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Author(s):

Sohaib Bin Wahid Muhammad Waqar
Zobaria Rehman Muhammad Wasim
Muhammad Idrees

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Prevalence of HCV-*Helicobacter pylori* Co-infection and its Effects on Liver Function Enzymes of Patients in Lahore, Pakistan

Sohaib Bin Wahid^{1,2*}, Muhammad Waqar³, Zobaria Rehman³, Muhammad Wasim³, Muhammad Idrees³

¹Department of Biology, Lahore Garrison University, Lahore Pakistan

²Liwah Hospital, Liwa-Madinat Zayed Road, Liwa, Abu Dhabi, United Arab Emirates

³Genome Centre for Molecular Based Diagnostics and Research, Lahore Pakistan

*Corresponding author: sohaibbinwahid@gmail.com

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Abstract

Helicobacter pylori and HCV are the most prevalent pathogens worldwide associated with high rates of morbidity and mortality. This study aims to determine the prevalence of *H. pylori* in HCV infected patients and compares changes in liver function tests (LFTs) of both HCV mono-infected and HCV/*H. pylori* co-infected patients. Eighty-seven participants participated in this study who were first screened for HCV using GeneXpert technology followed by *H. pylori* antigen stool test. A complete blood count (CBC) analysis and LFTs were performed to determine the impact of HCV and *H. pylori* on liver enzymes. Our findings suggested that 45% of patients had HCV mono-infection, whereas 55% were co-infected with both HCV and *H. pylori*. Furthermore, we also observed that ALT, AST, and ALP levels were significantly elevated in HCV/*H. pylori* co-infected patients as compared to HCV mono-infected patients. To the best of our knowledge, this is the first study to report the prevalence of *H. pylori* in HCV infected subjects of Lahori population.

1. Introduction

More than 10 million people of Pakistan are infected with HCV which is one of the major causes of morbidity and mortality. HCV frequently leads to hepatocellular carcinoma (HCC), liver fibrosis, and cirrhosis. Another common gastric disease inducing pathogen *H. pylori* is a bacterial pathogen in the developing world which is known as risk factor of liver diseases and it has been reported to induce hepatotoxicity. Several studies have also shown the high prevalence of *H. pylori* infection in patients with chronic liver disease (CLD). According to World Health Organization, *H. pylori* is a

carcinogenic factor that may cause cardiovascular diseases, metabolic diseases, and it also disturbs normal liver function. Furthermore, many observations suggest that *H. pylori* and HCV co-infection worsens liver inflammation, liver fibrosis, and liver cirrhosis [1, 2, 3].

Helicobacter pylori has been reported to cause diseases in the upper region of gastrointestinal tract, such as chronic and acute gastritis, mucosa associated lymphoid tissue, gastric adenocarcinoma, and gastric and duodenal ulcers. Moreover, *H. pylori* has been reported to induce hepatotoxicity in vitro. *H. pylori*

causes the production of ureases that neutralizes hydrochloric acid in the stomach which increases its pH that favors the growth of *H. pylori* [4, 5]. *Helicobacter pylori* has been reported to increase liver fibrosis in mice models [6].

There is a lack of robust epidemiological information and conclusive data regarding the prevalence of *H. pylori* in HCV infected population of Pakistan. Therefore, this is the first study that demonstrates the combined prevalence of both HCV and *H. pylori*. Liver function tests (LFTs) and complete blood count (CBC) in both HCV mono-infected patients and HCV/*H. pylori* co-infected patients.

2. Materials and Methods

2.1. Study Subjects

The current study was conducted on 87 patients who visited our laboratory 'Genome Centre for Molecular Based Diagnostics and Research' situated in Lahore, Pakistan on 2nd February, 2018. Informed consent was obtained from all patients. Demographic details regarding their age and gender were recorded. All patients were screened for HCV using GeneXpert® System IV- Cepheid (Germany). HCV negative patients were excluded from the study, whereas HCV positive patients were further tested to detect the presence of *H. pylori*.

2.2. HCV Detection

HCV RNA was detected using an advanced technology known as GeneXpert® that is used for rapid quantification of HCV RNA in human serum. About 3ml of serum sample was shifted to single use disposable GeneXpert cartridges that hold the RT-PCR reagents and perform RT-PCR.

2.3. Detection of *H. Pylori* Infection

All participants were screened for *H. Pylori* using QuickVue TLI *H. pylori* stool antigen test (USA) following manufacturers' instructions.

2.4. Assessment of Liver Function Tests (LFTs) and Complete Blood Count (CBC)

LFT was performed in the chemistry analyzer (Mindray BS 200, Shenzhen, China) and complete blood components were analyzed in BC-3000Plus (Shenzhen, China).

2.5. Statistics

Continuous variables are presented as mean value \pm standard deviation. Statistical analysis was performed using IBM SPSS Version 25 (SPSS Inc., Chicago, IL, USA), while *p*-value less than 0.5 was considered significant.

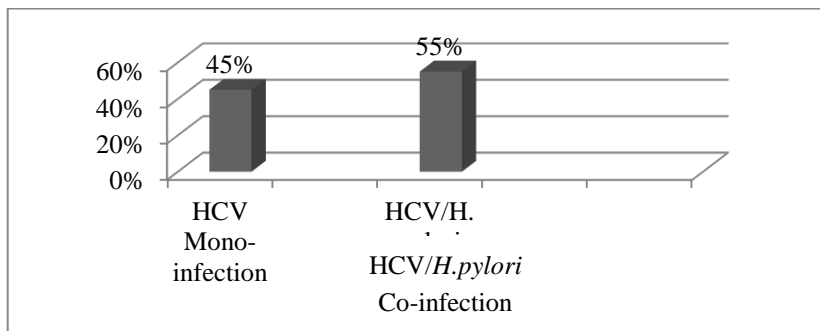


Figure 1. General prevalence of HCV mono-infection and HCV/*Helicobacter pylori* co-infection

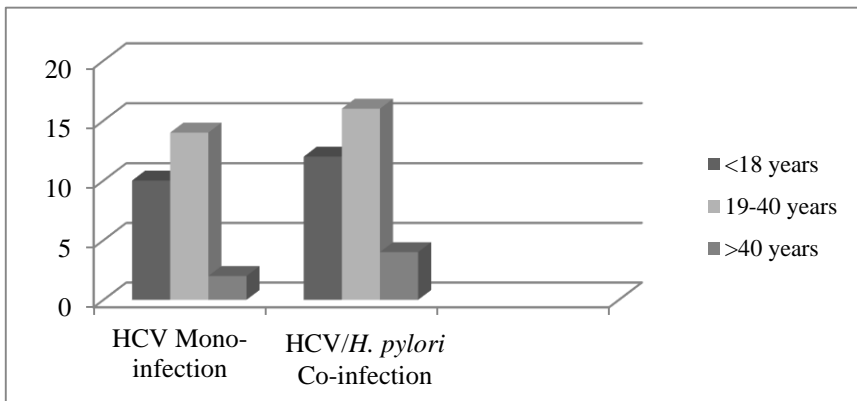


Figure 2. General prevalence of HCV and *Helicobacter pylori* in different age groups

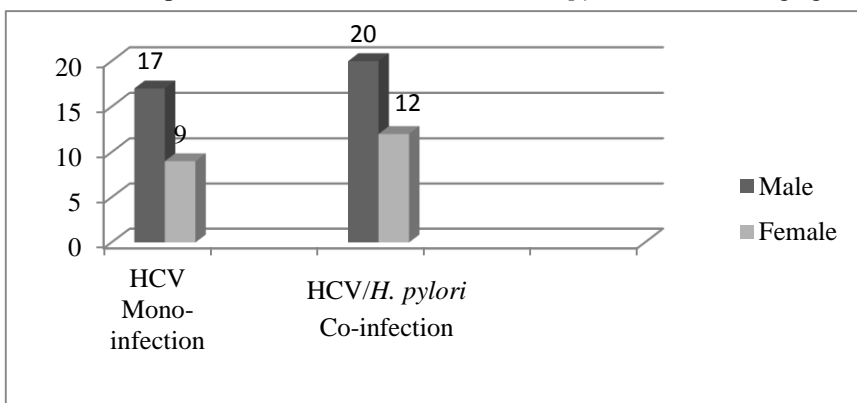


Figure 3. Genderwise distribution of HCV and *Helicobacter pylori*

3. Results

In this study, out of the total 87 patients 58 were found positive for HCV and their majority was found in males ($n=36$; 60%) as compared to females ($n=22$; 37.9%). Out of 58 HCV positive patients, 32 (55%) were co-infected with *H. pylori*, whereas 26 (45%) had HCV mono-infection (Figure 1).

We observed that the majority of patients were middle-aged, that is, between 19-40 years of age (fig 2) and the prevalence of HCV was found higher in males as compared to females (Figure 3).

CBC analysis showed a non-significant difference between blood components of HCV mono-infected and HCV/*H. pylori*

co-infected patients. The mean value of WBCs in HCV mono-infected patients was 9.24 ± 2.24 and it differed non-significantly from 9.54 ± 2.6 ($p=1$). A significant decrease in platelets ($p=0.06$) and hemoglobin ($p=0.14$) was observed in HCV/*H. pylori* co-infected patients as compared to HCV mono-infected patients (Table 1).

The findings of our study showed that ALT, AST, and ALP levels were found to be significantly higher in HCV/*H. pylori* co-infected patients as compared to HCV mono-infected patients (Table 2).

4. Discussion

This is the first study that highlighted the prevalence of HCV/*H. pylori* co-infection

Table 1. Complete Blood Count Analysis of Both Study Groups

Blood component	Mean \pm SD	Mean \pm SD	p-value
White blood cells ($10^9/L$)	9.24 \pm 2.24	9.54 \pm 2.6	1
Platelets (mm^3)	251.2 \pm 98.48	209.2 \pm 74.4	0.06
Hemoglobin (g/dl)	13 \pm 3.63	11.8 \pm 2.54	0.14

*SD: standard deviation

Table 2. Comparing Liver Function Tests in Both Study Groups

Liver function test	HCV mono-infection	HCV/ <i>H. pylori</i> co-infection	p-value
ALT (IU/L)	65.38 \pm 59.39	75.5 \pm 52.3	0.04
AST (IU/L)	47.34 \pm 29.88	40.86 \pm 17	0.03
ALP (IU/L)	112.19 \pm 43	121 \pm 37.3	0.04
Total Protein (g/dL)	8.08 \pm 0.59	8 \pm 0.51	0.058
Albumin (g/dL)	4.15 \pm 0.56	4.15 \pm 0.28	1
Total bilirubin (mg/dL)	0.38 \pm 0.17	0.38375 \pm 0.19	1
Direct bilirubin (mg/dL)	0.13 \pm 0.05	0.14 \pm 0.08	0.058

and compared the biochemical profile and CBC in both HCV mono-infected and HCV/*H. pylori* co-infected patients. We observed that the presence of *H. pylori* in HCV patients significantly elevated their liver function enzymes, whereas CBC analysis differed non-significantly in both groups. The prevalence of both infections was found to increase significantly with male gender and non-significantly with age. The overall prevalence of HCV was 66% and *H. pylori* and HCV co-infection was observed in 55% participants of the study. We observed that the prevalence of both infections was manifold higher than developed countries. The prevalence of *H. pylori* has been reported already to be 20% at 20 years of age and it increases to 50% at 50 years of age. Our findings comply with the findings of Pogorzelska J et al., who reported 46.9% prevalence of *H. pylori* in general population [3]. The findings of our study are also supported by Rocha M. et al., who reported an association between *Helicobacter* species' DNA in the liver and HCV cirrhosis, with

or without HCC [7]. Similarly, another study reported a higher frequency of *H. pylori* among HCV or HBV infected patients with post-inflammatory liver cirrhosis than in patients with alcoholic liver cirrhosis or primary biliary cirrhosis [3]. Salehi M et al., documented that *H. pylori* treatment results in a decrease in liver enzymes [8]. *H. pylori* resides in the epithelial cells of the mucous membrane of the prepyloric part of the stomach and it adheres to cell surface because of the presence of cilia. *H. pylori* infection causes disturbance in the metabolism of lipid that leads to hypercholesterolemia and hypertriglyceridemia and a decrease in high density lipoprotein. Moreover, the same bacterium also plays an important role in the metabolism of liver fibrosis, steatosis, and hepatocytes [9]. *H. pylori* infection may also cause injuries in multiple organs, such as chronic injury of pancreas which affects liver function.

Therefore, based on the analysis of biochemical tests, the results of the current study suggest that *H. pylori* is a new

etiologic agent which can also cause hepatocellular carcinoma and other liver complications and this finding corroborates the previously reported study that reported the presence of *H. pylori* in extra-hepatic tissues of human beings and mice [10].

5. Conclusion

In conclusion, our results highlight the very high prevalence of *H. pylori* among HCV-infected patients and a significant increase in liver function enzymes. Therefore, all HCV patients should be periodically screened for *H. pylori* to minimize its pathogenic influence on liver, gastric epithelium and the risk of malignant transformation. The study warrants future studies based on a larger sample size as well as on the mechanism behind the pathogenicity of *H. pylori* and HCV.

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