammation. They suggested the use of antioxidants in HCV infected patients treated with the interferon to get better outcomes. They demonstrated that the use of Vitamin-E in HCV patients have 2.4 times more chance of reducing viral load than patients not given vitamin E.²¹

CONCLUSION

In our, liver function tests and FRAP assay was performed to evaluate the status of liver health and serum antioxidant capacity. Study results revealed a significantly high HBV & HCV induced oxidative stress and deranged liver parameters in male and female patients, suggesting a possible association between viral pathogenesis and level of oxidative stress. Both, HBV and HCV infected patients showed almost similar levels of oxidative stress. Antioxidant supplement use in these patients may be more beneficial for better anti-viral therapy outcomes to treat HBV and HCV infection.

However, further investigations are needed to identify best antioxidant for cure of Hepatitis infection.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

GM: Conception & study design, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

ZS: Analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

MA: Acquisition of data, drafting the manuscript, approval of the final version to be published

KR: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request



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