Cross talk of serum elements, cardiac and liver enzymes in patients with HCV chronic hepatitis and hepatocellular carcinoma in Pakistani population

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ABSTRACT

HCV-associated hepatic pathologies are now the frequent inducer of cardiac abnormalities because of cardio-hepatic interaction. Studies are going on to elucidate and highlight the possible factors involved in this complex interaction, but this paradigm is still unclear. Here, we aimed to explore the interrelationship among electrolytes, cardiac, and liver enzymes in HCV-associated hepatic abnormalities, including chronic hepatitis and hepatocellular carcinoma in the Pakistani population. 100 Hepatitis C virus (HCV) infected patients with liver disorders and 50 healthy individuals were recruited in the present analysis. Trace elements, ions, and enzyme concentrations were quantified *via* an automatic analyzer, while HCV was confirmed by enzyme-linked immunosorbent assay (ELISA). Our results demonstrated that serum Ca^{++}, Mg^{++}, Fe^{++}, Cl^{-}, and PO_4^{-} levels were significantly increased in HCV patients with hepatic pathologies, including cirrhosis and carcinoma. Cardiac enzymes, including aspartate aminotransferase (AST), creatine kinase (CK_2), and lactate dehydrogenase (LDH) concentration, were also elevated in HCV patients. Furthermore, serum cholesterol and triglyceride levels significantly differed in HCV patients than in normal individuals. Impaired alkaline transaminase (ALT) and alkaline phosphatase levels in HCV patients further validate the HCV patients' hepatic pathologies. Interestingly, all these impaired factors were positively correlated with the progression of hepatic disorders. In conclusion, altered ionic concentrations, cardiac enzymes, and liver dysfunction markers suggest their significant relationship to HCV leading liver pathologies in the Pakistani population.

Key Words: HCV, Electrolytes, Cardiac enzymes, Liver enzymes, Hepatic pathologies

1. INTRODUCTION

The primary cause of both acute and chronic liver disease is the Hepatitis C virus (HCV). Worldwide, up to 170 million people are chronically infected by HCV. 80% of the HCV-infected individuals become chronic carriers who may then progress to severe liver diseases. Within 2-3 decades of infection, 10–20% of chronically HCV-infected individuals develop severe liver cirrhosis, and 1–5% may develop hepatocellular carcinoma (HCC). HCV is a hepatotropic flavivirus in the Hepacivirus genus. It is a positive-stranded RNA virus with a genome size of 9.6 Kb (Chen & Morgan, 2006; Modi & Liang, 2008; Zaltron, Spinetti, Biasi, Baiguera, & Castelli, 2012).

HCV infections are associated with severe alterations of host body redox status. HCV infection can leads to oxidative stress through multiple mechanisms that include chronic inflammation, iron overload, and liver injury by increasing oxidative stress markers in some HCV patients (Paracha et al., 2013). The HCV induces liver damage accompanied by Fe^{++} overload-induced oxidative stress, inflammatory responses, cytotoxicity induced by virus core proteins, immune-mediated processes that show a significant relationship between HCV and endogenous elements. (Arain et al., 2014; Choi & Ou, 2006; Guo, Chen, Lin, Shih, & Ko, 2012; Rahman, 2007).

Iron (Fe) is an essential component of catalase enzymes, hemoglobin, and myoglobin. It also plays as a pro-oxidant and creates oxidative stress in the presence of lipids. Individuals with high levels of lipids and serum iron are at increased risk of cancer. Iron accumulation, an outcome of viral and liver damage, is a common finding in chronic hepatitis C, but detailed mechanisms have not yet been fully elucidated (Berg et al., 2001; Kohgo, Ikuta, Ohtake, Torimoto, & Kato, 2007; Lambrecht et al., 2011; Nahon, Ganne-Carrié, Trinchet, & Beaugrand, 2010; Price & Kowdley, 2009). The high mutation rate of HCV and elevated liver enzyme ALT has been associated with the marked increase in Fe's levels in infected individuals (Rashed, 2011). Iron depletion maintained higher sustained virological response rates and significantly reduced ferritin levels and ALT activity (Franchini, Targher, Capra, Montagnana, & Lippi, 2008; Gentile et al., 2009; Tessman & Romani, 1998).

Similarly, alterations in cellular ions (i.e., Mg^{++}, Ca^{++}, Na^{+}, K^{+}) homeostasis influence biological membrane fluidity or produce reactive molecules (i.e., free radicals), which hamper the operation of signal transduction pathways in the liver, cardiac, and smooth muscle cells (Tessman & Romani, 1998). Exposure to toxic elements and alterations in essential elements homeostasis may be the risk factor for HCV infection (Lingala & Ghany, 2015). In the present study, the relationship of endogenous elements/electrolytes alteration is observed in the hepatic disorders common in the Pakistani population.

A condition termed cirrhotic cardiomyopathy, characterized by hyperdynamic circulation, increased cardiac output, reduced peripheral vascular resistance, and arterial pressure, represents the association between cirrhosis and cardiovascular abnormalities. Numerous cellular signaling pathways contribute to these abnormalities, including cardiovascular dysregulation, central nervous system, and humoral factors such as nitric oxide. Both endogenous and exogenous cannabinoids have significant cardiovascular effects. Certain evidence suggests that in cirrhosis at multiple levels, increased activity of the endocannabinoid system contributes to the development of both cardiac and vascular changes (Moezi, Gaskari, & Lee, 2008).

Previous studies have demonstrated liver and heart abnormalities' co-existence because both are systemic diseases (Xanthopoulos et al. 2019). Further elevated cardiac enzymes such as AST, LDH, and CK2 have been reported in different liver diseases (Neuschwander-Tetri 2017) but in Pakistani population it is essentially required to give some potential data showing interplay of cardiac, liver enzymes and essential elements in HCV pathologies. This study is designed to investigate this gap and to give some basic correlating data regarding altered levels of some ions, cardiac, and liver enzymes in liver diseases, specifically hepatitis C and hepatocellular carcinoma.

1. MATERIAL AND METHODS

2.1 Subjects

This is an observational and analytical study comprised of HCV patients with liver complications. Blood samples were collected from Benazir Bhutto Hospital (BBH) and Holy Family Hospital (HFH) Rawalpindi, Pakistan. A total of 150 subjects were selected (n =100, hepatitis with HCV patients, n = 50 were healthy individuals). Subjects were excluded if they were <20 or >80 of age. Prior informed consent was taken at the time of blood collection and questionnaire filling. The questionnaires were carefully filled, including personal data, past and present clinical history, family history (ultrasound and liver biopsy reports).

2.2 Ethical considerations

The present study was approved by the institutional ethical board and was conducted according to the Helsinki Agreement's guidelines.

2.3 Blood Sample Collection

Blood for biochemical and electrolyte analysis was collected in coagulant-containing gel tubes and immediately stored at -4 °C. The serum was then separated as supernatant from cells by centrifugation at 4000 RPM for 5 minutes and stored at -20 °C until analysis.

2.4 Diagnosis of HCV through ELISA

HCV was diagnosed by the Diagnostic kit for Antibody to Hepatitis C Virus (ELISA) for the qualitative detection of antibodies to hepatitis C in human serum as previously described (Afridi et al., 2014)

2.5 Biochemical Determinations

The concentration of liver enzymes, i.e., alanine aminotransferase (ALT), alkaline phosphatase (Arain et al.), and Cardiac enzymes, i.e., aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK_2), were determined in serum by the Enzymatic rate method with SYNCHRON CX9-PRO automatic analyzer (Beckmann Coulter, USA) using commercially available assay kits. Serum total bilirubin levels were determined by a timed endpoint diazo method in SYNCHRON CX9-PRO automated analyzer (Beckmann Coulter, USA) using a commercially available assay kit.

2.6 Electrolytes Determination:

Serum electrolytes i.e., Na^+, K^+, Cl^-, Ca^{++}, Mg^+, $Fe^{++,}$ and PO_4^- were determined using Ion-Selective Electrode (ISE) with SYNCHRON CX9 PRO automatic analyzer (Beckmann Coulter, USA). Manufacturer's instructions with commercially available Synchron buffers and reagents were utilized. An ISE (Ion-selective electrode), made of glass or validamycin, is a thin membrane with selective binding sites across which only the specific ion can be transported from higher to a lower concentration and creating a potential difference through binding these sites. The sample was diluted with a high ionic strength ISE electrolyte buffer, minimizing the variation in the specimens' activity coefficients to be analyzed. The ISE electrolyte buffer and ISE electrolyte

reference maintain ion activity constant on the electrodes. A potential was generated at the surface of ISE when the diluted sample passes through the flow cell. The magnitude of potential change was proportional to the concentration of the respective electrolyte. Nernst equation was used to determine the concentration of electrolyte from this potential (Albert, Subramanian, Rangarajan, & Pandey, 2011).

2.7 Statistical analysis

Data were subjected to analysis of variance (ANOVA) post by Turkey's multiple comparison test and Student's t-test using SPSS 16. All the data presented as mean ± SD 16. $p < 0.05$ was considered to be significant.

2. RESULTS

3.1 Impaired cardiac enzymes level correlates with HCV-associated hepatic abnormalities

In the present analysis 100 patients (HCV hepatitis) and 50 healthy control individuals were included (patient: age above 20 years, male: female 62: 48, Control age above 20 years, male: female 38: 12). The final diagnosis patients were: HCV + Chronic (n = 68), HCV + Cirrhosis hepatitis (n = 32).

After HCV validation in the patients with liver disorders, including chronic cirrhosis and carcinoma, cardiac enzymes (CK_2, AST, and HDL) were assessed in the serum of under observational subjects (Table 1). Interestingly, cardiac enzymes level was significantly higher in HCV patients with hepatic abnormalities than the control subject (Table 1). Further statistical analysis demonstrated that CK_2, LHD, and AST levels were significantly higher in patients with HCV-chronic conditions; however, in HCV-with cirrhosis patients, LHD and AST levels were significantly higher compared to the control subjects (Table 2). This finding suggests that HCV-associated hepatic pathologies could impair cardiac enzymes status and activities.

Table 1: Comparison of electrolytes, cardiac and liver enzymes between liver patients and healthy individuals

Parameters	**Patients (n = 100)**	**Controls (n = 50)**	***P* values**
Cardiac enzyme			
CK2 (IU/L)	155.6 ± 221.10	85.4 ± 32.18	0.0273
LDH (IU/L)	323.6 ± 163.60	133.88 ± 26.80	<0.0001
AST (IU/L)	82.9 ± 8.3	25.8 ± 10.18	<0.0001
Electrolytes			
Ca++ (mg/dl)	9.54 ± 0.51	8.9816 ± 0.629	<0.0001
Mg^{++}(mg/dl)	1.95 ± 0.40	0.7102 ± 0.26	<0.0001
Fe^{++}(ug/dl)	163.7 ± 56	90.755 ± 296.8	<0.0001
Na+ (mmol/l)	135.5 ± 6.8	138.76 ± 3.579	0.007
K+(mmol/l)	3.6 ± 0.66	4.39 ± 0.62	<0.0001

Cl- (mmol/l)	108.9 ± 10.9	101.67 ± 5.28	<0.0001
PO4-- (mg/dl)	3.95 ± 0.79	3.42 ± 0.524	0.002
Liver enzymes			
T.bil (mg/dl)	2.52 ± 3.18	0.5673 ± 0.309	<0.0001
ALT (IU/L)	181.80 ± 32.5	29.580 ± 11.86	<0.0001
ALP (IU/L)	251.1 ± 169.7	184.2 ± 64.2	0.046
Lipid profile			
Cholesterol (mg/dl)	148.7 ± 103.7	165.98 ± 21.6	<0.0001
Triglyceride (mg/dl)	211.7 ± 154.5	100.67 ± 35.5	<0.0001

Values are given as means ± SD. p values were calculated by using the Student's t-test.

Table 2: Multiple comparison of cardiac enzymes among chronic (n = 68), cirrhosis (n = 32) and normal (n = 50) subjects**.**

Parameters	***CK2 (IU/L)***	***LHD (IU/L)***	***AST (IU/L)***
Chronic+ HCV	169.1 ± 265.7	322.4 ±176.2	80.5 ± 68.3
Normal	85.4 ± 32.2	133.4 ± 26.7	25.8 ± 10.0
P values	0.0390	<0.0001	<0.0001
Cirrhosis+ HCV	126.9 ± 55.2	326.5 ±136.6	88.1± 109.8
Normal	85.4 ± 32.2	133.4 ± 26.7	25.8 ± 10.0
P values	0.5739	<0.0001	<0.0001

Values are given as means ± SD. *p* considered significant when less than 0.05**.**

3.2 HCV infection accelerates hepatoxicity

Retarded functioning of liver enzymes and alterations in their specified expression is reported in the liver abnormalities (Tolleson, 2018). Herein to validate the liver toxicity, we measured liver toxicity markers, including total bilirubin, ALT, and ALP. As expected, altered levels of liver enzymes were detected in the serum of patients with

hepatic pathologies compared to control subjects (Table 1). Notably, all the proteins mentioned above (ALT, ALP, and total bilirubin) were significantly impaired in the serum of the HCV patients with chronic hepatic pathology conditions. In contrast, only total bilirubin differed considerably in HCV with cirrhosis patients than normal individuals; however, we did not detect any significant change in ALT and ALP levels (Table 3).

Table 3: Multiple comparisons of liver enzymes and lipids level among chronic, cirrhosis, and normal subjects

Parameters	***liver enzymes (IU/L)***			***Lipids (mg/dl)***	
	Total bilirubin	ALP	ALT	Cholesterols	Triglycerides
Chronic + HCV	2.36 ± 2.59	263.7 ±170.7	190.9 ±348.6	144.3 ±79.74	211.8 ±149.5
Normal	0.56 ± 0.306	184.7 ±64.2	29.8 ± 11.9	165.7 ±21.45	100.3 ± 35.81
P values	0.001	0.01	0.005	0.54	<0.0001
Cirrhosis+ HCV	2.85 ± 4.21	224.7 ±164.9	162.8 ±275.9	158.2 ±143.1	211.5 ± 167.2
Normal	0.56 ±0.306	184.7 ±64.2	29.8 ± 11.9	165.7 ±21.45	100.3 ± 35.81
p values	0.001	0.61	0.08	1.00	0.001

P values were calculated by ANOVA post by Turkey's test. Values are given as means ± SD.

3.3 Serum electrolytes/elements status in HCV-associated hepatitis

Determination of the level of the electrolytes in the serum of patients and control subjects was carried out. Interestingly, we found an impaired electrolytes level between HCV patients and control subjects (Table 1). Ca^{++}, Mg^{++}, Fe^{++}, Cl^{-} and PO4 were significantly higher in HCV infected patients than in healthy individuals. However, serum K^{+} concentration was significantly decreased in HCV patients (Table 4). These findings may suggest the contribution of specific electrolytes/elements in HCV and liver disorders.

Table 4: Electrolytes concentration among HCV chronic, cirrhosis, and normal individuals

Electrolytes	***Normal***	***HCV + Chronic***	***p values***
		HCV + cirrhosis	

Ca $^{++}$ (mg/dl)	8.9920 ± 0.62754	9.5353 ± 0.51421	<0.0001
		9.5594 ± 0.53028	<0.0001
Mg $^{++}$ (mg/dl)	0.7120 ± 0.26927	2.0029 ± 0.42985	<0.0001
		1.9156 ± 0.34837	<0.0001
Fe^{++} (ug/dl)	90.4000 ± 29.4881	154.75 ± 55.6023	<0.0001
		180.91 ± 55.75138	<0.0001
Na^{+} (mmol/l)	138.70 ± 3.5642	135.56 ± 6.8423	0.016
		136.69 ± 6.81761	0.411
K^{+} (mmol/l)	4.3940 ± 0.62316	3.6838 ± 0.72824	<0.0001
		3.7281 ± 0.52685	<0.0001
Cl^{-} (mmol/l)	101.62 ± 5.24459	108.01 ± 10.07360	0.001
		111.06 ± 10.58586	<0.0001
PO4^{--} (mg/dl)	3.4240 ± 0.52121	3.8868 ± 0.78072	0.002
		4.0969 ± 0.82050	<0.0001

Values are given as means ± SD. *p* considered significant when less than 0.05

3.4 Cholesterols and triglyceride

The liver is an essential organ for lipid metabolism, and an impaired lipid profile could contribute to the progression of hepatic pathologies (Luo, Pu, Wang, & Xu, 2010). Herein, we found a significantly altered level of both cholesterol and triglycerides in patients' serum compared to the normal subjects (Table 1). Cholesterol level (144.3 ± 79.7) was significantly decreased in HCV chronic conditions compared to normal (165.7 ± 21.45). In contrast, the triglyceride levels were significantly increased in both HCV with chronic (211.8 ± 149.5) as well as in cirrhosis (211.5 ± 167.2) liver abnormalities compared to normal (100.3 ± 35.81) subjects (Table 3). The results may indicate the deregulation of lipids in HCV-induced liver damage conditions.

3. DISCUSSION

Here, we demonstrated that HCV infection accelerates liver toxicity by increasing hepatotoxicity markers impairment. HCV infection could also enhance cardiac enzymes during liver cirrhosis condition, supporting the cardio-hepatic interaction. Impaired lipid profile (cholesterol and triglycerides) supports this notion. Furthermore, HCV-associated hepatitis aggravates the imbalance in electrolytes concentration, which may further contribute to the dysregulation of the biological process involved in the hepatic pathologies.

Hepatitis C virus (HCV) infection is the major risk factor that can lead to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Lingala & Ghany, 2015). Enhanced AST activity, LDH, and CK_2 are

general cardiac pathologies markers reported in specific hepatic pathologies (Neuschwander-Tetri, 2017). Additional parameters analysis in the coexisted liver and cardiac pathologies have also been carried out in other populations (Xanthopoulos et al., 2019). However, the expression and mechanism of serum up-regulation of CK_2 and its isoforms are still under discussion. The elevated CK_2 level has also been reported as a functional contributor in chronic HCV hepatitis (Anderson, Zeng, Rock, & Yoshida, 2000; Faloppi et al., 2014). In the present study, a significant (p = <0.0001) increase in cardiac enzyme levels in HCV patients, specifically with chronic hepatitis compared to healthy individuals, suggests an interrelationship of cardiac enzymes and HCV. The upregulated level of dysfunctional liver markers, including bilirubin, ALP, and ALT, confirmed liver abnormalities in agreement with previously documented studies (Wahib, Seif El Nasr, Mangoud, El Shazly, & Morsy, 2005).

Electrolytes play essential roles in various biological processes, and their homeostasis is critical for life (Bertini & Cavallaro, 2008). The ions act as cofactors such as iron, an integral component of catalase enzymes, hemoglobin, and myoglobin (Kohgo et al., 2007; Nahon et al., 2010). It is also well known that the high mutation rates of HCV and raised liver enzyme ALT levels have been associated with markedly increased Fe^{++} in infected individuals (Gentile et al., 2009). Fe^{++} ions in NADPH's presence can also induce Ca^{++} ions from the liver microsome damaging Ca^{++} and Mg^{++} ATPase (Rolfs & Hediger, 1999). In the present study, we detected a significant up-regulation of ions, including Fe^{++}, $Ca^{++,}$ and $Mg^{++,}$ in patients diagnosed with HCV hepatitis. The absence of K^+ and LDH release in the perforated or the extracellular compartment suggests that the release of Mg^{++} induced by ethanol occurs through the operation of a specific transport process (Onji et al., 1992). A significant decrease in the K^+ ion concentration in sera of the patients has suggested its role and is in agreement with previous findings. Under the umbrella of the mentioned shreds of evidence, the current findings may present a significant link between altered ionic concentrations in HCV and liver abnormalities.

5. CONCLUSION

In summary, we conclude that cardiac enzymes (CK_2, AST, and LDH), electrolytes/elements, liver enzymes (ALT and ALP), and total bilirubin deregulation play a significant role in HCV-allied chronic hepatitis as well as in cirrhosis carcinoma. This study also suggests a relation between electrolytes, lipids, cardiac and liver enzymes in hepatitis. They may hold the key to understanding the unique biological roles of these factors in different liver disorders and malignant transformation.

Declarations

Ethical Approval and Consent to participate : Yes

Consent for publication. Yes

Availability of data and materials. All data generated or analyzed during this study are included in this article.

Competing interests: The authors declare no competing financial interests.

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