

Assessment of *Helicobacter pylori* Serology in Relation to Hepatitis C Virus Infection

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Abstract

Helicobacter pylori infection is highly prevalent worldwide and has been implicated in extra-gastric manifestations, including chronic liver disease. Hepatitis C virus (HCV) remains a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Emerging evidence suggests a potential association between *H. pylori* infection and the progression of HCV-related liver pathology.

This study aimed to assess the seroprevalence of *H. pylori* in patients infected with HCV compared to non-HCV controls in selected tertiary care hospitals of the capital territories of Pakistan.

A total of 80 serum samples were collected, comprising 40 HCV-positive and 40 HCV-negative individuals, from four major hospitals in Rawalpindi and Islamabad. Serological analysis was performed to detect *H. pylori* infection. Gender-based distribution and prevalence rates were calculated and compared between groups.

Among HCV-positive individuals, 50% (20/40) were seropositive for *H. pylori*. Of these, 43.33% (13/30) were males and 70% (7/10) were females. In contrast, among HCV-negative individuals, 25% (10/40) were positive for *H. pylori*, with a prevalence of 15% (3/20) in males and 35% (7/20) in females.

The overall prevalence of *H. pylori* infection was significantly higher in HCV-infected patients compared to controls. The findings also suggest gender-related differences in coinfection patterns. The increased prevalence of *H. pylori* among HCV-infected patients supports previous reports indicating a possible role of *Helicobacter* species in hepatic inflammation and disease progression. Chronic HCV infection is characterized by persistent inflammatory responses mediated by cytokines such as IL-1, IL-6, and TNF-alpha, while *H. pylori* is a potent inducer of inflammatory cascades and may exacerbate hepatic injury. Socioeconomic and environmental factors, including overcrowding and poor sanitation, may

contribute to higher coinfection rates in the studied population.

The study demonstrates a higher seroprevalence of *H. pylori* infection among HCV-positive individuals compared to non-infected controls, suggesting a potential association between *H. pylori* and progression of HCV-related liver disease. These findings highlight the need for further prospective studies to evaluate the impact of *H. pylori* eradication on clinical outcomes in patients with chronic HCV infection.

Keywords

Helicobacter pylori; Hepatitis C virus; Seroprevalence; Coinfection; Chronic hepatitis; Liver cirrhosis; Hepatocellular carcinoma; Inflammatory cytokines; Pakistan

1. INTRODUCTION

Viral hepatitis continues to pose a substantial threat to public health systems across the globe. Among the various hepatitis viruses, hepatitis C virus (HCV) has emerged as a particularly challenging pathogen due to its ability to establish persistent infections that frequently progress to severe liver complications (Afzal MS *et al.*, 2026). The global burden of this disease remains considerable, with estimates suggesting that approximately 160 million individuals worldwide carry the virus, representing roughly 3% of the global population (Averhoff F *et al.*, 2012). Chronic HCV infection serves as the underlying cause for about one-third of all cirrhosis cases and hepatocellular carcinoma (HCC) diagnoses annually (Lavanchy D. *et al.*, 2011).

The geographical distribution of HCV varies considerably. While the industrialised nations have

observed declining infection rates through improved screening and public health measures, the mortality associated with HCV-related liver disease is projected to continue rising over the coming decades (Razavi H *et al.*, 2013). In the South Asian context, Pakistan faces an especially heavy burden. Current estimates indicate that roughly 6% of the Pakistani population carries HCV, with approximately 10 million infected individuals nationwide (Kumar T *et al.*, 2017). This places Pakistan second only to Egypt in terms of absolute HCV burden (Afzal MS. *et al.*, 2016). A nationwide survey conducted during 2007-2008 reported a prevalence of 4.8%, though more recent data from certain regions suggest figures exceeding 8% (Qureshi H. *et al.*, 2010). From a virological perspective, HCV belongs to the genus Hepacivirus within the Flaviviridae family (Smith DB *et al.*, 2014). Its genome consists of a positive-sense single-stranded RNA molecule approximately 9.6 kilobases in length, encoding a single polyprotein that undergoes processing into functional viral proteins (Cuevas JM *et al.*, 2009). Seven major genotypes have been identified through phylogenetic analysis, with these genotypes further divided into 67 confirmed subtypes (Messina JP. *et al.*, 2015). The genotype distribution carries clinical significance, as it influences both treatment response and the rate of fibrosis progression (Thein HH. *et al.*, 2008).

Alongside viral hepatitis, another pathogen of considerable global importance is *Helicobacter pylori*. This Gram-negative, microaerophilic bacterium colonises the gastric mucosa and has been recognised as a class I carcinogen by the World Health Organization (Hooi JKY. *et al.*, 2017).

First successfully cultured by Warren and Marshall in 1983, *H. pylori* has since been firmly established as the causative agent of chronic active gastritis and is strongly associated with peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (Yan TL *et al.*, 2013).

The prevalence of *H. pylori* infection shows marked variation across different geographical regions and socioeconomic settings. In developing countries, infection rates can reach 80-90% of the population, whereas developed nations typically report rates below 50% (Mitchell H. *et al.*, 2016). Several factors contribute to this disparity, including differences in sanitation infrastructure, household crowding, and access to clean water. In Pakistan specifically, *H. pylori* infection is widespread, with studies reporting prevalence rates ranging from 74% to over 84% in certain populations (Rasheed F. *et al.*, 2012). The bacterium's ability to form biofilms and coccoid variants enables it to persist in the hostile gastric environment, while its flagella facilitate penetration of the mucus layer and attachment to epithelial cells (Bhimani DR. *et al.*, 2013).

The possibility of an association between *H. pylori* and liver disease has attracted increasing research attention. Several mechanisms have been proposed through which *H. pylori* might influence hepatic pathology, including the release of inflammatory mediators, molecular mimicry, and the potential migration of the bacterium or its products from the gastric lumen to the liver via the portal circulation (Rocha M. *et al.*, 2005). Chronic HCV infection itself produces a pro-inflammatory milieu characterised by elevated levels of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-alpha) (Balkwill *et al.*, 2001).

The additional inflammatory stimulus provided by *H. pylori* coinfection may potentially amplify hepatic injury and accelerate disease progression.

Coinfection with HCV and *H. pylori* presents important clinical considerations. Both infections are highly prevalent in Pakistan, and their overlapping distribution raises questions about potential interactions that could affect patient outcomes. While some investigators have reported higher *H. pylori* prevalence among HCV-positive individuals, the evidence remains inconclusive and geographically variable (El-Masry S. *et al.*, 2010). Understanding whether such an association exists in the Pakistani population could have implications for clinical management, particularly regarding the potential benefits of *H. pylori* eradication therapy in patients with chronic hepatitis C.

Against this background, the present study was designed to investigate the seroprevalence of *H. pylori* among HCV-positive patients attending tertiary care hospitals in Rawalpindi and Islamabad. By comparing *H. pylori* infection rates between HCV-positive and HCV-negative individuals, we sought to determine whether a significant association exists between these two infections in the Pakistani context.

2. MATERIALS AND METHODS

2.1. Study Design and Setting

This hospital-based cross-sectional study was conducted at four tertiary care facilities in the capital territories of Pakistan: Pakistan Institute of Medical Sciences (PIMS) Islamabad, Holy Family Hospital Rawalpindi, District Headquarters (DHQ) Hospital Rawalpindi, and Benazir Bhutto Hospital Rawalpindi. Laboratory analyses were performed at the Health Sciences Laboratory,

Comwave Institute Islamabad.

2.2. Sample Collection

A total of 80 serum samples were collected, comprising 40 from HCV-positive patients and 40 from HCV-negative controls. Approximately 5 mL of venous blood was drawn from each participant using standard aseptic technique. Samples were transported to the laboratory and processed within two hours of collection. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20 degrees C until analysis.

2.3. Inclusion and Exclusion Criteria

Individuals of both sexes and various age groups with elevated serum ALT/SGPT levels above the normal reference range during liver function testing were included in the study. Subjects with normal ALT/SGPT levels were excluded.

2.4. HCV Detection

Initial screening for HCV antibodies was performed using a rapid immunochromatographic test kit (Healgen Scientific LLC, USA) following the manufacturer's instructions. Samples showing positive results were subsequently confirmed by enzyme-linked immunosorbent assay (ELISA) using a first-generation HCV ELISA kit.

2.5. H. pylori Serology

Detection of anti-H. pylori IgG antibodies was carried out using the EUROIMMUN ELISA kit, a semi-quantitative in vitro assay. The procedure involved coating microtiter strips with H. pylori antigens, followed by incubation with diluted patient serum samples. After washing, enzyme-conjugated anti-human IgG was added, and the reaction was developed using chromogen/substrate solution. Colour intensity was measured photometrically at 450/630 nm using a BioTek microplate reader. Results were interpreted according to the manufacturer's cut-off values: less than 16 RU/mL negative, 16-22 RU/mL borderline, and greater than or equal to 22 RU/mL positive.

2.6. Statistical Analysis

Data were analysed using descriptive statistics. Prevalence rates were calculated as percentages, and gender-based distributions were compared between HCV-positive and HCV-negative groups.

3. RESULTS AND DISCUSSION

3.1. Sample Distribution

A total of 80 serum samples were collected from four hospitals in the capital territories, with 40 HCV-positive and 40 HCV-negative samples. The distribution across participating centres is presented in Table 1.

Table 1. Distribution of samples collected from hospitals in the capital territories

Hospital	HCV Positive	HCV Negative
District Headquarters Hospital, Rawalpindi	10	10
Holy Family Hospital, Rawalpindi	10	10
Benazir Bhutto Hospital, Rawalpindi	10	10
Pakistan Institute of Medical Sciences, Islamabad	10	10
Total	40	40

3.2. H. pylori Prevalence in HCV Positive Patients

Among the 40 HCV-positive individuals tested, 20 (50%) were found to be seropositive

for H. pylori. Breaking this down by gender, 13 out of 30 male patients (43.33%) and 7 out of 10 female patients (70%) showed positive results for H. pylori antibodies. These findings are summarised in Table 2.

Table 2. Gender-wise distribution of H. pylori among HCV-infected population

Characteristics	HCV Positive	H. pylori Positive
Male	30/40 (75%)	13/30 (43.33%)
Female	10/40 (25%)	7/10 (70%)
Total	40/40 (100%)	20/40 (50%)

3.3. H. pylori Prevalence in HCV-Negative Controls

In the HCV-negative control group, 10 out of 40 individuals (25%) tested positive for H.

pylori. The gender distribution showed 3 out of 20 males (15%) and 7 out of 20 females (35%) were seropositive. The data are presented in Table 3.

Table 3. Gender-wise distribution of H. pylori among non-HCV infected population

Characteristics	HCV Negative	H. pylori Positive
Male	20/40 (50%)	3/20 (15%)
Female	20/40 (50%)	7/20 (35%)
Total	40/40 (100%)	10/40 (25%)

3.4. Comparative Analysis

The overall seroprevalence of H. pylori was markedly higher in HCV-positive patients (50%) compared to HCV-negative controls (25%). Additionally, female participants in both groups showed higher H. pylori positivity rates than their male counterparts.

The findings of this investigation demonstrate a notably higher rate of H. pylori seropositivity among patients with chronic HCV infection when compared to controls without a

hepatitis C. This pattern aligns with several previously published reports that have explored the relationship between these two pathogens (Queiroz DM. *et al.*, 2006). Understanding why such an association might exist requires consideration of both the biological mechanisms involved and the environmental factors that could promote coinfection.

One line of reasoning focuses on the immunological changes that occur during chronic

HCV infection. Persistent viral replication in the liver triggers ongoing inflammatory activity, with consequent release of multiple cytokines including IL-1, IL-6, and TNF-alpha (Heim MH. *et al.*, 2014). This altered immune environment could theoretically create conditions that favour *H. pylori* colonisation or persistence. At the same time, *H. pylori* itself is a powerful stimulus of gastric and systemic inflammation, with the potential to amplify the overall inflammatory burden through pathways involving NF-kappa B activation and pro-inflammatory gene expression (Crabtree J. *et al.*, 1995).

An alternative perspective considers whether *H. pylori* infection might actively contribute to liver damage. Experimental work has shown that certain *Helicobacter* species can produce hepatotoxic substances capable of inducing necrosis in cultured hepatocytes (Meyer-ter-Vehn T. *et al.*, 2000). While the evidence for direct involvement of *H. pylori* in human liver disease remains preliminary, the possibility that gastric *Helicobacter* species or their products might reach the liver through portal venous drainage has been suggested by several research groups (Waluga M. *et al.*, 2015).

The gender differences observed in our sample deserve comment. Female participants in both HCV-positive and HCV-negative groups showed higher *H. pylori* positivity compared to males. This finding contrasts with some previous reports that identified male sex as a risk factor for *H. pylori* acquisition (Pellicano R. *et al.*, 2015). However, given the relatively small sample sizes involved, particularly in the female subgroup, these observations should be interpreted cautiously. Larger studies would be needed to establish whether gender truly

modifies the risk of *H. pylori*-HCV coinfection in this population.

When considering the broader context of *H. pylori* prevalence in Pakistan, our data fit within the range reported by other investigators working in this region. Rasheed and colleagues documented an *H. pylori* prevalence of 74.4% in their study population (Rasheed F. *et al.*, 2012). While other groups have reported figures ranging from 76.7% to 84.6%. The variation between studies likely reflects differences in diagnostic methods, population characteristics, and socioeconomic profiles of the samples examined.

The relatively high background prevalence of *H. pylori* in Pakistan may partially explain the coinfection patterns we observed. Factors such as household overcrowding, limited access to sanitation facilities in certain areas, and practices that facilitate faecal-oral or oral-oral transmission create an environment where *H. pylori* spread readily (Bardhan PK. *et al.*, 1997). When these conditions coincide with a similarly high prevalence of HCV, the probability of encountering individuals infected with both pathogens increases substantially.

From a clinical standpoint, if *H. pylori* coinfection does indeed accelerate HCV-related liver disease, then screening for and treating *H. pylori* in patients with chronic hepatitis C could represent a relatively simple intervention with potentially meaningful benefits. Some researchers have already begun exploring this question, though randomised controlled trials specifically examining whether *H. pylori* eradication improves liver-related outcomes in HCV patients remain scarce (Ponzetto A *et al.*, 2000).

Our study has several limitations that should

be acknowledged. The sample size, while adequate for preliminary observations, was not large enough to permit extensive subgroup analyses or multivariate adjustment for confounding variables. We relied on serological testing rather than direct detection methods such as culture or polymerase chain reaction, which means we could not distinguish between active and past *H. pylori* infection. Additionally, our cross-sectional design does not allow us to establish temporal relationships or infer causality.

Future research in this area would benefit from longitudinal designs that track HCV-infected patients over time, comparing liver disease progression between those with and without *H. pylori* coinfection. Studies incorporating direct detection methods for *H. pylori* and measuring inflammatory biomarkers would help clarify the mechanistic relationships involved. Investigation of other enterohepatic *Helicobacter* species, such as *H. hepaticus* and *H. bilis*, may also provide valuable insights given their potential role in hepatobiliary pathology (Pisani P. *et al.*, 2008).

4. CONCLUSION

The present study found that *H. pylori* seroprevalence was substantially higher among HCV-positive patients (50%) compared to HCV-negative controls (25%) attending tertiary care hospitals in Rawalpindi and Islamabad. This observation supports the hypothesis that an association exists between *H. pylori* infection and chronic HCV, though the nature of this relationship remains to be fully elucidated. The findings suggest that clinicians managing patients with chronic hepatitis C should consider evaluating for *H. pylori* coinfection, particularly given the potential therapeutic implications.

Prospective studies examining whether *H. pylori* eradication influences the clinical course of HCV-related liver disease would be of considerable interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Acknowledgments

The authors gratefully acknowledge the administration and laboratory staff of District Headquarters Hospital Rawalpindi, Holy Family Hospital Rawalpindi, Benazir Bhutto Hospital Rawalpindi, and Pakistan Institute of Medical Sciences (PIMS) Islamabad for their cooperation in sample collection and laboratory facilitation. The authors also extend their appreciation to all study participants for their voluntary participation.

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