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BIOCHEMICAL MARKERS OF LIVER TOXICITY AMONG COAL MINE WORKERS OF PUNJAB, PAKISTAN SUFFERING FROM HCV

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ABSTACT

Hepatitis C is among the leading hepatic disorders in current period through which about 3 % world population has been anguish among them 170 million were diagnosed as persistent carriers. A great range of alteration in liver biochemical parameters were found to be allied with HCV infestation. Current study was designed to evaluate the extent of HCV mediated abnormalities in liver biochemical markers which includes ALT (alanine aminotransferase), AST (Aspartate transaminase), ALP (Alkaline Phosphatase) and serum bilirubin. The study was conducted on coal miners of Punjab province, Pakistan. HCV was primarily diagnosed through one step rapid test device after which positive samples were confirmed through ELISA. Biochemical markers were determined through Autoanalyzer by using standard procedure provided with spinreact kits. Simple linear regression analysis significantly explained 24 %, 56.2 %, 68.8 % and 56 % variance in ALT (alanine aminotransferase), AST (Aspartate transaminase), ALP (Alkaline Phosphatase) and serum bilirubin level among HCV positive coal mine workers respectively. Results have clearly indicated significant correlation between HCV seropositivity and liver biochemical markers. Findings of present study conclude monitoring of liver biochemical markers is crucial during HCV infectivity as it represents the degree of impairment in liver functioning. In addition to this elevation in these diagnostic markers could points toward the presence of HCV in respective individual.

Keywords: Liver biochemical markers, alanine aminotransferase, aspartate transaminase, alkaline phosphatase, coaldust.

INTRODUCTION

Nearly 3 % of the world's population has been suffering from hepatitis C infectivity whereas amongst them 170 million are persistent carriers while hepatic fibrosis probably develop among 20-30 % of HCV infected individuals over an interlude of 20-30 years (Afdhal and Nunes, 2004). 7.1 million Pakistanis (10 %) have hepatitis C virus (HCV). HCV genotype 3 is the most common in Pakistan. Risk factors for contracting hepatitis in coal miners of Pakistan include, but are not limited to, sharing razors, using niswar, injecting drugs, and being homosexual (Batool et al.,2017). The lack of patient awareness about disease causes and transmission, affordability for investigations and drug treatment, and experienced healthcare professionals all hinder HCV elimination from Pakistan(Waheed and Siddiq, 2018).Prolonged exposure to coal dust is responsible for oxidative stress that involved in the pathogenicity of many diseases (Batool et al., 2020).Chronic HCV viral infection results in the recruitment of inflammatory cells toward the liver, where they can clear out infected hepatocytes.Thus, chronic suppression of these intrahepatic inflammatory cells

promotes tissue injury, which in turn initiates liver fibrosis that modifies liver morphology, ultimately leading to the destruction of liver function. Connective tissue growth factor (CTGF) and transforming growth factor (TGF-1) are both upregulated by HCV, which contributes to fibrogenesis (Shin et al., 2005). The chief co-factors in HCV suffering patients to develop hepatic fibrosis are alcohol and immunodeficiency. 10-20 % chronic hepatitis C patients possibly will develop cirrhosis in about 20 years after which there is 7 % panorama for switching into hepatocellular carcinoma in about 5 years (Chen & Morgan, 2006; Fattovich et al., 1997).

There is a growing requirement for non-invasive indicators of hepatic injuries due to the limitations of liver biopsies, such as high cost, risks, and a lack of dynamic findings that can serve as surrogates for ongoing pathogenic mechanisms. Superlative serum markers have to be unswerving, cost-effective and suitable for varied cases of chronic liver disease and demonstrate a numerical involvement in diagnosis of hepatic damage. The hepatotoxins fabricate a wide-ranging concoction of irrefutable and histopathological marker of hepatic grievance. There are certain biochemical markers that, when elevated, indicate liver damage. These include bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. Increases in serum enzyme levels are used as the applicable indicators of liver toxicity, whereas increases in direct and indirect bilirubin levels are considered evidence of overall liver job.A combination of an increase in transaminase levels and bilirubin levels that exceeds the standard upper limit is regarded as an ominous indicator of hepatotoxicity (Reuben, 2004). Efforts to evaluate more than a few markers from the same individual assure a better probability of sensation in discerning nominal from rigorous fibrosis and in predicting cirrhosis (Gebo et al., 2002). Given the complexity and diversity of the liver's role, it is understandable that no single test can identify a liver function disorder. The serum ALT (alanine aminotransferase) level is the most commonly considered, readily available, and inexpensive surrogate marker for evaluating the predictive value of liver histology (Kim et al., 2008). Liver enzyme plays a very important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate. The assessment of this enzyme is a accurate test for identifying liver abnormalities since it is mainly found in the liver (Dufour et al., 2000); (Amacher, 2002). Elevated level of ALT (alanine aminotransferase) was observed in hepatic infection like hepatitis C, diseases of muscles and traumatisms.

Serum glutamic oxaloacetate transaminase (SGOT) is another liver enzyme that aids in protein synthesis.AST (aspartate aminotransferase) catalyses the reductive transfer of aspartic acid to -ketoglutarate in order to produce oxaloacetate and glutamate, thereby allowing citric acid cycle entry. In addition to the liver, it is also present in organs such as the brain, heart, muscle, and kidney (Lin et al., 2011).

Damage to any of these tissues can lead to an increase in blood concentration (Nathwani et al., 2005). These enzymes will leak into the blood if the liver cell membrane is damaged. It will increase bloodstream enzyme activity and may indicate liver cell membrane breakdown. The normal origin of its elevation from liver source is excessive alcoholism, which can also cause infiltrative liver damage. An elevated AST (Aspartate transaminase) level can be used to diagnose hepatocellular necrosis (Ozer et al., 2008), as it can also indicate abnormalities in the heart, muscles, brain, or kidney. The bile is the organ that is responsible for the

excretion of the hydrolase enzyme known as alkaline phosphatase. In an alkaline pH environment, it hydrolyzes monophosphates. Primarily, it can be found in the cells of the liver's biliary canal, which are lined with these cells. In addition to this, it can be found in other organs such as bone, placenta, kidneys, and intestines. Alkaline phosphatase is an enzyme that is involved in the process of bile production in the body. The presence of cholestasis is indicated by an elevated alkaline phosphatase level, which is evidence of a dysfunctional hepatobiliary process. Cholestasis is characterised by high levels of cholesterol. Having an increase in alkaline phosphatase or bilirubin despite having a relatively small or nonexistent increase in ALT is primarily considered to be a biomarker of hepatobiliary effects and cholestasis (Ramaiah, 2007). There is a correlation between increased levels of ALP (Alkaline phosphatase) and drug-induced cholestasis in human subjects (Wright & Vandenberg, 2007) . Bilirubin is a naturally occurring anion secreted by the liver in the form of bile after haemoglobin in red blood cells has been degraded. It's a chemical that the liver uses to make bile, and it's already present in the blood in trace amounts. Lower hepatic function can cause jaundice and other symptoms of hepatotoxicity by increasing bilirubin levels. Total bilirubin, rather than ALT (alanine aminotransferase), may serve as a more accurate indicator of the severity of illness following severe hepatic injury in humans (Dufour et al., 2000).

Commencement of HCV infectivity is typically non-investigative and infrequent assessment has been carried out concerning the abnormality in liver biochemical parameters including transaminases, alkaline phosphatase and serum bilirubin level. Hence current study is designed to determine the association of liver biochemical markers with HCV infectivity among coal miners.

MATERIALS AND METHODS

The study was conducted on coal mine workers of district Khushab and Chakwal, Punjab Pakistan. Blood samples were collected in 5 mL uncontaminated non- reusable syringes through venipuncture technique. Blood samples were centrifuged instantly subsequent to collection according to the method of (Dzoma et al., 2010) at 10,000 rpm for 3-5 minutes to separate the serum. Serum was collected with the help of micropipette and stored in properly labeled eppendrophs and frozen immediately for further analysis. HCV diagnosis was performed through HCV one step rapid test device which is a quick chromatographic immunoassay designed for qualitative detection of Hepatitis C Virus in serum or plasma. Positive samples were confirmed through ELISA. Assay for ALT (alanine aminotransferase), AST (Aspartate transaminase), ALP (Alkaline Phosphatase) and serum bilirubin was performed on MINDRAY BS-120 / BS-200E Autoanalyzer by using standard procedure provided with kit prepared by spinreact cooperation following the installed software in computer. The temperature adjusted for the reaction was 37 °C at primary wavelength of 340nm. 40 U/L and 46 U/L were taken as reference limit for ALT (alanine aminotransferase) and AST (Aspartate transaminase) respectively. While 250 U/L was reference value for ALP (Alkaline phosphatase), while 0.2-1.0 were standard limit for serum bilirubin. Regression analysis was performed through SPSS (v. 20^{th}) to estimate HCV mediated biochemical abnormalities in hepatic markers.

RESULTS

HCV is an unrelenting and quiet infection which makes the crucial identification as intricate process. Hepatitis C viral infection considerably affects all

major hepatic biochemical markers including transaminases, alkaline phosphatase and serum bilirubin. As the hepatic injury proceeds elevated serum enzymes level was observed among infected individuals. Linear regression analysis was conceded out to ascertain this premise. ALT(alanine aminotransferase) level was found to be elevated as a consequence of HCV infectivity among coal mine workers as it is most sensitive biochemical marker of hepatic illness.

i. ***HCV and ALT*** (alanine aminotransferase)

According to our best estimates, the equation for the straight line that connects ALT(alanine aminotransferase) and HCV reads as follows: ALT = (40.5788) + (37.9328)*HCV (n = 104).The correlation among ALT and HCV is β= 0.490 (P= 0.0000) while probable value of ALT(alanine aminotransferase) when HCV is zero, is (y-intercept) 40.579 ± 8.246. The slope of the line that represents the anticipated change in ALT (alanine aminotransferase) per unit change in HCV is 37.933 ± 6.690 (Figure 1.). The percentage of the difference in ALT (alanine aminotransferase) that can be explained by the fluctuation in HCV is 0.240 (Table 1.).The slope has a 95 % confidence interval that spans from 24.663 to +51.203. The lower 95 % confidence interval limit for the intercept is 24.223 and the higher 95 % confidence interval limit is 56.935(Table 2.).

Table 1. Model Summary of regression analysis among HCV and ALT.

					Change Statistics			
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df2	Sig. F Change
1	.490[a]	.240	.232	25.81076	.240	32.149	103	.000

a. Predictors: (Constant), hepatitis c

Table 2. Coefficients of regression analysis among HCV and ALT.

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0 % Confidence Interval for B	
Model		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	40.579	8.246		4.921	.000	24.223	56.935
	hepatitis c	37.933	6.690	.490	5.670	.000	24.663	51.203

a. Dependent Variable: ALT

ii. ***HCV and AST(*** Aspartate transaminase)***:***

Hepatitis allied liver injury is promising source of increase in serum AST (Aspartate transaminase) level, to approximate the impact of HCV on Aspartate aminotransferase (AST) following linear regression analysis was carried out. The correlation between AST (Aspartate transaminase) and HCV is β=0.750. Straight line

estimate for AST and HCV is: AST = (26.798) + (49.934)*HCV (n=104).When HCV is set to zero, the y-intercept represents the estimated value of AST, and it is 26.798 with a standard error of 5.379 (95 % CI= 16.1288, 37.4681).Slope (95 % CI= 41.278, 58.591) is the estimated proportional change in AST(Aspartate transaminase) with respect to a unit change in HCV, and it is 49.934 (Fig 2.).The coefficient of determination (R-Squared) between AST and HCV is 0.562, meaning that 56 % of the variation in AST (Aspartate transaminase) can be attributed to differences in HCV (Table 3.).A t-value of 11.4417 was obtained from testing the hypothesis that the slope is zero. This t-test has a significance level of 0.0000 (Table 4).

Table 3. Model Summary of regression analysis among HCV and AST level.

					Change Statistics			
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df2	Sig. F Change
1	.750[a]	.562	.558	16.83741	.562	130.913	103	.000

a. Predictors: (Constant), hepatitis c

Table 4. Coefficients of regression analysis of HCV and AST.

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
Model		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	26.798	5.379		4.982	.000	16.129	37.468
	hepatitis c	49.934	4.364	.750	11.442	.000	41.278	58.591

a. Dependent Variable: AST

iii. *HCV and ALP (*Alkaline phosphatase)

Alkaline phosphatase level is also influenced by HCV infectivity. To appraise this hypothesis linear regression analysis was performed. The results clearly evaluate our hypothesis that alkaline phosphatase level elevated as a result of hepatitis viral load (Figure 3.). The correlation between ALP (Alkaline phosphatase) and HCV is β=0.829. Linear regression between ALP (Alkaline phosphatase) and HCV is estimated to have the following equation: ALP = (51.309) + (89.540)*HCV (n=104).Using a standard error of 7.360, we can estimate that ALP (Alkaline phosphatase) is 51.309 when HCV is equal to zero. Standard error for the slope (the predicted amount by which ALP changes for each unit change in HCV) is 5.972, as in Figure 3. R-Squared equals 0.688, which is the percentage of shifts in ALP (Alkaline phosphatase) that can be explained by shifts in HCV (Table 5).A t-value of 14.994 was obtained after conducting a significance test to determine whether or not the slope is zero. This t-test has a significance level of 0.0000.Since 0.0000 is less than 0.0500 we cannot accept the null hypothesis that the slope is zero. The value 77.695 is the value at the lower end of the confidence interval for the slope, and the value 101.385 is the value at the upper end.

Table 5. Model Summary of regression analysis of HCV and ALP.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics			
					R Square Change	F Change	df2	Sig. F Change
1	.829[a]	.688	.685	23.03861	.688	224.833	103	.000

a. Predictors: (Constant), hepatitis c

Table 6. Coefficients of regression analysis of HCV and ALP.

Model		Unstandardized Coefficients		Standardized Coefficients			95.0 % Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
1	(Constant)	51.309	7.360		6.971	.000	36.710	65.908
	hepatitis c	89.540	5.972	.829	14.994	.000	77.695	101.385

a. Dependent Variable: ALP

Table 7. Model Summary of regression analysis among HCV and serum bilirubin

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics			
					R Square Change	F Change	df2	Sig. F Change
1	.748[a]	.560	.556	.16807	.560	129.830	102	.000

a. Predictors: (Constant), hepatitis c

Table 8. Coefficients of regression analysis among HCV and serum bilirubin level.

Model		Unstandardized Coefficients		Standardized Coefficients			95.0 % Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
1	(Constant)	.185	.054		3.446	.001	.079	.292
	hepatitis c	.496	.044	.748	11.394	.000	.410	.583

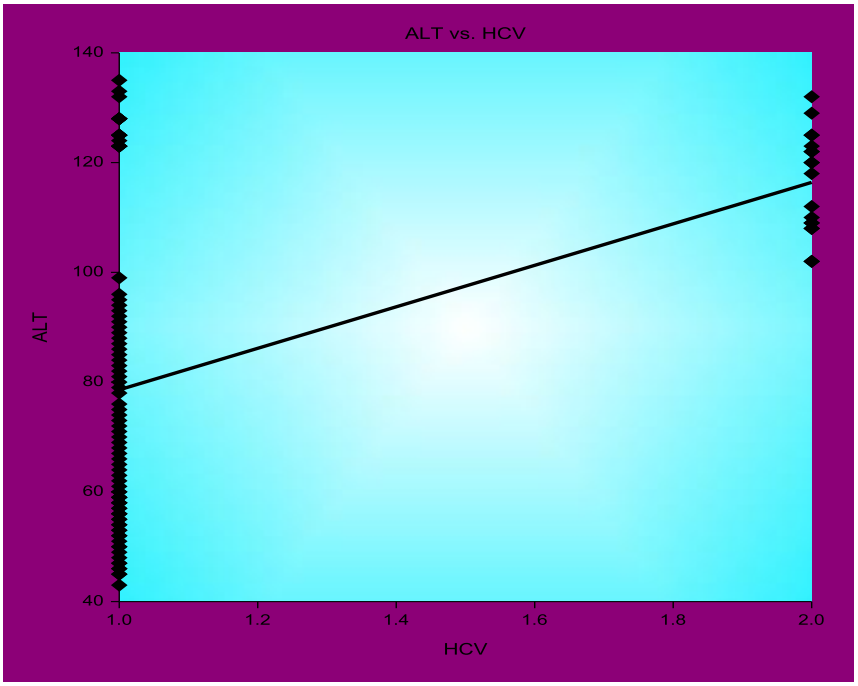

Figure 1: Regression analysis among HCV vs. ALT level. The value of 36.71 is considered to be the lower limit of the 95 % confidence interval for the intercept, while 65.908 is considered to be the upper limit (Table 6.).

iv. HCV and Serum Bilirubin

Elevated serum bilirubin level is also an authentic marker for identifying the presence of HCV. Results of linear regression analysis too provide evidence for this assumption (Fig 4.). It is estimated that the equation of the straight line that establishes a relationship between serum bilirubin and HCV is as follows:Serum bilirubin = (0.1850) + (0.4964)*HCV (n=104).

A t-value of 11.3943 was obtained after conducting a significance test on the hypothesis that the slope is zero.This t-test has a significance level of 0.0000 .According to Table 8, the y-intercept, which represents the estimated value of serum bilirubin when HCV is equal to zero, is 0.1850.054 (95 % confidence interval = 0.079, 0.292).The slope, which can be thought of as the estimated change in serum bilirubin for each unit change in HCV, is found to be 0.4960.044 (95 % confidence interval = 0.410, 0.583).The value of R-Squared, which is the proportion of the variation in serum bilirubin that can be accounted for by variation in HCV, is 0.560. This value represents the proportion of the variation in HCV that can be accounted for (Table 7.).There is a 0.748 correlation between the levels of serum bilirubin and HCV.

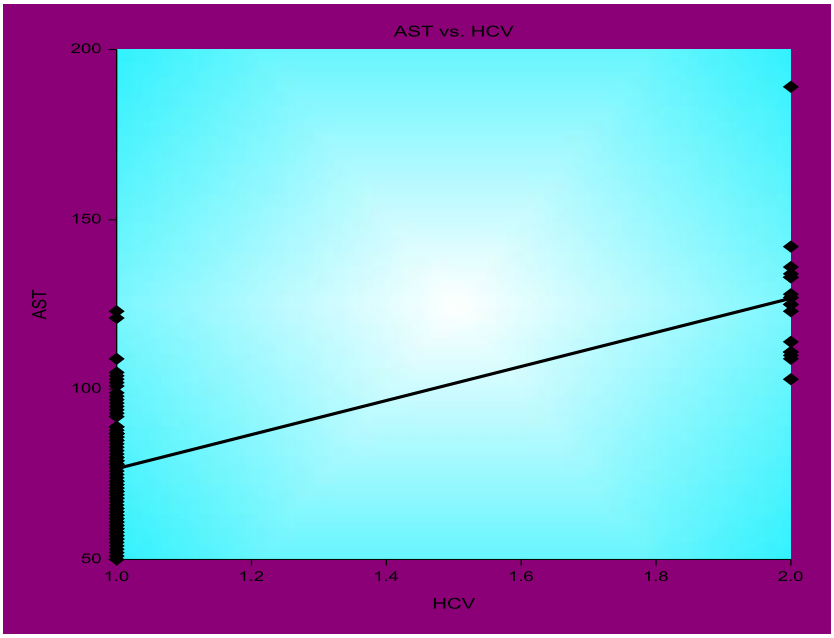

Figure 2: Regression analysis among HCV vs. AST level

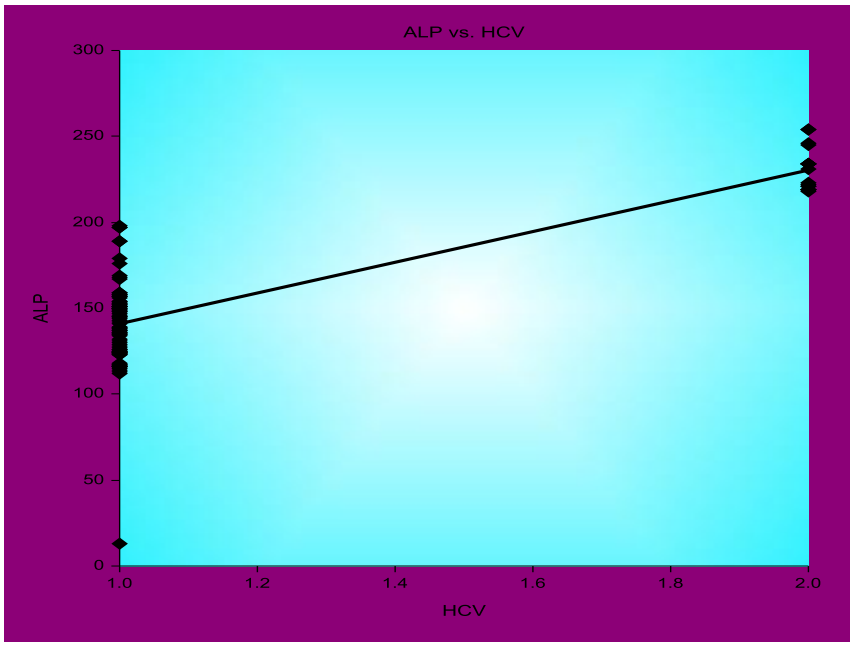

Figure 3: Regression analysis among HCV vs. ALP level

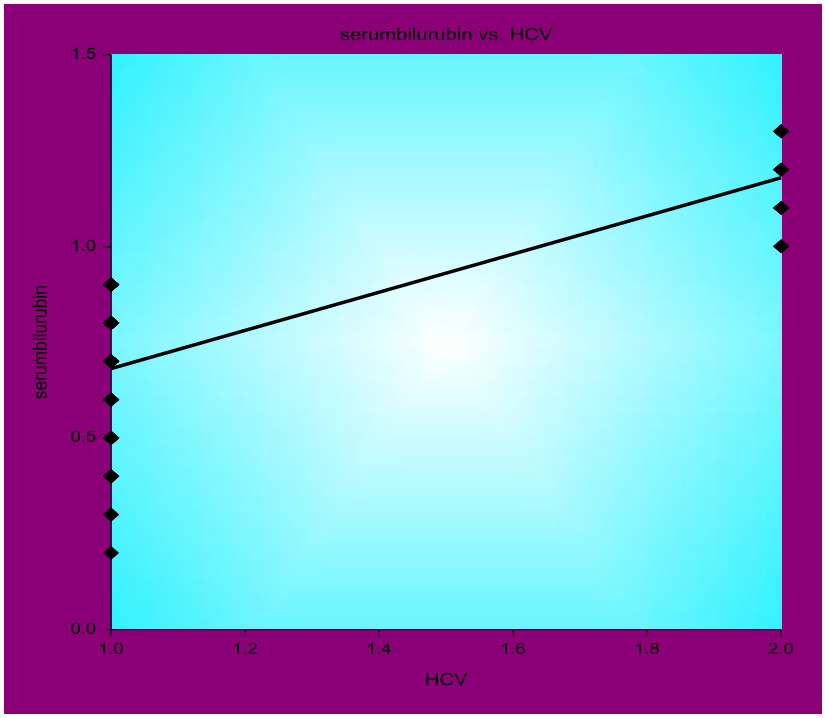

Figure 4: Regression analysis among HCV vs. Serum Bilirubin

DISCUSSION

Till now HCV is considered to be responsible for progression of liver disease. In order to appraises the correspondence among unusual clinical liver markers in coal workers effected with hepatitis C virus, four basic biochemical markers ALT(Alanine aminotransferase), AST(Aspartate transaminase), ALP (Alkaline phosphatase) and bilirubin level were estimated. Significant correlation was observed among serum bilirubin, ALT (alanine aminotransferase), ALP (Alkaline phosphatase) and AST (Aspartate transaminase) levels and HCV infectivity while level of AST (Aspartate transaminase) was even high in comparison to ALT (alanine aminotransferase) which points toward liver fibrosis due to extreme HCV viral load. Elevated bilirubin value is typically coupled with liver metastases along with liver tumor association leading toward hepatocellular carcinoma and liver cirrhosis through HCV, while elevated aminotransferases levels act as markers of liver cell injury (Tazawa et al., 2004), (Ong et al., 1999). Viral hepatitis infectivity is the foremost reason of amendment in ALT (alanine aminotransferase) among wide-reaching populaces (Clark et al., 2003), (Pendino et al., 2005), (Chen & Morgan, 2006). Elevated levels of ALP (Alkaline phosphatase) are generally associated with increasing age, high BMI, serum uric acid, accumulation of heavy metals, smoking, alcohol abuse (Hsu et al., 1996) extra-hepatic bile impediment, crucial biliary cirrhosis, intrahepatic cholestasis, infiltrative liver infection, hepatitis, cirrhosis, main sclerosing cholangitis and hepatic lymphoma. (Asghar et al., 2011) reported elevated transaminase and ALP (Alkaline phosphatase) level among individuals infected with HCV which is in agreement to our results.

An alteration in ALP (Alkaline phosphatase) levels larger than normal can be pinpointing toward advanced infection succession (Lee et al., 2007), (Saif et al., 2005), (Wiwanitkit, 2001). The increased AST (Aspartate transaminase) level had been attributed to mitochondrial injury associated with HCV infection and progression of liver fibrosis (Okuda et al., 2002). Findings of (De Ritis et al., 1965)

were in consistent to our results reporting increase in level of liver enzymes in presence of viral hepatitis. Highly significant and elevated level of ALP (Alkaline phosphatase) and serum bilirubin was observed during the incidence of acute viral hepatitis by a study carried out through (Rekha & Murthy, 2011) which is in agreement to current study findings. Increase in alkaline phosphatase is due to enhanced production through liver along with subsequently impaired bile duct excretion. Elevated level of bilirubin in circulation represents defective liver function in clearing bilirubin through bile secretions resulting in jaundice. These findings lead to conclusion that a significant alteration in liver enzymes in associated with HCV infectivity which further points toward defective liver functioning.

CONCLUSION

Liver health is an important parameter of human health and physiology. Hepatitis being a chronic condition should be detected earlier in order to combat this disease. Hence along with the test for hepatitis, the liver health biomarkers such as ALT (alanine aminotransferase), AST (Aspartate transaminase), ALP (Alkaline Phosphatase) and serum bilirubin level can be effective parameters to check the seropositivity and extent of liver damage as a result of hepatitis as they have shown positive correlation with hepatitis induced liver damage. These biomarkers are easily measured and cost effective respectively.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CONSENT FOR PUBLICATION

All authors agreed to the publication of the manuscript.

AUTHOR'S CONTRIBUTION

All authors equally participated in this research study. Dr. Aima Iram Batool, Dr. Muhammad Fayyaz Ur Rehman and Fariha designed the study and analysis. Asma Noreen carried out lab works. Naima Huma Naveed, Dr.Iram Inayat and Dr. Muhammad Ali Kanwal did the final preparation of draft. Hakim Bibi and Humaira Jabeen carried out statistical analysis.

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