Prevalence and association of HBV and HCV infection with cardiovascular disease risk factors in a peri-urban population
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Objective: To investigate the possible associations of hepatitis B and C virus infection with cardiovascular disease risk factors in a peri-urban population.

Methods: The cross-sectional study was conducted from February to December 2016 in the peri-urban low-resource locality of Bin Qasim Town in Karachi. Serum samples were screened for hepatitis B surface antigen and hepatitis C virus antibodies. Anthropometric measurements were taken and markers related to cardiovascular disease were examined. Association of the two hepatitis virus infections with cardiovascular disease were investigated by analyzing the data using SPSS 16.

Results: There were 691 subjects. Serum triglyceride levels were significantly low in patients with hepatitis B virus (p<0.05). Those with hepatitis C virus had markedly low total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride levels (p<0.05 each), whereas random blood sugar and body mass index values were significantly high (p<0.05 each). Hepatitis C virus infection was positively associated with body mass index and random blood glucose, and inversely associated with total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol (p<0.05 each).

Conclusion: Hepatitis B virus infection showed a significant inverse association with triglyceride levels. However, hepatitis C virus infection was positively associated with body mass index and random blood sugar, and inversely associated with total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels.

Keywords: Hepatitis B, Hepatitis C, Coronary heart disease, Lipoproteins, Cholesterol.

Introduction
Hepatitis is a major global health issue, especially in the developing countries such as Pakistan,¹ where it is found mostly in the rural population.² Hepatitis is an inflammation of the liver, principally caused by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, and is responsible for minute disorders to liver cirrhosis or hepatocellular carcinoma(HCC).³ The prevalence of HBV and HCV worldwide is 10-15%.² Pakistan is the world’s sixth largest country in terms of HBV and HCV prevalence at around 2.5% and 4.8%, respectively.³

Chronic infection and associated inflammation may be involved in the initiation and progression of atherosclerosis,⁴ leading to cardiovascular diseases (CVDs). According to the World Health Organisation (WHO), till 2017 approximately 17.7 million people had died because of CVDs, representing 31% of global deaths, and, among those deaths, 7.4 million people died because of coronary heart disease (CHD).⁵ Main factors related with pathophysiology are age, male gender, family history, smoking, hypertension (HTN), diabetes mellitus (DM), obesity, high total cholesterol (TC), low-density lipoprotein cholesterol(LDL-C) and triglyceride (TG), as well as low high-density lipoprotein cholesterol(HDL-C) and less physical activity.⁵ HBV may lead to chronic liver disease by dyslipidaemia.⁶ Su et al.⁷ investigated the association of a lower level of HDL-C and TC with asymptomatic chronic HBV infection. HCV is an independent risk factor for CVDs owing to its core protein nature. HCV can also play a crucial role in promoting insulin resistance (IR), oxidative stress (OS), and liver steatosis. Moreover, several direct and indirect
mechanisms are assumed to be involved in the development of atherogenes is by HCV, including replication and colonisation in the arterial walls, fibrosis, increased levels of inflammatory markers and metabolic syndrome (MS).\(^7\) Association of HBV and HCV infection with CVD among the Pakistani population are not clear. The current study was planned to discern not only the prevalence of HBV and HCV infection and related determinants, but also to investigate the possible association of HBV and HCV infection with CVD risk factors in the target population.

Subjects and Methods
The cross-sectional study was conducted from February to December 2016 in the peri-urban low-resource locality of Bin Qasim Town in Karachi. Only those living in the selected community and willing to participate were included, and those who refused to participate were excluded. Convenient sampling was used and consent was obtained from each subject. The sample size was calculated considering mean cholesterol among Pakistani HCV patients to be 218.38±1.54 mg/dl,\(^8\) and using 99% confidence interval (CI) and 0.2 bound of error. Blood samples were drawn from each non-fasting participant, and the sample was centrifuged. Serum was isolated and stored at -80°C. A self-designed questionnaire was used to acquire demographic information, including age, gender, family history, medication history, and tobacco consumption. Anthropometric measurements, including subject’s body weight, height, body mass index (BMI), waist and hip circumferences, and systolic and diastolic blood pressures, were also taken. Data relevant to the coronary artery disease (CAD) prevalence was also collected. The advance quality single-step test strip (In-Tec Products, Incorporation, Xiamen, China) was used for the visual detection of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies in serum samples. Biochemical analysis was performed for quantitative measurement of serum cholesterol, HDL-C, LDL-C, TG, and glucose for all participants. All biochemical tests were performed based on the routine enzymatic colorimetric method by automatic analyser (Hitachi 902, Hitachi High-Technologies Corporation, Tokyo Japan).

According to the Centre for Disease Control (CDC) prevention, waist-to-hip ratio (WHR) >0.90 for males and >0.80 for females is considered an indicator of the risk of heart disease in South Asian population.\(^9\) BP measurements were performed in a standardised manner.\(^10\) BMI was calculated using the standard formula weight (kg)/height (m\(^2\)).\(^9\) TC ≥200 mg/dL, LDL-C ≥130 mg/dL, reduced HDL-C ≤40 mg/dL in males and ≤50 mg/dL in females, and TG ≥150 mg/dL were considered abnormal values as recommended by the American Heart Association (AHA).\(^11\) DM was defined as random blood glucose (RBG) ≥140 mg/dL and or with the treatment of hypoglycaemic agents as suggested by the American Diabetes Association (ADA).\(^12\)

Data was analysed using SPSS 16. To check the null hypothesis that there is no relationship between HBV or HCV infection and CVD risk factors, univariate (chi square test and t-test) and multivariate (linear regression model) analyses was done. T-test and Chi-square tests were applied for comparison between sero-positive and sero-negative groups with p<0.05 being significant. To define the strength of association of HBV or HCV infection with CVD risk factors, linear regression model was used. In multivariable analysis, gender, DM, HTN, tobacco consumption and BMI were used for adjustment.

Results
Of the 3,000 individuals approached, 691 (23%) agreed to participate, among those two were co-infected patients and excluded from the analysis and finally presenting the total of 689. The prevalence percentages of HBV and HCV infection were 75 (10.9%) and 43 (6.2%), respectively (Table 1). The prevalence of HBV infection was predominant in males (p<0.05). Conversely, the prevalence of HCV infection was high in females though the difference was not significant (p>0.05). The prevalence of HBV infection was high in subjects aged 21-40 years 44 (14.3%) compared to those aged ≤20 years 19 (8.4%) and ≥41 years 12 (7.6%). The highest prevalence of HCV infection was in those aged ≥41 years 30 (19.1%) followed by ≤20 years 0.4% (1) and 21-40 years 3.9% (12). Both HBV and HCV infections were considerably high in married individuals and (p<0.05). HBV-infected subjects had a lower mean WHR, TC, LDL-C, RBS and, to some extent, low HDL-C compared to those for HBV-negative subjects (p>0.05). In contrast, TG levels were significantly low in HBV-infected subjects than in HBV-negative subjects (p<0.05). Also 57.3% HBV-infected subjects were married compared to 45.2% among the
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Characteristics Controls HBsAg (+) Anti-HCV (+) p-value p-value
Gender (%) Male 39.6(226) 60.0(45) 0.001 34.9(15) 0.543
Mean-Age (years) 28.8 ±15.1 29.7 ±13.6 0.617 46.3 ±11.6 0.001
DM (%) 2.8(14) 6.8(5) 0.070 11.4(4) 0.006
Hypertension (%) 11.4(57) 11.0(8) 0.921 20.0(7) 0.127
CAD (%) 0.2(1) 1.4(1) 0.112 0.0(0) 0.792
Family History of CAD (%) 1.0(5) 4.1(3) 0.034 2.9(1) 0.311
Stroke (%) 0.2(1) 0.0(0) 0.589 0.0(0) 0.708
CLD (%) 0.0(0) 1.4(1) 0.009 0.0(0) 0.924
Tobacco use (%) 46.2(264) 64.0(48) 0.008 48.8(21) 0.464
Systolic blood pressure (mmHg), Mean ± SD 122.1±15.1 120.1±12.6 0.660 124.1±15.0 0.463
Diastolic blood pressure (mmHg), Mean ± SD 79±7.6 82±9.0 0.228 83.1±18.7 0.036
Cholesterol (mg/dL), Mean ± SD 197.4±63.8 185.8±48.2 0.127 162.2±64 0.001
Triglyceride (mg/dL), Mean ± SD 110.2±64.2 54.8±15.4 0.006 90.1±48.6 0.001
HDL-C (mg/dL), Mean ± SD 109.4±42.2 116.2±50.4 0.338 119.2±53.4 0.038
LDL-C (mg/dL), Mean ± SD 197.4±63.8 185.8±48.2 0.006 162.2±64 0.001
Total cholesterol (mg/dL), Mean ± SD 57.1(34) 67.1(37) 0.099 57.1(20) 0.226
BMI, Mean ± SD 24.4±6.8 26±10.0 0.117 31.1±17.4 0.001
WHR, Mean (SD) 0.0±1.0 0.4±0.1 0.271 0.9±0.1 0.129
Marital status% 45.2(258) 57.3(43) 0.037 74.4(32) 0.000

Percentages and Mean ± standard deviation (SD) are calculated by Chi-square and T-test respectively. p-values <0.05 are shown as statistically significant, DM (Diabetes mellitus), CAD (Coronary artery disease), CLD (Chronic liver disease), RBG (Random blood glucose), BMI (Body mass index), WHR (Waist-hip ratio).

Table-2: Unadjusted and adjusted linear regression beta-coefficients of cardiovascular disease (CVD) risk factors in hepatitis B virus (HBV)-positive individuals.

<table>
<thead>
<tr>
<th>Risk factors for CVDs</th>
<th>Unadjusted Coefficients</th>
<th>p-value</th>
<th>Adjusted Coefficients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>-17.64 (-27.55-(-7.73))</td>
<td>0.001</td>
<td>-17.89 (-29.29-(-6.51)</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-9.10 (-22.89-4.69)</td>
<td>0.196</td>
<td>-16.48 (-32.31-0.64)</td>
<td>0.042</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-2.71 (-5.23-(-0.18))</td>
<td>0.036</td>
<td>-1.29 (-4.01-1.44)</td>
<td>0.354</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-13.01 (-20.89-(-5.29))</td>
<td>0.001</td>
<td>-13.43 (-22.32-(-4.54)</td>
<td>0.003</td>
</tr>
<tr>
<td>RBG</td>
<td>6.47 (0.35-12.59)</td>
<td>0.038</td>
<td>5.13 (-0.99-11.24)</td>
<td>0.100</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.99 (-1.65-3.62)</td>
<td>0.463</td>
<td>-0.29 (-2.96-2.37)</td>
<td>0.826</td>
</tr>
<tr>
<td>WHR</td>
<td>0.01 (-0.004-0.031)</td>
<td>0.129</td>
<td>0.04 (-0.01-0.02)</td>
<td>0.659</td>
</tr>
<tr>
<td>BMI</td>
<td>1.32 (1.96-4.67)</td>
<td>0.000</td>
<td>2.99 (1.66-4.32)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Percentages and Mean ± standard deviation (SD) are calculated by Chi-square and T-test respectively. p-values <0.05 are shown as statistically significant, Confidence interval (CI) 95%.

Table-3: Unadjusted and adjusted linear regression beta-coefficients of cardiovascular disease (CVD) risk factors in hepatitis C virus (HCV)-positive individuals.

<table>
<thead>
<tr>
<th>Risk factors of CVDs</th>
<th>Unadjusted Coefficients</th>
<th>p-value</th>
<th>Adjusted Coefficients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>-11.68 (-26.68-3.33)</td>
<td>0.060</td>
<td>-14.95 (-30.51-0.62)</td>
<td>0.060</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-29.89 (-51.02-(-8.77))</td>
<td>0.006</td>
<td>-32.82 (-54.78-(-10.87)</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.69 (2.16-5.55)</td>
<td>0.388</td>
<td>1.39 (-2.39-5.18)</td>
<td>0.469</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-7.36 (-19.3-4.57)</td>
<td>0.226</td>
<td>-9.67 (-21.98-2.62)</td>
<td>0.123</td>
</tr>
<tr>
<td>RBG</td>
<td>-4.59 (-14.0-4.82)</td>
<td>0.338</td>
<td>-8.33 (-16.67-(-0.006)</td>
<td>0.050</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-1.59 (-8.69-5.51)</td>
<td>0.660</td>
<td>-2.29 (-9.34-4.74)</td>
<td>0.521</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.015 (-0.04-0.001)</td>
<td>0.271</td>
<td>-0.02 (-0.05-0.003)</td>
<td>0.085</td>
</tr>
<tr>
<td>BMI</td>
<td>1.43 (0.36-3.21)</td>
<td>0.117</td>
<td>0.90 (-0.83-2.63)</td>
<td>0.308</td>
</tr>
</tbody>
</table>

Percentages and Mean ± standard deviation (SD) are calculated by Chi-square and T-test respectively. p-values <0.05 are shown as statistically significant, Confidence interval (CI) 95%.

Non-infected with a low prevalence of CAD and family history of coronary heart disease. In addition, only tobacco consumption (64.0% vs.45.5%) was substantially high in HBV-infected subjects. HCV-infected subjects had markedly lower mean serum TC (162.2±64 vs. 197.4±63.8 mg/dL), LDL-C (90.1±48.6 vs.

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116.2±50.4mg/dL), HDL-C (47.7±18.7 vs. 53.1±16.0 mg/dL), and TG (122.0±68 vs. 140.0±90.2 mg/dL) than HCV-negative subjects. By contrast, RBS levels and BMI were significantly high in HCV-infected subjects (p<0.05 each). Furthermore, most HCV-infected subjects were married and the prevalence of DM (11.4% vs. 2.8%) was higher than in HCV-negative subjects. Tobacco consumption was comparably high but was not significant (p>0.05).

Unadjusted coefficient of CVD risk factors showed that HBV infection was inversely associated with TG even after adjustment (p<0.05). Unadjusted coefficient of CVD determinants demonstrated that HCV infection was positively associated with BMI and RBS, but it was inversely associated with TC, HDL-C and LDL-C (p<0.05). Similarly, adjusted coefficients of CVD risk factors showed a significant positive link with BMI (p<0.05). Considerably inverse associations were observed among HCV infection and cholesterol level, TG, and LDL-C (p<0.05 each) (Tables 2-3).

### Discussion

The continuous rise of HBV and HCV infection is an alarming situation in Pakistan. Several international reports have supported the association of HBV and HCV infection with CVD, but not a single report regarding this association from Pakistan is available.

The current study included 691 individuals from a population belonging to the periphery of Karachi. The prevalence of HBV infection is notably high in males mostly middle-aged. Males are more prone to have chronic HBV infection compared to females. HBV can be transferred through sexual activity, and in males, HBV can be transferred through homo- as well as heterosexual activities, especially in the middle-age group.

In addition, a high carrier rate among people aged 40-50 years was observed. In the current study, HBV infection is highly prevalent in married persons, which is in line with the findings of a previous study. This study determined that the prevalence of HCV infection was prominently high in the old age group (≥41 years). The same trend has been described, but slight variation was observed as the highest prevalence of HCV infection was detected in people aged 50-59 years. In our study, HCV infection was more prevalent in female participants, but was not statistically different, and comparable results have been reported in the past.

We found that HBV-infected subjects had low TC, TG, LDL, and RBS mean values compared with healthy participants. These outcomes are comparable with previous studies. Current results indicated that HCV-infected patients had significantly low TC, LDL-C, and TG levels compared with HCV-negative individuals; similarly, low TC, LDL-C, and TG levels were determined in HCV-infected patients.

The reduced levels of serum LDL-C, HDL-C, and TC were positively associated with the severity of liver disease. Significant reduction in TC and TG levels in cirrhotic patients has been confirmed, which is because liver biosynthesis is reduced. Studies have mentioned that lipids have a significant function in responding to any infection because lipoproteins can bind various viruses to diminish their toxic effects.

We found that HBV infection was inversely associated with TG. Studies have supported the inverse association of HBsAg sero-positivity with hypertriglyceridemia and decreased HDL-C after adjustments. The inverse correlation between chronic hepatitis B(CHB) and MS may be accredited as favourable consequences to the lipid profiles of infected subjects. In contrast, no significant association between the risk of CAD and HBV infection was found. The reason for the inverse association is unclear, but a low inflammatory burden of CHB infection may be a possible reason.

Another possibility is the low prevalence of traditional risk factors for CAD, such as DM, HTN and hyperlipidaemia. Possible association between HCV infection and CVD risk has been concluded with different results. Our findings demonstrated that HCV infection was positively associated with BMI and RBS, but was inversely associated with TC, TG, LDL-C, and HDL-C. After adjustment for CVD risk factors, HCV infection was positively associated with BMI and inversely associated with TC and LDL-C. These results are comparable with a study that demonstrated that HCV infection seems to be an independent risk factor of CVD because of its association with steatosis, DM and IR. A study showed that chronic HCV infection appears to predispose the infected individuals to developing atherosclerosis, which directs them toward alterations in carotid plaques, although blood lipid levels and the prevalence of MS are low in HCV-infected subjects. A study observed an alteration in the glucose breakdown in HCV-infected population. The core protein of HCV can degrade insulin receptor substrates (IRS) 1 and 2 by enhancing the
expression of tumour necrosis factor-alpha (TNF-α) and the inhibition of the cytokine signaling-3, leading to defective phosphorylation of PI3K and Akt. It might be possible that decreased TC, LDL and HDL levels in the serum of cirrhotic patients may be related to the further development of cirrhosis. Therefore, establishing a mean range of lipid profile that can be used as a prognostic marker for patients at risk of liver cirrhosis is crucial.

The outcomes of the current study are not very close to the past studies as our study has certain limitations. For example, we were unable to collect fasting samples. Alanine transaminase (ALT) levels of the participants were also not measured. Besides, the time and logistics did not allow us to continue the clinical/collection unit for the required time. Longitudinal follow-up studies may help to better understand the association of HBV and HCV with CVD.

Conclusion
Most HBV and HCV-infected patients were asymptomatic and had low awareness about the disease and the risk. Such individuals not only serve as carriers but may experience extremely serious consequences that are costly as well as untreatable. Moreover, the horizontal transmission was found to be an essential mode of transfer of HBV and HCV infection. Proper screening, vaccination, and treatment need to be addressed urgently.

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References


