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Investigating the Role of Stress Enzymes in the Pathogenesis and Progression of Hepatitis B and C

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Abstract

Hepatitis is the most common disease all over the world This review examines the epidemiology, transmission, and the critical role of oxidative stress and related enzymes in the pathogenesis of these diseases. Hepatitis B, which infects an estimated 350 million people worldwide, is primarily transmitted through blood, sexual contact, and vertical transmission from mother to child. The virus's resilience in external environments exacerbates its transmission risk. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, plays a pivotal role in liver damage associated with these infections. Enzymes such as cytochrome P450 and glutathione S-transferases (GSTs) are integral to the detoxification of harmful substances. However, variations in these enzymes' activity can influence individual susceptibility to drug toxicity and disease progression. This review highlights the significance of understanding these enzymatic processes for better management and treatment of Hepatitis B and C, emphasizing the need for continued research into oxidative stress and its role in liver pathology.
Keywords: HCV, HBV, Stress Enzyme, P450

INTRODUCTION

Hepatitis is the most common disease all over the world. There are different types of hepatitis i.e., hepatitis B, C, D, E, and F but hepatitis B and C are the most common. It is estimated that 350 million people are infected with hepatitis B all over the world (Lavanchy, 2004). There are roughly 1.25 million hepatitis B carriers which are positive for hepatitis B surface antigen (HBsAg) (Mast et al., 2005; Mcquillan et al., 1999). The carriers of HBV have huge danger of increasing cirrhosis, hepatic breakdown, and hepatocellular carcinoma (HCC) (Beasley, 1956). Only 15% to 40% carriers develop serious infection during their lifetime

(Bosch et al., 2005). Proper treatment and management for hepatitis B is required in patients waiting for liver transplantation and post liver transplant cases (Terrault et al., 2005).

HBV is transmitted by injection in skin and veins, and sexual exposure. (Mastee et al., 2005). The virus of Hepatitis B can remain alive in external environment for long time (Bond et al., 1981; Petersen et al., 1976). The new born and infants of hepatitis B mother are at 90% and 25-30% risk of getting HBV infection respectively while adult progeny are 5% susceptible to HBV infection (Beasley et al.,1982; Beasley et al., 1983; Coursaget et al.,1987; Mcmahonbj et al., 1985; Tassopoulos et al., 1987). The person whose immune system is not properly workin has greater chance to develop HBV after a mild HBV infection (Bodsworth & Raffanti, 1989; Horvath et al., 1994). All age groups are vaccinated in United States that's why the chances of occurring hepatitis B are so less (Chevillotte, 1983; Villaerl et al., 1982). According to the research conducted in 1999 chronic hepatitis, HBsAg, hepatitis anti-HBc, and hepatitis C antibody (anti-HCV) were positive in 56.2%, 70%, and 40.7% cases, respectively (Bagheri et al., 1999). The occurance of antibody to HCV (anti-HCV) in patients of chronic liver disease (CLD) in Saudi Arabia was 30.4% (Al-Karawi et al., 1992). In Pakistani patients with chronic liver disease and hepatocellular carcinoma, 24% of the patients had HCV infection (Tong et al., 1996). In dialysis patients the occurrence of HCV infectivity in north-western Europe is 3% (Schneeberger et al., 2000) and 76% in Asia Pakistan (Soetjipto *et al.*, 1996).

It was found by enzyme linked immune sorbent assay (ELISA) test that 45% HCV patients were intravenous drug abusers while 12% were affected by jaundice previously. It is found that 54% of intravenous drug abusers (IDUs) with positive HCV have the history of blood donation (Zali et al., 2001) HCV core protein alters mitochondrial function and engages in oxidative stress. A bit of the expressed HCV core protein on the mitochondria which rearrange the cytochrome c from mitochondrial to cytosolic fractions and increases ROS resulting to increase the oxidative stress. HCV core expression equally increased ROS and lipid per oxidation products and it also stimulate antioxidant gene expression. A mitochondrial electron transport inhibitor prevents the core-induced increase in ROS in this way oxidative stress can be decreased by antioxidant enzymes (Okuda et al., 2002). Numerous enzymatic and antioxidant activities present inside the body which function as a protector so, when oxidative stress occur due to pathogenic activity then these enzymes express and regulate the homeostasis of the body (Mates et al., 1999). The enzymes which are involved in phase I transformation reactions are collectively called the cytochrome P450

(CYP). Cytochrome P450 enzymes are comparatively non-specific to identify and can be change numerous different toxins. Cytochromes of humans have the ability to detoxify all the foreign materials of body (Redlich et al., 2008). It metabolizes toxins very slowly as compared to other enzymes (Dai, 2001). CYPs are the 5% of total liver proteins. CYPs are amongst the most well studied proteins due to their critical and major role in detoxification and metabolism (Lardone et al., 2009). Cytochrome p450 is a type of monooxygenases. Monooxygenases are the enzymes that add one hydroxyl group into substrates in many metabolic pathways. (Harayama et al., 1998; Schreuder et al., 1999). The persons with alcoholic liver disease have higher monooxygenase and reductase value than control group (Hoensch et al., 1979). Due to multiple form of haematoprotein cytochrome P450 drug responses vary from individual to individual (Comai & Gaylor, 1973).

The hepatic monooxygenases consist of a chain of cytochrome P450-dependent enzyme system and their main function is the metabolism of large amount of exogenous and endogenous compounds. Cytochromes P450 play important role in detoxification of foreign substances (Ahmad et al., 2024). However, these enzymes can also activate those compounds which can cause toxicity and also alter the cells which result in cancer (Gonzalez, 2005). The human cytochrome P450 enzyme system metabolizes the broad group of xenobiotics. Devastation of cytochrome P450 activity may lead to toxicity. Difference in the regulation and expression of the drug metabolizing enzymes may play a key role in both inter individual difference in sensitivity to drug toxicity and tissue-specific injury (Park et al., 1995). Glutathione-s-transferases (GSTs) also known as antioxidant enzymes, it detoxifies the free radicals and oxidized lipids or DNA (Sheehan et al., 2001; Ketterer, 1998). Glutathione S-transferases can als catalyze the transfer of glutathione to phase I products. The main function of GSTs is the metabolism of several endobiotic, (van, 2009). GSTs are soluble enzymes and in humans, and comprises the 4% of the soluble protein in the human liver and present in several other tissues (Hayes & Strange, 2000; van, 2000). Esterases also perform a role in detoxification of xenobiotics (Shen & Dowd, 1991; Sattar et al., 2024). The estimation of plasma estrases is the most accurate among all the liver proteins. Nonspecific esterases (NSEs) are widely distributed in many types of cells. They are demonstrated and assayed by the cleavage of a short-chain acyl group from a chromogenic leaving group. Their actual function is poorly understood, however, although a list of liver esterase substrates, carboxylic acid esters, has been assembled (Wynne et al., 1973). cytochemistry is used for the

differentiation of leukocytes estrases and its granular activities (Bozdech & Bainton, 1981; Monahan et al., 1981; Grossi et al., 1982; Monahan et al., 1981).

The objective of our study is to examine the correlation between stress enzyme activity levels and the severity of liver damage in patients with Hepatitis B and C. This objective aims to provide insights into the potential involvement of stress enzymes in the progression of these viral infections and their impact on liver health. By understanding this relationship, the research seeks to contribute to the development of targeted interventions for managing Hepatitis B and C.

MATERIALS AND METHODS

Population studied

All procedures were in agreement with the declaration of Helsiki. Protocol of present study was approved by the Advance Study and Research Board, University of Sargodha. Ethical Committee, University of Sargodha granted permission for the start of work. Subjects of research included three groups. First group called as Hepatitis B group called as hepatitis B patients and the second is Hepatitis C group called as hepatitis C patients and the third is control group included age and sex matched healthy individuals. Study included 300 subjects in total, out of which 100 were hepatitis B patients and 100 were hepatitis C patient and 100 were healthy individuals.

Sample collection:

Samples were collected from September 2014 to February 2015. Individuals involved in sample collection were introduced about the research and ethical criteria were fulfilled. Hepatitis B and C patients were confirmed by Hepatitis test and healthy individuals were selected for the control group sampling. Healthy patients are also screened for diabetes and hypertension. Sterilized syringes (BD, USA) were used to puncture cubital vein for drawing blood. 5cc blood was taken from each individual and blood samples were shifted to a plane tube (BD, USA). Before further analysis, the serum was separated from all samples by centrifugation and the serums were kept at -18°C for further study.

Questionnaire for data collection:

A Form was designed to keep records of name, age, gender, smoking, hypertension, diabetes and hepatitis status and type of hepatitis etc related to individual.

Data collection performa

Sr. No:_____Name:_____Age:_____ Gender:_____Type of Hepatitis: _____		
Status	Yes	No
Hypertension		
Diabetes		

Hypertensive individuals were defined on the basis of systolic and diastolic blood pressure. These readings were taken the experts hired by the hospitals and laboratories from where samples were collected. Having more than 140 mmHg systolic blood pressure and more than 90 mmHg diastolic blood pressure was taken as criteria for hypertension patients. Peoples were considered as diabetic on the basis of their blood sugar level. If blood sugar level was higher than 120 mg/dl (fasting), individuals were considered as diabetic.

Enzyme preparation

Estimation of detoxifying enzymes, serum was separated from the blood by the centrifugation process. For separating serum from the blood sterile plane tube was used without any anticoagulant, after taking sample, leave the tube in a standing position for about 20-30 minutes (it can take shorter time than this so check it periodically) after that blood is clotted , centrifuge it at 20C degree , 1500g for 10 minutes then remove the serum very quickly and flash freeze it in -18 C to preserve for next assay. This serum was used as enzyme source for total protein estimation, biochemical estimation of non-specific esterases (α and β esterases), Glutathione-S-transferases (GSTs) and Monooxygenases.

Biochemical estimation of detoxifying enzymes

To measure the activity of non-specific esterase's method of Barker et al (1998) was followed. Alpha naphthyl acetate (Substrate A) and Beta naphthyl acetate (Substrate B) were used as substrates. Optical density of reference was subtracted from the optical density of solution containing serum. The resulting optical densities (OD) were compared with standard curves to convert the absorbance to product concentrations. The enzyme activities were expressed as mM of product formed/min/mg of protein.

Biochemical estimation of monooxygenases

The activity of monooxygenases was determined by following the method described by Vulule et al (1999). Reaction mixture comprised of 20 µl of homogenate (serum), 200 µl of 3,3, 5',5'- Tetramethyl benzidine (TMBZ) solution. Reference solution for reaction mixture

contained 200 µl TMBZ, 120 µl 0.625 M potassium phosphate buffer (PPB) at pH 7.0 and 30 µl 3% hydrogen peroxide. After ten minutes readings were recorded at the wavelength of 620 nm. The quantity of monooxygenases was calculated from a standard curve of cytochrome C.

Total protein assay

Estimation of the total protein contents was done by using dye binding method of Bradford (1976). The reaction mixture contained 10 µl of serum, 90 µl phosphate buffer (100 mM, pH 7.0) and 100 µl of Bradford dye reagent and mixed by shaking and incubated for 15 minutes. When incubation was over solutions were kept for five minutes to develop color then absorbance was recorded at the wavelength of 595 nm on micro plate reader (Thermo scientific Multiskan FC Microplate photometer). The amount of absorbance observed was proportional to the amount of protein present. By using the standard curve of Bovine Serum Albumin (BSA) the amount of protein in the sample was estimated.

Biochemical estimation of glutathione-s-transferases

The activity of Glutathione S-trasferases towards 1-chloro-2,4-dinitrobenezene (CDNB) was estimated according to the method of Habig et al (1974). The total volume of the mixture was comprised of 40 µl 1.0 mM reduced Glutathione, 20 µl 1.0 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 200 µl phosphate buffer (100 mM, PH 7.0) and 20 µl of serum. Reference solution for reaction mixture contained 20 µl 1.0mM CDNB, 200 µl phosphate buffers (100 mM, PH 7.0) and 40 µl 1.0 mM reduced Glutathione. The absorbance was measured at 340 nm after five minutes of the reaction. Absorbance values were converted to units of concentration using a molar extinction coefficient (ε) of 9.6 mM cm^{-1} for CDNB-GSH conjugate.

Statistical analysis

For comparison of activity of different enzymes in males and females of hepatitis B and C Sample Man-whitney test was applied by using Minitab Software (version 14.1).

RESULTS AND DISCUSSION

This study measured serum level of Total protein content, monooxygenases, glutathione-s-transferases, and non specific β estrases in patients suffering from hepatitis B and C and healthy volunteers by using Enzyme linked immune sorbent assay (ELIZA).

Biochemical estimation of total proteins (HCV male)

In HCV male hepatitis C patients and healthy individuals, the level of total protein was 289.0mg/ml and 257.7mg/ml, respectively. The amount of total protein in hepatitis B and C

individuals was non significantly higher as compared with the control groups (*U*= 2563.0; P =0.2970) (Table 3.1).

TABLE 3.1:

Level of total proteins (mg/ml) in control group and HCV male patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	28	257.7	31	2563	0.297
HCV patients	57	289	21.9		

HCV female patient

The level of total proteins were 115.7mg/ml and.175.7mg/ml in the healthy individuals group and HCV female hepatitis C patients, respectively. The amount of total protein in diseased individuals was significantly higher compared to the control (*U*= 1011.5; P = 00254) (Table 3.2).

TABLE 3.2:

Activity of total protein (mg/ml) in control and HCV female patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	35	115.7	17.7	1011.5	0.0254
HCV Patients	32	175.7	19.6		

HBV male patients

The total protein contents in HBV males were 278.9mg/ml and in control group was 266.1mg/ml. The amount of total proteins in diseased individuals was non significantly higher as compared with the control group (*U*= 1317.0; P =0.8960) (Table 3.3).

TABLE 3.3:

Level of total proteins (mg/ml) in control group and HBV male patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	27	266.1	29.4	1317	0.896
HCV male patients	39	278.9	13.1		

HBV female patients

The total proteins were 269.2mg/ml and 130.6mg/ml in HBV female and control group respectively. The amount of total protein in hepatitis B female patients were significantly higher compared to the control group (*U*= 1237.0; P =0.000) (Table 3.4).

TABLE 3.4:

Activity of total protein (mg/ml) in control group and HBV female patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	31	130.6	18.4	1237.0	0.0000
HBV patients	30	269.2	17.9		

Biochemical estimation of monooxygenases

The activity of monooxygenases in HCV male patients and control group individuals was 22.28μg/min/mg and 25.93μg/min/mg, respectively. Statistically the difference was significant (U =1458.0; P=0.0083); (Table 3.5).

TABLE 3.5:

*Activity of monooxygenases (*μg/min/mg*) in control group and HCV male patients.*

Group studied	Number	Mean	S.E	U value	P-value
Control goup	29	25.93	1.36	1458	0.0083
HCV patients	45	22.88	1.34		

HCV female patients

The activity of monooxygenases in control group was 60.33 µg/min/mg and in HCV female patients its value was 59.04 µg/min/mg. statistically the difference was not significant (U =1254.0; P=0.3767); (Table 3.6)

TABLE 3.6:

*Activity of monooxygenases (*µg/min/mg*) in control group and HCV female patients*

Group studied	Number	Mean	S.E	U value	P-value
Control group	25	60.33	4.74	1254	0.3767
HCV patients	40	59.04	3.24		

HBV male patients

In HBV male patients and control group the activity of monooxygenases was 28.32 µg/min/mg and 27.68µg/min/mg respectively. Statistically the difference was not significant (U =2478.5; P=0.1730); (Table 3.7).

TABLE 3.7:

*Activity of monooxygenases (*µg/min/mg*) in control group and HBV male patients*

Group studied	Number	Mean	S.E	U value	P-value
Control group	29	28.32	1.06	2478.5	0.1730
HBV patients	55	27.68	1.21		

HBV female patients

The activity of monooxygenases in HBV female patients weres 60.33µg/min/mg and 39.93µg/min/mg in control and HBV females group, respectively. Statistically the difference was significant (U =690.0; P=0.0015); (Table 3.8).

TABLE 3.8:

*Activity of monooxygenases (*µg/min/mg*) in control group and HBV female patients.*

Group studied	Number	Mean	S.E	U value	P-value
Control group	25	60.33	4.70	690.0	0.0014
HBV patients	31	39.93	4.10		

Biochemical estimation of Glutathione-S-transferases

The level of Glutathione-S-transferases was 0.627 *nM/mg protein/min* and 0.860 *nM/mg protein/min* in the control and HCV male patients, respectively. Statistical analysis shows that the level of Glutathione-S-transferases between two groups differed significantly (U =1456.5; P =0.0018); (Table 3.9).

TABLE 3.9:

Activity *of glutathione-s-transferases (nM/mg protein/min) in control and HCV male patients.*

Group studied	Number	Mean	S.E	U value	P-value
Control group	28	0.627	0.248	1456.5	0.0018
HCV patients	37	0.860	0.113		

HCV female patients

The levels of Glutathione-S-transferases were 0.815 *nM/mg protein/min* and 3.55 *nM/mg protein/min* in the control group and HCV female patients, respectively. Statistically the level of Glutathione-S-transferases between two groups differed significantly (U =797.0; P =0.0220); (Table 3.10).

TABLE 3.10:

Activity *of glutathione-s-transferases (nM/mg protein/min) in control group and HCV females' patients*

Group studied	Number	Mean	S.E	U value	P-value
Control group	32	0.815	0.202	797.0	0.0220
HCV patients	26	3.55	1.36		

HBV male patients

The value of Glutathione-S-transferases was 0.2212 nM/mg protein/min and 1.391 nM/mg protein/min in the control group and HBV male patients, respectively. Statistically the level of Glutathione-S-transferases between two groups differed significantly (U =997.0; P =0.0031) (Table 3.11).

TABLE 3.11:

Activity *of glutathione-s-transferases (nM/mg protein/min) in control group and HBV male patients.*

Group studied	Number	Mean	S.E	U value	P-value
Control group	37	0.2212	0.0330	997.0	0.0031
HBV patients	27	1.391	0.313		

HBV female patients

The value of Glutathione-S-transferases in HBV female patients was 0.3238 nM/mg protein/min and 0.815 nM/mg protein/min in the control group. The level of Glutathione-S-transferases between two groups was not different significantly (U =738; P =0.2767); (Table 3.12).

TABLE 3.12:

Activity *of glutathione-s-transferases (nM/Mg protein/min) in control group and HBV female patients.*

Group studied	Number	Mean	S.E	U value	P-value
Control group	27	0.3238	0.0393	738.0	0.2767
HBV patients	32	0.815	0.202		

Biochemical estimation of non specific beta esterases

HCV male patients

The activity of β-esterases in control group was 10.03Mm/min/mg protein. However in HCV male patients the activity was 14.020Mm/min/mg protein. statistically the level of β-esterase was higher in control group than hepatitis C male patients (U=2044; P-Value =0.0016); (Table 3.13).

TABLE 3.13:

Activity of beta β esterases (mM/min/mg protein) in control and HCV male patients..

Group studied	Number	Mean	S.E	U value	P-value
control group	27	10.03	1.05	2044	0.0016
HCV patients	47	14.020	0.562		

HCV female patients

The activity of β-esterases in control group was 10.25Mm/min/mg protein. However in HCV female patients the activity was 13.146Mm/min/mg protein. statistically the level of β-esterase was higher in control group than hepatitis C female patients (U=2094; P-Value =0.0479); (Table 3.14).

TABLE 3.14:

Activity of beta β esterases (mM/min/mg protein) in control and HCV female patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	35	10.25	1.09	2094	0.0479
HCV patients	46	13.146	0.843		

HBV male patients

In HBV male patients the activity of β-esterases in control group is lower than disease patients. The value was 10.03Mm/min/mg protein in control patients. However in HBV male patients the activity was 13.857Mm/min/mg protein. statistically the level of β-esterase was greater in control group than HBV male individuals (U=2036; P-Value =0.002); (Table 3.15).

TABLE 3.15:

Activity of beta β esterases (mM/min/mg protein) in control and HBV male patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	27	10.03	1.05	2036.0	0.0022
HBV patients	47	13.857	0.442		

HBV female patients

In HBV male patients the activity of β-esterases in control group is lower than disease patients. The value was 9.991Mm/min/mg protein in control patients. However in HBV male patients the activity was 14.393Mm/min/mg protein. statistically the level of β-esterase was significant greater in control group than Hepatitis B female patients (U=1511; P-Value =0.0023); (Table 3.16).

TABLE 3.16:

Activity of beta β esterases (mM/min/mg protein) in control and HBV female patients.

Group studied	Number	Mean	S.E	U value	P-value
Control group	67	9.991	0.729	1511.0	0.0023
HBV patients	25	14.393	0.679		

DISCUSSION

Hepatitis is a viral infection that causes liver toxicity due to increase in reactive oxygen species (ROS). Oxidative stress produced in response of ROS is found to affect levels of detoxifying enzymes in hepatocytes particularly GST, monooxygenases and non specific beta esterases in Hepatitis B and C. Activity of these enzymes is found to be changed in many liver diseases specifically Hepatitis B and Hepatitis C.

Hughes et al. (1997) and Loguercio et al. (1998) reported that high concentration of GST can be used as biomarker in diagnosis of breast cancer and liver disease on the basis of their significantly higher value ($p<0.01$) in hepatocytes and serum. In present study the GST values in serum were determined and found to be increased ($p<0.05$) in patients of hepatitis B and C as compared with the control group. These results are in accordance with Hughes et al. (1997) and Loguercio et al (1998). However female patients with hepatitis B did not show high levels of GST which is contradictory to their findings (Bilal[a,b], 2021).

Harrison et al. (1989) found that in humans the cells of renal proximal tubule have significantly high ($p<0.05$) concentrations of alpha GST, while distal tubular cells have pi GST. According to the findings of Harpur et al. (2011) and Bailey et al. (2012) in rodents urinary and serum alpha GST levels are significantly higher as compared with other body tissues which can be used as bioindicator of necrosis in kidney and liver cells. Giannini et al. (2011) evaluated chronic Hepatitis C patients for iron accumulation and GST. Results show that there was more damage of liver in patients which has high value of GST in serum. The result of Helaly & Mahmoud (2003) showed that alpha-GST values were significantly high ($p<0.05$) in HCV infected individuals than healthy individuals. Similar results were found in the present study.

Monooxygenases (cytochrome p450) might be poor in individual who do not take sufficient dietary proteins and have great degree of hepatotoxicity. There was significantly lower ($p<0.05$) value of monooxygenases in the epithelial cells of digestive tract in the patients with cancer of large intestine and pathology due to pharmaceutical drugs (Michael & Mcevoy,

2011). According to Otani et al. (2005) expression of cytochrome P450 (Type of monooxygenases) has found to increase total ROS by 3 times as compared with cells which did not express cytochrome P450. The result of present study are in accordance with these scientist. The result of present study showed that the value of monooxygenases are not significantly lower ($p>0.05$) in HCV positive females and HBV positive male patients but its value was significantly lower ($p<0.05$) in HCV positive males and HBV positive females patients. In contrary with these results, Mcfadyen (2001) showed that this enzyme also demonstrates a significantly high level of enhanced expression in metastatic disease (Sajjad et al., 2024).

Polymorphism in cytochrome enzyme is not found to be cause of hepatotoxicity (Aitha et al., 2000). Mohamed et al. (2012) has reported that cytochrome p450 is significantly vital in development of non-alcoholic hepatitis by promoting oxidative stress and its value is significantly increased ($p<0.05$). According to the present study there is a significant ($p<0.05$) decrease in value of monooxygenases in Hepatitis B and C male and female patients as compared with healthy individuals. Michael & Mcevoy (2011) also reported that infection of Hepatitis B and C virus cause hepatotoxicity and this toxicity inhibit and significantly decrease the value of monooxygenases. Findings of the present study are not in accordance with Aithal et al (2000) and Mohamed et al (2012) who reported that monooxygenases were found to be increased due to liver toxicity.

Results of present study showed that the activity of Monooxygenases was greater in females as compared to male. Levels of monooxygenases were highest in HCV positive females and lowest in HCV males due to the high degree of toxicity in females than males because females exposure to the toxic material is higher because of menstral problem than males.

The esters of short chain fatty acids are hydrolysed by non specific esterases, and their quantity was significantly increased ($p<0.05$) by the injection of drug sodium taurocholate (Gomori, 1949; Nachlas & Seligman, 1949). In contrary with the result of present study Gomori (1949) reported that in serum samples of Hepatitis C the value of non specific esterases was not changed. Some drugs or infections such as Taurocholate decrease the activity of non specific esterases. Present results showed increased esterase activity but 9.2% serum samples showed decreased activity of non specific β esterases, this may be due to exposure of these samples to such unknown drugs or infection which decreases their activity (Bilal and Fatimah, 2021).

It is known that females are facing the problem of menopause and some other problems related to menstrual cycle. In old age among females the levels of esterases are found to be decreased due to menopause. It can be the reason for lowest activity of esterases in HCV female patients as compared with hepatitis C male patients but in hepatitis B +ve females the activity of beta esterases was highest which may be due to the exposure of such reagents with hepatitis B virus.

Monooxygenases might be poor in individual who do not take sufficient dietary proteins and having great degree of hepatotoxicity. The activity of Monooxygenases was greater in females as compared with males. Monooxygenases level was highest in HCV female and lowest in HCV male individuals due to the high degree of toxicity in females than males because females' exposure to the toxic material is higher because of menstrual problems than males

Results of present study suggested that hepatitis B and C virus can induce detoxifying enzymes by producing the toxicity and oxygen reactive species. Present study showed that GST can be used as indicator of infection of hepatitis B and C in males and female patients.

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