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Assessment Of Helicobacter Pylori Serology In Relation To Hepatitis C Virus Infection

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Abstract

Hepatitis C virus (HCV) infection and Helicobacter pylori infection are major global health concerns, particularly in developing countries where infectious diseases continue to impose a significant burden on public health systems. Both infections are associated with considerable morbidity and may adversely affect the health status of infected individuals. In recent years, coinfection has emerged as an important subject of clinical and epidemiological interest because the coexistence of multiple infectious agents in the same host may influence disease progression, immune response, diagnosis, and treatment outcomes. The present study was conducted to assess the serological prevalence of Helicobacter pylori in patients infected with HCV and to compare its frequency with that in non-HCV individuals. A total of 80 participants were included in this study, comprising 40 HCV-positive patients and 40 non-HCV individuals who served as controls. Blood samples were collected from all participants and screened for anti-Helicobacter pylori antibodies using immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA). The collected data were analyzed to determine the prevalence of Helicobacter pylori in both study groups and to evaluate its distribution according to gender. The findings revealed that 50% (20/40) of HCV-positive

individuals were positive for Helicobacter pylori antibodies, whereas only 25% (10/40) of individuals in the non-HCV control group were found positive. This difference indicates a higher seroprevalence of Helicobacter pylori among patients with HCV infection. Gender-wise analysis showed that in the HCV-positive group, 43.33% (13/30) of males and 70% (7/10) of females were positive for Helicobacter pylori. In the non-HCV group, 15% (3/20) of males and 35% (7/20) of females were positive. These results demonstrate that Helicobacter pylori infection was more frequent in HCV-infected individuals than in healthy controls, while female participants showed relatively higher seropositivity than males in both groups. The study indicates a significant association between Helicobacter pylori infection and HCV infection. The higher prevalence of Helicobacter pylori among HCV-positive individuals suggests that coinfection may have important clinical implications in the diagnosis and management of affected patients. Routine screening of Helicobacter pylori in HCV-infected patients may therefore be beneficial for better clinical evaluation and proper management of coinfections. Further large-scale studies are needed to explore the underlying biological relationship and clinical significance of Helicobacter pylori and HCV coinfection.

1 Introduction

Hepatitis C virus (HCV) infection remains one of the most important global public health concerns and continues to impose a major burden on healthcare systems, particularly in developing countries. It is a blood-borne viral infection that primarily affects the liver and is widely recognized as a major cause of chronic hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma. The long-term nature of HCV infection makes it especially dangerous, as many infected individuals remain asymptomatic for years before developing severe hepatic complications. In regions with limited healthcare resources, poor screening practices, unsafe blood transfusion procedures, reuse of contaminated needles, and inadequate infection-control measures continue to facilitate the spread of HCV. As a result, HCV infection not only represents a serious clinical problem but also a significant socioeconomic challenge. In addition to its hepatic manifestations, HCV infection has also been associated with a variety of extrahepatic disorders, which may further complicate disease progression and patient management. These complications often arise because chronic viral infection can alter host immunity, promote persistent inflammation, and weaken overall physiological status [1]. Therefore, understanding the association of HCV with other infectious agents is important for improving diagnosis, patient care, and long-term therapeutic outcomes. Coinfection with bacterial pathogens may enhance disease burden and may also affect the general health status of infected individuals. For this reason, recent attention has increasingly focused on the possible relationship between HCV and other chronic infections. Helicobacter pylori is a gram-negative, spiral-shaped bacterium that colonizes the gastric mucosa and is one of the most prevalent bacterial infections worldwide. It is strongly associated with several gastrointestinal disorders, including chronic gastritis, peptic ulcer disease, gastric mucosal inflammation, and gastric malignancy. The prevalence of Helicobacter pylori infection is particularly high in low- and middle-income countries, where poor sanitation, overcrowded living conditions, low socioeconomic conditions, and limited access to clean water support its transmission. Because the organism may persist in the body for long periods, it often causes chronic infection and contributes substantially to disease burden in affected populations [2]. The coexistence of HCV infection and Helicobacter pylori infection has become an area of growing scientific interest because both infections are widespread and may coexist in the same patient. This possible coinfection may carry important clinical implications, as both conditions are associated with chronic inflammation and prolonged pathological effects. Some previous observations have

suggested that Helicobacter pylori infection may occur more frequently in individuals with chronic liver diseases, including those infected with HCV. However, the available literature remains inconsistent, and the biological and clinical relationship between these two infections has not yet been fully clarified. This uncertainty highlights the need for further investigation, particularly in local populations where epidemiological data are limited and where both infections are common. Serological analysis is considered an effective and practical approach for the detection of Helicobacter pylori infection, especially in population-based and comparative studies. Laboratory methods such as immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA) are frequently used for screening anti-Helicobacter pylori antibodies because they are simple, rapid, economical, and suitable for processing multiple samples [3]. These techniques are particularly useful in settings where advanced invasive diagnostic procedures are not readily available. In the present context, serological assessment provides an appropriate way to examine the frequency of Helicobacter pylori infection among HCV-positive individuals and to compare it with non-HCV controls. The major characteristics and clinical relevance of both HCV and Helicobacter pylori infection are summarized in Table 1.

Table 1: Comparative overview of Hepatitis C virus and Helicobacter pylori infection

Feature	Hepatitis C virus (HCV)	Helicobacter pylori
Nature of pathogen	Virus	Bacterium
Main site of infection	Liver	Gastric mucosa
Major associated diseases	Chronic hepatitis, cirrhosis, hepatocellular carcinoma	Gastritis, peptic ulcer disease, gastric cancer
Usual mode of transmission	Blood-borne transmission	Oral-oral or fecal-oral transmission
Clinical pattern	Chronic viral liver infection	Chronic gastric bacterial infection
Public health importance	Major cause of chronic liver disease worldwide	Major cause of chronic gastrointestinal infection worldwide
Common detection methods	Serology, PCR, liver function tests	Serology, urea breath test, stool antigen test, endoscopy
Importance in coinfection studies	May influence immune status and chronic inflammation	May increase disease burden in HCV-infected individuals

Considering the widespread prevalence of both infections and the possible consequences of coinfection, it is important to assess whether Helicobacter pylori infection is more frequent in HCV-positive patients than in non-infected individuals. Such an investigation may provide useful epidemiological evidence and may help in improving screening strategies and clinical management practices. Therefore, the present study was conducted to assess Helicobacter pylori serology in relation to HCV infection and to compare the prevalence of anti-Helicobacter pylori antibodies between HCV-positive patients and non-HCV controls. In addition, the study also aimed to evaluate the gender-wise distribution of Helicobacter pylori seropositivity in order to provide a clearer understanding of the infection pattern in both groups.

2 Hepatitis C Virus Infection and Its Clinical Significance:

Hepatitis C virus (HCV) infection is recognized as one of the most serious global infectious diseases and remains a leading cause of chronic liver-related morbidity and mortality worldwide. It is an enveloped, single-stranded RNA virus that is transmitted primarily through blood and blood-derived exposures, with the liver serving as its principal target organ. After entering the host, the virus infects hepatocytes and initiates a complex process of viral persistence, immune activation, and progressive tissue injury. A major challenge associated with HCV infection is that its early clinical phase is frequently asymptomatic or associated with only mild and non-specific symptoms. Consequently, a substantial proportion of infected individuals remain undiagnosed for prolonged periods, allowing the infection to silently progress toward chronic liver damage. This delayed recognition significantly increases the likelihood of severe hepatic complications and makes HCV infection a major concern in both clinical medicine and public health practice [4]. The epidemiological burden of HCV remains considerable, particularly in developing countries where healthcare systems often face limitations in routine screening, diagnostic infrastructure, infection-control practices, and access to antiviral therapy. Transmission is strongly associated with exposure to contaminated blood, including unsafe blood transfusions, reuse of contaminated syringes and needles, poorly sterilized medical or dental instruments, and other invasive procedures carried out under inadequate hygienic conditions. In some settings, additional risk factors such as unsafe therapeutic injections, traditional scarification practices, and lack of awareness regarding blood-borne infections may further contribute to its persistence. The continuing transmission of HCV in resource-limited environments reflects not only a biomedical issue but also a broader public health and socioeconomic problem, as the long-term consequences of infection place a substantial burden on affected individuals, families, and healthcare institutions. From a pathological perspective, the clinical significance of HCV infection lies in its remarkable ability to establish chronicity. Unlike many acute viral infections that are effectively cleared by the host immune response, HCV often evades complete immune elimination and persists within the liver for years or even decades. This prolonged viral persistence stimulates continuous hepatic inflammation and immune-mediated hepatocellular injury. Over time, recurrent cycles of inflammation and repair lead to progressive fibrosis, distortion of hepatic architecture, and gradual deterioration of liver function [5]. If left untreated, this process may culminate in cirrhosis, liver failure, portal hypertension, and hepatocellular carcinoma. Thus, HCV infection is not merely an acute viral illness but a long-term pathological condition with potentially life-threatening outcomes. The progression of HCV-related liver disease is influenced by multiple host and environmental factors, including age, duration of infection, immune status, nutritional condition, alcohol use, metabolic disturbances, and the coexistence of other infections. This multifactorial progression makes HCV a clinically complex disease whose course may vary significantly among individuals. In some patients, hepatic injury advances slowly over many years, while in others the disease progresses more aggressively and leads to advanced liver pathology within a relatively shorter time. This variability underscores the importance of careful clinical assessment and continuous monitoring of infected patients, particularly in populations at risk of coinfection or compromised immune function. In addition to its direct hepatic consequences, HCV infection is increasingly recognized as a systemic disease with a wide range of extrahepatic manifestations. Chronic infection may affect multiple organs and biological systems through persistent immune stimulation, inflammatory signaling, and immune-complex mediated mechanisms. These extrahepatic effects may involve renal, hematological, endocrine, dermatological, metabolic, and immunological abnormalities, thereby expanding the clinical burden of the disease beyond the liver alone. The recognition of HCV as a multisystem disorder has significantly broadened its clinical relevance and has highlighted the importance of investigating the infection

in relation to broader physiological disturbances. This systemic dimension is particularly important when considering the interaction of HCV with other chronic infections, as such coexistence may intensify inflammatory burden and alter disease behavior. Another important dimension of HCV infection is its impact on host immunity and inflammatory regulation. Persistent viral replication may lead to chronic immune activation, dysregulation of cytokine signaling, and altered host defense mechanisms. These immunological changes may influence the body's susceptibility to secondary or concurrent infections and may also modify the clinical expression of coinfecting pathogens. In this regard, HCV-positive individuals constitute an especially important group for the study of coinfection patterns [6]. The coexistence of HCV with chronic bacterial infections may aggravate systemic inflammation, complicate disease presentation, and interfere with effective clinical management. Such interactions are particularly relevant in the context of Helicobacter pylori, a chronic bacterial pathogen that also induces prolonged inflammatory responses and may coexist in vulnerable patient populations. The broader clinical importance of HCV therefore extends beyond its established role in chronic liver disease. It represents a persistent viral condition that combines silent progression, long-term hepatic destruction, immune dysregulation, and multisystem effects. These characteristics make HCV a medically significant infection not only because of its direct pathogenicity but also because of its capacity to interact with other infectious and non-infectious conditions. The study of HCV in relation to bacterial coinfections is especially valuable, as it may reveal additional dimensions of disease burden that are not fully captured by liver-focused evaluation alone. The conceptual progression and clinical impact of HCV infection can be illustrated in Figure 1, beginning with blood-borne exposure and viral entry, followed by hepatocellular infection, chronic hepatic inflammation, fibrosis, cirrhosis, hepatocellular carcinoma, and associated extrahepatic manifestations. Such a framework highlights the dynamic and progressive nature of the infection and supports its characterization as a chronic systemic disease rather than an isolated hepatic disorder.

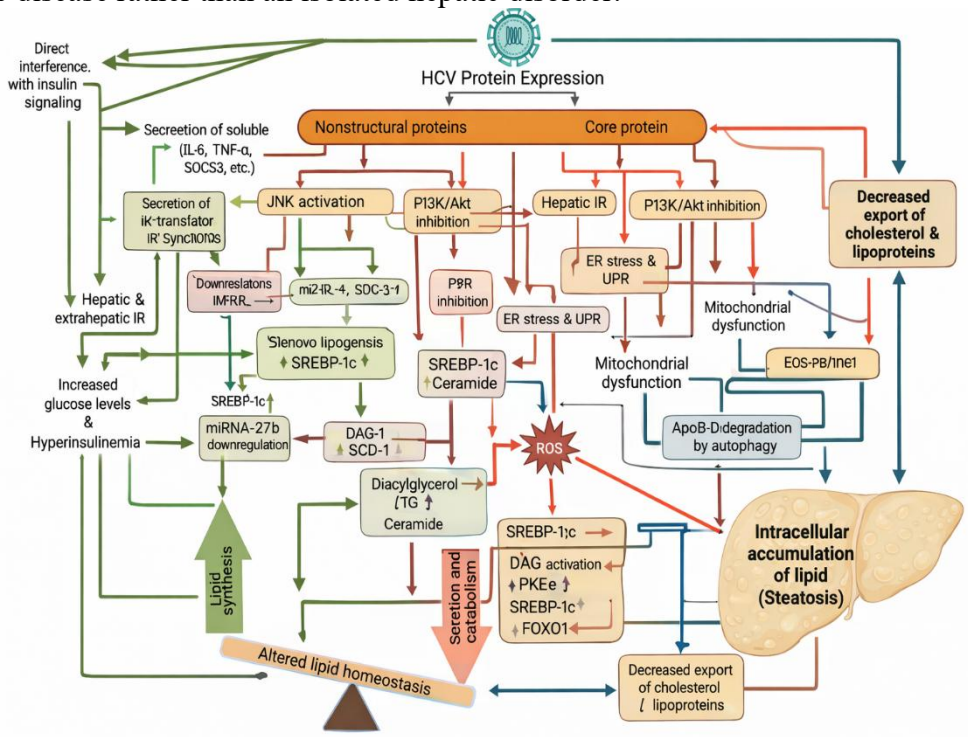

Figure 1: Clinical progression and broader significance of Hepatitis C virus infection.

HCV infection remains a major challenge in modern medicine because of its silent onset, high potential for chronic persistence, progressive liver damage, and broad systemic consequences. Its complex pathogenesis and long-term effects make it an

important subject of clinical, epidemiological, and laboratory investigation. A deeper understanding of the clinical significance of HCV is essential not only for improving diagnosis, prevention, and treatment strategies, but also for clarifying its possible association with other chronic infectious agents. This perspective is particularly relevant for the present study, which seeks to examine the relationship between HCV infection and Helicobacter pylori seropositivity in order to better understand the burden and implications of coinfection.

2 Helicobacter pylori Infection and Serological Assessment:

Helicobacter pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the gastric mucosa and is recognized as one of the most common chronic bacterial infections in humans. It is widely distributed across the world and is particularly prevalent in developing countries, where poor sanitation, overcrowding, low socioeconomic conditions, and limited access to clean drinking water facilitate its transmission. Infection is usually acquired during childhood and may persist for many years or even throughout life if not properly treated. Because of its persistent colonization and chronic inflammatory effects, Helicobacter pylori has become an important pathogen in both clinical medicine and public health research. The clinical significance of Helicobacter pylori infection is strongly linked to its role in gastric disease. It is well known for its association with chronic gastritis, peptic ulcer disease, gastric mucosal injury, atrophic gastritis, and gastric carcinoma [7]. In many infected individuals, the organism remains asymptomatic for long periods, but in others it may contribute to progressive gastric pathology and long-term complications. The ability of Helicobacter pylori to survive in the highly acidic environment of the stomach is one of its most remarkable biological features. Through the production of urease and other adaptive mechanisms, it is able to neutralize gastric acidity locally, penetrate the protective mucus layer, and establish long-term colonization on the gastric epithelium. This persistence contributes to continuous inflammatory stimulation and makes the organism medically important even in the absence of obvious early symptoms. In recent years, Helicobacter pylori has also been studied beyond its conventional role as a gastric pathogen. Chronic infection may influence systemic inflammatory responses, alter immune regulation, and potentially contribute to broader physiological disturbances. For this reason, scientific attention has expanded toward exploring its relationship with extra-gastric conditions and coinfections. The possible coexistence of Helicobacter pylori with chronic viral infections, including Hepatitis C virus infection, is therefore of considerable interest. Since both conditions involve prolonged inflammatory processes and chronic persistence in the host, their combined presence may have implications for disease burden, diagnosis, and patient management. The diagnosis of Helicobacter pylori infection can be carried out using invasive and non-invasive methods. Invasive techniques, such as endoscopic biopsy, histopathology, bacterial culture, and rapid urease testing, provide direct evidence of gastric colonization but are often expensive, technically demanding, and less practical for large-scale screening studies [8]. In contrast, non-invasive approaches such as stool antigen testing, urea breath testing, and serological assays are more feasible for epidemiological and comparative investigations. Among these methods, serological analysis is particularly useful in studies involving a larger number of participants because it is simple, economical, less time-consuming, and suitable for assessing exposure patterns in different study groups. Serological assessment is based on the detection of antibodies against Helicobacter pylori in blood samples. This method is widely used in prevalence studies because it allows researchers to estimate the proportion of individuals who have been exposed to the organism. Although antibody-based methods may not always distinguish active infection from previous exposure, they remain highly informative in comparative studies where the main objective is to examine frequency and distribution of seropositivity. In the context of the present study, serological testing is appropriate

because the aim is to compare Helicobacter pylori seropositivity in HCV-positive patients and non-HCV controls rather than to establish invasive clinical diagnosis of gastric disease. Among serological methods, the immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA) are commonly employed. ICT is a rapid and convenient screening tool that is easy to perform and particularly useful in settings with limited laboratory infrastructure. ELISA, in contrast, is generally considered more sensitive and reliable for detecting anti-Helicobacter pylori antibodies and is commonly used in research-based laboratory analysis [9]. The use of both ICT and ELISA in combination provides a practical strategy for serological screening, enabling rapid preliminary detection along with more dependable antibody assessment. This combined approach enhances the value of serological investigation in studies exploring possible infection patterns and associations. The major biological, clinical, and diagnostic characteristics of Helicobacter pylori relevant to the present study are present in Table 2**.**

Table 2: Biological, clinical, and serological profile of Helicobacter pylori relevant to the present study.

Parameter	**Description**	**Relevance to present study**
Microbiological nature	Gram-negative, spiral-shaped, microaerophilic bacterium	Confirms the bacterial identity of the organism under investigation
Site of colonization	Gastric mucosa	Indicates the primary biological niche of infection
Infection pattern	Usually chronic and long-lasting	Supports its importance in long-term coinfection studies
Main diseases associated	Chronic gastritis, peptic ulcer disease, gastric atrophy, gastric carcinoma	Highlights its clinical significance
Transmission-related factors	Poor sanitation, overcrowding, contaminated environment, low socioeconomic conditions	Explains its high prevalence in developing populations
Host response	Persistent inflammation and antibody production	Justifies the use of serological methods
Serological marker assessed	Anti-Helicobacter pylori antibodies	Provides the basis for infection screening in this study
ICT role	Rapid and simple preliminary screening method	Useful for quick detection in a laboratory setting
ELISA role	Sensitive and more reliable antibody detection method	Suitable for confirmatory serological assessment
Value in comparative studies	Helps determine prevalence and compare exposure across groups	Directly supports comparison between HCV-positive and control groups
Limitation of serology	Does not always clearly separate current from	Important for interpretation of study

	past infection	findings

The diagnostic pathway and research relevance of serological testing for Helicobacter pylori are further presented in Figure 2. *The figure illustrates the sequence of Helicobacter pylori serological assessment, beginning with sample collection, followed by antibody detection through ICT and ELISA, identification of seropositive cases, and comparison of prevalence between HCV-positive patients and non-HCV controls.*

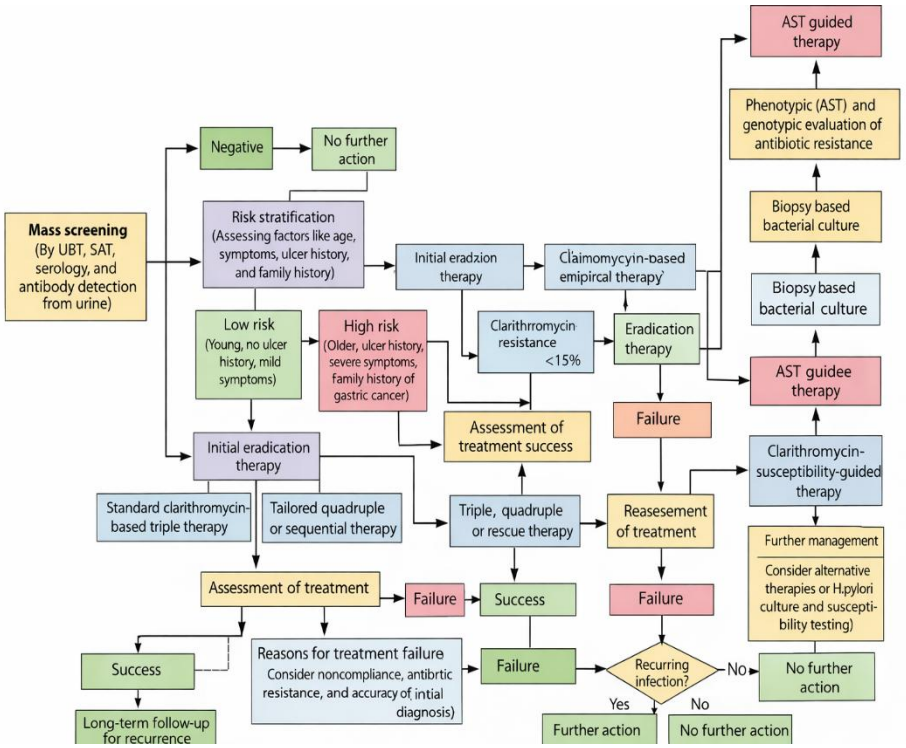

Figure 2: Serological assessment pathway of Helicobacter pylori infection for comparative study analysis.

Helicobacter pylori infection remains a medically significant chronic bacterial disease with substantial diagnostic and epidemiological importance. Its widespread prevalence, ability to persist for long durations, and association with chronic inflammation make it highly relevant in studies of coinfection and disease burden. Serological techniques such as ICT and ELISA provide an effective means of evaluating Helicobacter pylori exposure in research settings, particularly when the goal is to compare infection frequency across clinical groups. Therefore, the serological assessment of Helicobacter pylori forms a central component of the present study and provides an appropriate basis for examining its relationship with HCV infection.

3 Association between Hepatitis C Virus and Helicobacter pylori:

The possible association between Hepatitis C virus (HCV) infection and Helicobacter pylori infection has emerged as an important topic of clinical and epidemiological interest due to the chronic and persistent nature of both pathogens. HCV is a long-standing viral infection that primarily affects the liver and causes progressive hepatic injury, whereas Helicobacter pylori is a chronic bacterial infection of the gastric mucosa that induces prolonged local and systemic inflammatory responses. Although these two pathogens affect different primary organ systems, both are capable of producing sustained immune activation, chronic inflammation, and long-term pathological consequences [10]. This shared chronicity has led researchers to investigate whether a meaningful association exists between HCV infection and Helicobacter pylori seropositivity, and whether coinfection may contribute to an increased burden of disease. The clinical importance of this possible association lies in the fact that patients with HCV infection often exhibit impaired physiological status due to chronic liver

inflammation, altered metabolism, and changes in immune function. Persistent HCV infection may influence host defense mechanisms and may create a biological environment that is more favorable for the persistence or acquisition of other infections. In such circumstances, Helicobacter pylori infection may occur more frequently or may have a greater clinical impact in HCV-positive individuals than in the general population. The coexistence of these two infections may therefore represent more than a simple coincidence and may instead reflect overlapping pathological or immunological mechanisms that deserve careful investigation. Several mechanisms have been proposed to explain why Helicobacter pylori infection might be more common in HCV-infected patients. One possible explanation is that chronic HCV infection leads to immune dysregulation, which may reduce the host's ability to control bacterial colonization effectively. Another possibility is that long-term hepatic disease associated with HCV results in broader physiological weakness and inflammatory imbalance, thereby increasing vulnerability to coinfection. In addition, socioeconomic and environmental factors may contribute to the simultaneous occurrence of both infections, particularly in developing countries where poor sanitation, limited healthcare access, and inadequate infection-control measures increase overall exposure to infectious agents [11]. Therefore, the relationship between HCV and Helicobacter pylori may involve biological, clinical, and environmental dimensions rather than a single isolated factor. A number of previous studies have explored this possible relationship and have reported varying findings. Some investigations have shown that Helicobacter pylori seropositivity is significantly higher in patients with chronic liver disease, especially among those infected with HCV. These studies suggest that HCV-positive patients may constitute a higher-risk group for Helicobacter pylori infection and that coinfection may be linked with greater disease burden. In some reports, the presence of Helicobacter pylori has also been discussed in relation to worsening hepatic inflammation, progression of liver dysfunction, or increased systemic inflammatory activity. Such observations have strengthened the hypothesis that coinfection may have clinical relevance beyond simple coexistence. Despite these observations, the relationship between HCV and Helicobacter pylori remains inconsistent in the available literature. Some studies have demonstrated a strong association, whereas others have reported weak, statistically insignificant, or contradictory findings. These inconsistencies may arise from differences in study population, sample size, geographical location, diagnostic method, age distribution, socioeconomic background, and the criteria used to define infection status. In addition, some researchers have used serological approaches, while others have relied on invasive or non-invasive diagnostic methods with different levels of sensitivity and specificity. Such methodological variation makes direct comparison across studies difficult and contributes to the lack of uniform consensus regarding the strength and nature of the association. From a clinical perspective, the coexistence of HCV and Helicobacter pylori may have important implications for patient care. If Helicobacter pylori infection is more common among HCV-positive individuals, routine screening may become relevant in selected patient groups, particularly in settings where gastrointestinal symptoms, chronic inflammation, or unexplained disease burden is present. Coinfection may complicate clinical presentation and may require broader diagnostic evaluation than liver-focused management alone. Moreover, identifying Helicobacter pylori in HCV-positive patients may help clinicians adopt a more comprehensive approach to patient assessment and disease control. The association between HCV and Helicobacter pylori remains an important but not yet fully resolved area of scientific inquiry. Available evidence suggests that coinfection may be more frequent in HCV-infected individuals, but inconsistencies in reported findings indicate the need for additional studies using well-defined populations and appropriate diagnostic methods. Understanding this relationship is important not only for clarifying epidemiological patterns but also for improving screening, diagnosis, and patient management strategies. For this reason, the

present study seeks to evaluate Helicobacter pylori seropositivity in HCV-positive individuals and compare it with non-HCV controls in order to contribute to a clearer understanding of the possible association between these two clinically significant infections.

2 Methodology:

This chapter presents the methodological framework adopted for the present study. It explains the overall research design, study setting, participant selection criteria, sample collection procedures, laboratory methods used for the detection of Hepatitis C virus and Helicobacter pylori, and the approach used for data interpretation. The methodology was developed to provide a clear and systematic basis for examining the serological relationship between HCV infection and Helicobacter pylori infection among the selected study population.

2.1 Study Design and Study Area:

The present study was designed as a hospital-based comparative serological investigation to evaluate Helicobacter pylori infection in relation to Hepatitis C virus (HCV) infection. The comparative nature of the study made it possible to examine the serological frequency of Helicobacter pylori among HCV-positive individuals and to compare these findings with those observed in non-HCV controls. This design was considered appropriate because it allowed the researcher to assess the possible association between the two infections through systematic laboratory-based evaluation of blood samples obtained from the selected study population. The study was conducted in the metropolitan region of Islamabad and Rawalpindi, Pakistan, which represents an important urban healthcare zone with access to multiple tertiary and secondary care institutions. These cities serve a large and diverse population from different socioeconomic, educational, and residential backgrounds. Because of this demographic diversity, the study area provided a suitable setting for the collection of clinically relevant samples from individuals presenting with different health conditions. Conducting the study in this geographical region also increased the practical value of the findings, as both HCV infection and Helicobacter pylori infection remain important public health concerns in Pakistan. Clinical samples were collected from several major healthcare institutions, including Pakistan Institute of Medical Sciences (PIMS), Ali Medical Center, Polyclinic Hospital Islamabad, Holy Family Hospital, District Headquarters (DHQ) Hospital, Benazir Bhutto Hospital Rawalpindi, and Health Sciences Laboratory at Comwave Institute Islamabad. The involvement of these institutions strengthened the study by enabling sample collection from a broader patient base rather than relying on a single hospital or laboratory [12]. This multi-center approach enhanced the diversity of the study population and improved the representativeness of the collected samples. It also reduced the possibility of location-specific bias and allowed the data to reflect a wider clinical background. A hospital-based setting was especially appropriate for this investigation because the study required the inclusion of participants undergoing clinical evaluation, laboratory screening, or diagnostic assessment related to liver abnormalities and infectious conditions. Hospitals and diagnostic laboratories provided direct access to patients with suspected or confirmed HCV infection, as well as to non-HCV individuals who could serve as controls for comparative analysis. In addition, the hospital environment ensured the availability of trained personnel, standard sample collection procedures, and proper laboratory handling facilities, all of which were essential for maintaining the quality and reliability of the study. The flow of the study design and institutional sample collection process is illustrated in Figure 3. *The figure illustrates the hospital-based comparative design of the study, beginning with the selection of healthcare institutions in Islamabad and Rawalpindi, followed by participant recruitment, blood sample collection, serological testing, and comparative analysis of HCV-positive and*

non-HCV groups.

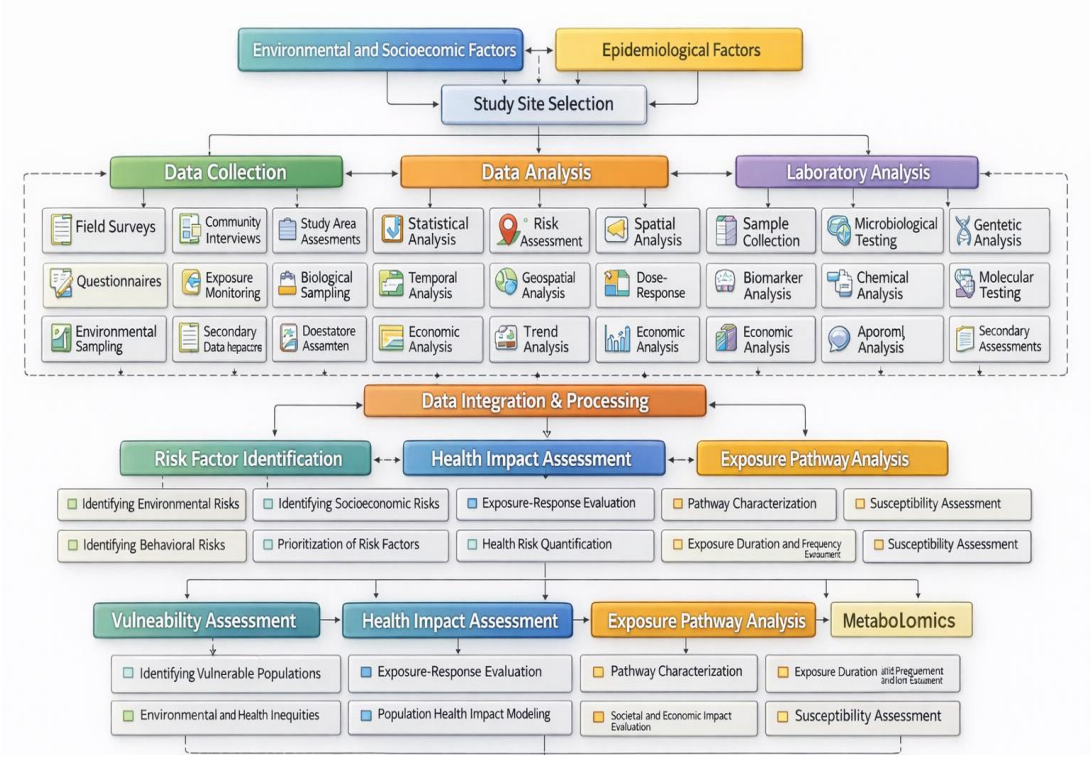

Figure 3: Overview of the study design and study area framework.

The chosen study design and study area provided a strong methodological foundation for the present investigation. The inclusion of multiple healthcare institutions from Islamabad and Rawalpindi improved the diversity and representativeness of the selected sample population, while the hospital-based comparative serological approach ensured that the relationship between HCV infection and Helicobacter pylori infection could be examined in a structured and clinically relevant manner. Therefore, this study setting was considered suitable for generating meaningful findings regarding the serological association between these two clinically significant infections.

2.2 Sample Collection, Inclusion Criteria, and Processing:

A total of 80 blood samples were collected from the selected study participants for serological investigation. The study population consisted of 40 HCV-positive individuals and 40 non-HCV controls, allowing a comparative evaluation of Helicobacter pylori seropositivity between the two groups. Blood collection was carried out under standard clinical and aseptic conditions to ensure sample quality and patient safety. Approximately 5 cc of venous blood was obtained from each participant using sterile disposable syringes and properly labeled collection tubes. The use of standard blood collection procedures was essential for minimizing contamination, preserving sample integrity, and ensuring the reliability of subsequent laboratory analysis. The samples were collected from participants attending the selected hospitals and diagnostic centers included in the study area. Efforts were made to obtain samples from different healthcare institutions so that the study population would represent a broader clinical background rather than a single-center sample source. After collection, all blood specimens were carefully transported to the Health Sciences Laboratory under appropriate handling conditions for further serological processing. Proper transportation and handling of blood samples were important to avoid degradation, clotting irregularities, or other pre-analytical errors that could affect the validity of the test results. Following transportation to the laboratory, the collected blood samples were processed for serum or plasma separation. Each sample was centrifuged according

to standard laboratory procedures to obtain a clear supernatant suitable for serological testing [13]. Only clear and non-hemolyzed serum or plasma samples were selected for analysis, while compromised or poor-quality samples were excluded from diagnostic use. This step was necessary because hemolyzed or contaminated samples may interfere with antigen-antibody reactions and reduce the accuracy of immunological assays such as ICT and ELISA. Therefore, careful sample processing formed an essential part of the study methodology and contributed to the reliability of the generated data. The inclusion criteria were established to ensure that the selected participants were relevant to the objectives of the study. Individuals of different age groups and both genders were considered for inclusion. Participants showing elevated serum alanine aminotransferase (ALT/SGPT) levels above the normal range during liver function testing were regarded as more appropriate for screening, as raised ALT/SGPT values may indicate hepatic inflammation or liver-related abnormality. Such individuals were considered suitable for evaluation of HCV infection and its possible relationship with Helicobacter pylori seropositivity. The inclusion of participants with suspected liver dysfunction strengthened the clinical relevance of the investigation. In contrast, individuals with normal ALT/SGPT values were excluded from the primary study selection criteria used for suspected hepatic involvement. This exclusion helped narrow the study population to participants who were more likely to show liver-associated clinical abnormalities, thereby improving the relevance of the comparison between infected and non-infected groups. In addition, the control group comprised non-HCV individuals who were included for comparative purposes so that the prevalence of Helicobacter pylori antibodies could be assessed against a non-HCV background. This comparative grouping was essential for evaluating the serological association between the two infections. The major components of sample collection, participant selection, and laboratory processing are shown in Table 3.

Table 3: Summary of sample collection, inclusion criteria, and processing procedures

Component	Description
Total study participants	80 individuals
Study groups	40 HCV-positive participants and 40 non-HCV controls
Sample type	Venous blood
Quantity collected	Approximately 5 cc from each participant
Collection conditions	Standard aseptic and clinical procedures
Source of samples	Selected hospitals and diagnostic laboratories in Islamabad and Rawalpindi
Laboratory destination	Health Sciences Laboratory for serological analysis
Processing method	Centrifugation for serum or plasma separation
Acceptable sample quality	Clear and non-hemolyzed serum/plasma
Inclusion criteria	Different age groups, both genders, elevated ALT/SGPT levels
Exclusion criteria	Individuals with normal ALT/SGPT values
Methodological significance	Improved relevance for liver-related screening and reliable serological testing

Sample collection, inclusion criteria, and processing procedures were carefully structured to support the objectives of the present study. The use of clearly defined eligibility criteria ensured that the selected participants were clinically relevant to the investigation, while standardized blood collection and processing methods enhanced

the quality and reliability of the laboratory findings. By maintaining careful control over the pre-analytical phase, the study established a strong methodological basis for the serological assessment of Helicobacter pylori in relation to HCV infection.

2.3 Screening and Confirmation of Hepatitis C Virus Infection:

The detection of Hepatitis C virus (HCV) infection in the present study was carried out in two sequential stages, namely initial screening by immunochromatographic test (ICT) followed by confirmatory analysis using enzyme-linked immunosorbent assay (ELISA). This two-step laboratory approach was adopted to improve the reliability and accuracy of HCV diagnosis among the selected participants. The use of ICT provided a rapid and practical preliminary screening tool, whereas ELISA served as a more sensitive and dependable method for confirming the serological status of the samples. Together, these techniques formed an effective diagnostic strategy for identifying HCV-positive individuals included in the study. For initial screening, an HCV ICT kit manufactured by Healgen Scientific LLC, USA, was used according to the standard protocol provided by the manufacturer. Before performing the test, serum or plasma was separated from whole blood through centrifugation [14]. This step was necessary to obtain a suitable sample matrix free from cellular components that could interfere with the immunological reaction. Once the serum or plasma had been prepared, one drop of the sample was dispensed into the sample well of the ICT cassette. Immediately afterward, one drop of the supplied buffer solution was added to facilitate the capillary movement of the sample across the membrane. The test result was then observed and interpreted within 15 minutes, as recommended by the kit protocol. The interpretation of ICT results was based on the appearance of colored lines in the designated regions of the cassette. The appearance of two colored lines, one in the control region and one in the test region, was considered indicative of a positive result for HCV infection. In contrast, the appearance of only one colored line in the control region indicated a negative result. If the control line failed to appear, the test was considered invalid regardless of the presence or absence of a test line, and such results were excluded or repeated as required. The control line served as an internal quality indicator to confirm the proper functioning of the test cassette and the adequacy of sample migration. Although ICT is a useful and rapid screening method, it is generally considered more appropriate for preliminary identification rather than final confirmation of infection. For this reason, positive or suspected samples were further evaluated using ELISA, which offers improved sensitivity and specificity for serological detection [15]. ELISA was performed using a standard HCV detection kit following the protocol recommended by the manufacturer. Positive and negative controls were included in each assay run to validate the test performance, while blank wells were maintained for calibration and background correction. The inclusion of these controls was essential for ensuring the accuracy and reproducibility of the assay. In the ELISA procedure, specimen diluent and serum samples were dispensed into the assigned wells of the microtiter plate. The plate was then incubated at 37°C for the specified duration to allow antigen-antibody interaction to occur. After incubation, the wells were washed thoroughly with the appropriate wash buffer in order to remove any unbound substances. Subsequently, horseradish peroxidase (HRP)-conjugate was added to each well, followed by a second incubation period. This step allowed the enzyme-labeled conjugate to bind to the immune complexes formed during the first reaction phase. After another washing step, chromogen solutions were added to produce a measurable color reaction. The enzymatic conversion of the substrate generated a color intensity proportional to the amount of bound analyte present in the sample. Finally, stop solution was added to terminate the reaction, and the absorbance was measured at 450 nm with a reference wavelength of 630 nm using an ELISA plate reader. The optical density readings obtained from the samples were compared with the calculated cut-off value, and the final results were interpreted in accordance with the manufacturer's

instructions. This approach enabled the identification of confirmed HCV-positive and HCV-negative samples on the basis of standardized serological criteria. The confirmatory role of ELISA was especially important in the present study because accurate classification of HCV infection status was essential for subsequent comparison with Helicobacter pylori seropositivity. The major features of HCV screening and confirmation used in the study are summarized in Table 4.

Table 4: Screening and confirmatory methods used for HCV detection

Method	Purpose	Procedure summary	Interpretation basis	Importance in study
Immunochromatographic test (ICT)	Initial screening	One drop of serum/plasma and one drop of buffer added to cassette	Two lines = positive; one control line = negative; no control line = invalid	Rapid preliminary identification of HCV infection
Enzyme-linked immunosorbent assay (ELISA)	Confirmatory testing	Serum, controls, and reagents added to microtiter wells followed by incubation, washing, conjugate reaction, and absorbance reading	Results interpreted using cut-off values and optical density readings	More sensitive and reliable confirmation of HCV status

The screening and confirmation of HCV infection were carried out through a structured and reliable serological approach. The use of ICT as a rapid screening tool, followed by ELISA as a confirmatory method, strengthened the diagnostic accuracy of the study and ensured appropriate classification of participants into HCV-positive and non-HCV groups. This methodological step was essential because the entire comparative framework of the study depended on the accurate identification of HCV infection status before the assessment of Helicobacter pylori serology.

2.4 Assessment of Helicobacter pylori by ELISA:

The serological assessment of Helicobacter pylori infection in the present study was performed by enzyme-linked immunosorbent assay (ELISA) using the EUROIMMUN ELISA kit for the detection of IgG antibodies against Helicobacter pylori antigens in human serum or plasma. This assay was selected because it provides a practical, reliable, and widely accepted serological approach for evaluating exposure to Helicobacter pylori in comparative and epidemiological studies. ELISA is particularly useful in studies involving multiple samples because of its sensitivity, reproducibility, and ability to generate semiquantitative as well as quantitative data. In the context of the present investigation, the use of ELISA made it possible to determine the serological status of Helicobacter pylori among both HCV-positive patients and non-HCV controls in a standardized laboratory setting. The EUROIMMUN ELISA kit used in the study

was based on microtiter wells pre-coated with specific Helicobacter pylori antigens. These antigen-coated wells served as the reaction surface for the binding of anti-Helicobacter pylori antibodies present in patient serum or plasma [16]. Prior to the assay, serum or plasma samples were prepared under proper laboratory conditions to ensure their suitability for immunological testing. Diluted patient samples, calibrators, and controls were then dispensed into separate wells according to the assay protocol. The inclusion of calibrators and controls was essential for maintaining assay validity, ensuring standardization, and allowing accurate interpretation of the obtained results. During the first incubation step, the samples were allowed to remain in contact with the antigen-coated wells at room temperature for the required period. If a patient sample contained specific IgG antibodies against Helicobacter pylori, these antibodies bound to the immobilized antigens present on the well surface, resulting in the formation of antigen-antibody complexes. Samples lacking these specific antibodies did not form such complexes to a significant extent. This stage represented the primary immunological reaction of the assay and formed the basis for the subsequent detection process. After the first incubation, the wells were thoroughly washed to remove unbound substances, including excess serum components and non-specifically attached materials. Proper washing was a critical step in the assay because inadequate removal of unbound material could lead to background interference and inaccurate results [17]. Following the washing step, enzyme conjugate was added to each well. This conjugate was designed to bind specifically to the antigen-antibody complexes formed during the first reaction phase. The plate was then subjected to a second incubation period to allow adequate interaction between the conjugate and the bound antibodies. After the second incubation, the wells were washed again to remove any unbound enzyme conjugate. Subsequently, a chromogen/substrate solution was added to each well to initiate a color-producing enzymatic reaction. In wells containing bound enzyme conjugate, the substrate underwent conversion to produce a visible color change. The intensity of this color was directly proportional to the amount of anti-Helicobacter pylori IgG antibodies present in the sample. Therefore, samples with higher antibody concentrations produced a stronger color reaction, whereas samples with little or no antibody activity showed minimal color development. The enzymatic reaction was terminated by the addition of stop solution, which stabilized the developed color and allowed accurate measurement of the reaction endpoint. The optical density of each well was then measured using a microplate reader at 450 nm with a reference wavelength of 630 nm. These absorbance values were used to estimate the level of anti-Helicobacter pylori antibodies present in each sample. Because ELISA provides measurable optical density values, it offers an advantage over simpler rapid tests by allowing more precise evaluation of antibody response and more dependable classification of serological status. The interpretation of the ELISA results was performed according to the criteria provided by the kit manufacturer. Samples with values below 16 RU/ml were considered negative, indicating the absence of significant detectable antibody levels. Values from 16 to less than 22 RU/ml were categorized as borderline, suggesting an intermediate result that may require cautious interpretation. Samples with values equal to or greater than 22 RU/ml were considered positive for anti-Helicobacter pylori IgG antibodies. This classification system allowed the study population to be divided into negative, borderline, and positive categories for subsequent comparison between HCV-positive patients and non-HCV controls [18]. The stepwise process of Helicobacter pylori detection by ELISA is illustrated in Figure 4**.** *The figure illustrates the serological detection pathway of Helicobacter pylori by ELISA, beginning with serum or plasma preparation, followed by addition of samples to antigen-coated wells, incubation, washing, conjugate reaction, chromogenic color development, optical density measurement, and interpretation of results as negative, borderline, or positive.*

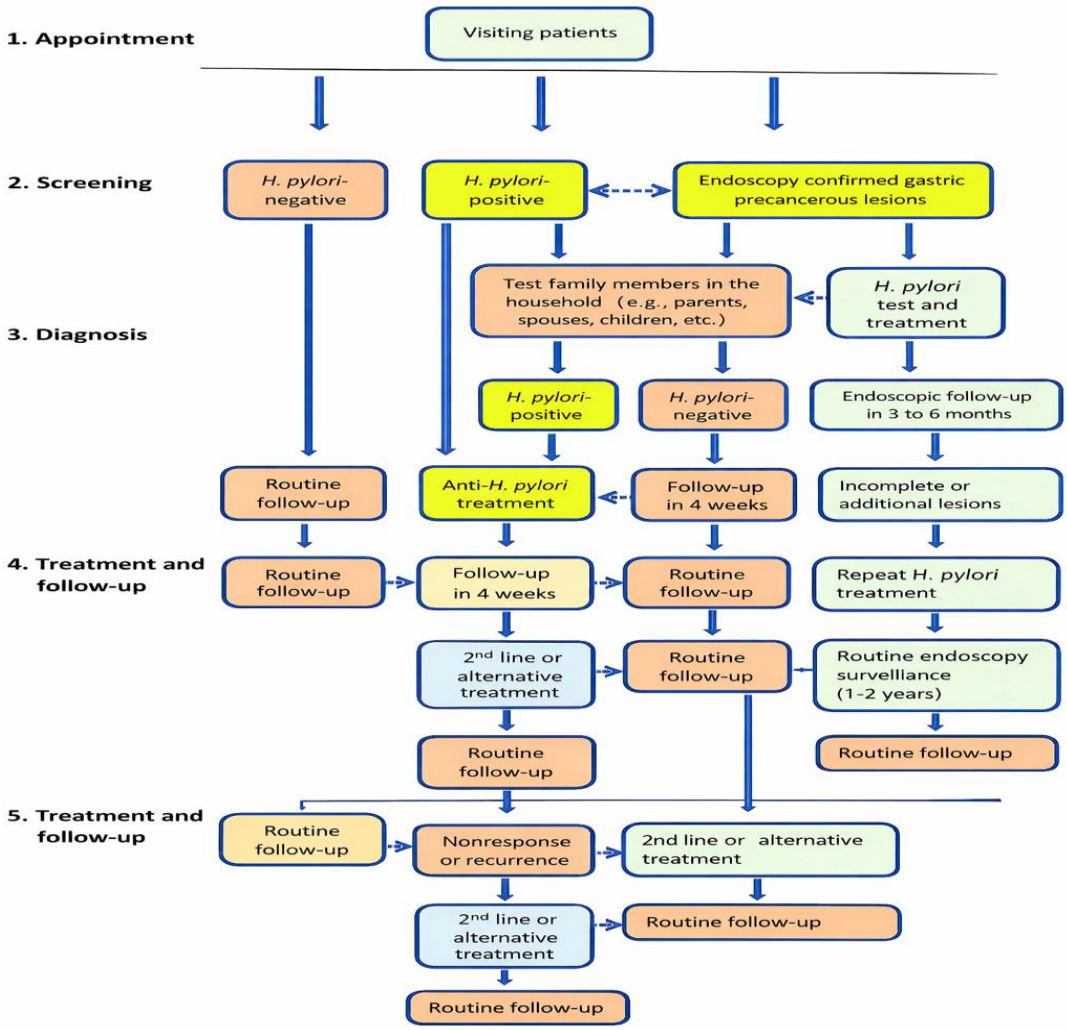

Figure 4: Flow diagram of ELISA-based assessment of Helicobacter pylori infection.

The assessment of Helicobacter pylori by ELISA provided a reliable and scientifically appropriate method for determining antibody status in the selected study population. The use of a standardized commercial kit, together with defined calibration and interpretation criteria, improved the accuracy and reproducibility of the assay. This method was particularly suitable for the objectives of the study because it enabled clear comparison of Helicobacter pylori seropositivity between HCV-positive individuals and non-HCV controls. Therefore, ELISA-based serological analysis formed a central laboratory component of the present research and provided the necessary basis for evaluating the possible relationship between Helicobacter pylori infection and HCV infection.

2.5 Materials Used and Data Analysis:

A variety of laboratory materials and equipment were used in the present study to ensure proper sample collection, handling, processing, screening, and serological analysis. These materials were essential for maintaining the accuracy, consistency, and reliability of the experimental procedures carried out during the investigation. The major materials included specimen collection containers, sterile syringes, centrifuge, pipettes, disposable tips, immunochromatographic test cassettes, desiccants, buffer solution, ELISA kits for HCV and Helicobacter pylori detection, ELISA plate reader, microcentrifuge tubes, adhesive foil or paper tape, tissue paper, and other standard laboratory consumables. Each of these items played a specific role in supporting the different phases of the study, beginning from blood collection and extending to

serological testing and final result interpretation. The specimen collection containers and sterile syringes were used for safe and hygienic collection of venous blood samples from the selected participants. Proper labeling and handling of these containers were important for maintaining sample identity and preventing mix-up during transportation and processing [19]. After collection, the centrifuge was used for the separation of serum or plasma from whole blood. This step was crucial because the serological assays performed in the study required clear serum or plasma samples for accurate antigen-antibody reactions. Pipettes and disposable tips were employed for precise measurement and transfer of serum samples, diluents, controls, and reagents during ICT and ELISA procedures. The use of accurate liquid-handling devices was especially important for minimizing procedural error and ensuring reproducibility of results. The ICT test cassettes, desiccants, and buffer solution formed the core materials used for the preliminary screening of Hepatitis C virus infection. These components allowed rapid serological testing and helped identify samples that required further confirmation. Similarly, ELISA kits for HCV and Helicobacter pylori provided the necessary reagents, coated wells, controls, calibrators, enzyme conjugates, chromogenic substrates, and stop solutions required for detailed serological analysis. The ELISA plate reader was used to measure optical density values at the specified wavelengths, thereby enabling objective interpretation of the assay results. Additional materials such as microcentrifuge tubes, adhesive foil or paper tape, and tissue paper supported sample preparation, reagent handling, sealing of microtiter plates, and maintenance of a clean working environment throughout the testing process. The principal materials and their functions in the study are present in Table 5**.**

Table 5: Major materials used in the study and their functions

Material/Equipment	**Function in the study**
Specimen collection containers	Collection and storage of blood samples
Sterile syringes	Venous blood withdrawal from participants
Centrifuge	Separation of serum or plasma from whole blood
Pipettes and disposable tips	Accurate measurement and transfer of samples and reagents
ICT test cassettes	Preliminary screening of HCV infection
Buffer solution	Facilitation of sample migration in ICT procedure
Desiccants	Maintenance of ICT kit quality and moisture protection
ELISA kit for HCV	Confirmatory serological detection of HCV infection
ELISA kit for Helicobacter pylori	Detection of anti-Helicobacter pylori antibodies
Microcentrifuge tubes	Temporary holding and handling of processed samples
ELISA plate reader	Measurement of optical density during ELISA analysis
Adhesive foil or paper tape	Covering ELISA plates during incubation
Tissue paper and other consumables	Maintenance of cleanliness and routine laboratory support

In addition to the materials used, data analysis formed an equally important component

of the methodological framework. The results generated from ICT and ELISA testing were carefully recorded and organized in a systematic manner for interpretation and comparison. The primary purpose of data analysis was to determine the frequency of HCV infection and Helicobacter pylori seropositivity among the selected study participants. Since the study was based on a comparative design, the obtained results were evaluated separately for HCV-positive individuals and non-HCV controls so that the serological distribution of Helicobacter pylori could be examined in relation to HCV infection. The analytical process involved calculation of frequencies, percentages, and group-wise comparisons. The number of seropositive and seronegative individuals in each study group was identified, and the distribution of Helicobacter pylori antibodies was assessed accordingly. In addition to overall comparison between the two main groups, the data were also interpreted with reference to gender and other relevant demographic or clinical variables where applicable. This helped provide a broader understanding of infection distribution and strengthened the interpretation of the findings in relation to the objectives of the study. The comparative analysis was particularly important because the main purpose of the investigation was not only to detect infection status, but also to assess whether Helicobacter pylori seropositivity was more frequent among HCV-infected individuals than among non-HCV controls [20]. By arranging the laboratory results in a structured form and comparing the proportions between groups, the study was able to evaluate the possible serological association between these two infections. The analytical approach therefore served as the basis for presenting meaningful findings and drawing conclusions regarding the relationship between HCV infection and Helicobacter pylori infection. The materials used in the study provided the practical foundation for sample collection, serological testing, and laboratory accuracy, while the data analysis process provided the interpretive framework for understanding the obtained results. The combination of appropriate materials, standardized laboratory procedures, and structured analytical evaluation strengthened the methodological quality of the research and supported a reliable assessment of Helicobacter pylori serology in relation to HCV infection.

3 Result and Discussion:

The present study was conducted to assess Helicobacter pylori serology in relation to Hepatitis C virus (HCV) infection and to compare its prevalence between HCV-positive individuals and non-HCV controls. A total of 80 blood samples were included in the investigation, comprising 40 HCV-positive participants and 40 HCV-negative individuals taken as controls. The samples were collected from major hospitals of the capital region, including District Headquarters Hospital Rawalpindi, Holy Family Hospital Rawalpindi, Benazir Bhutto Hospital Rawalpindi, and Pakistan Institute of Medical Sciences Hospital Islamabad. Equal representation of HCV-positive and HCV-negative samples was maintained in order to ensure balance in the comparative analysis and to provide a clear basis for evaluating the serological association between HCV infection and Helicobacter pylori infection. The hospital-wise representation of collected samples is presented in Table 6.

Table 6: Representation of samples collected from participating hospitals

Hospital	**HCV-positive samples**	**HCV-negative samples**
District Headquarters Hospital Rawalpindi	10	10
Holy Family Hospital Rawalpindi	10	10
Benazir Bhutto Hospital Rawalpindi	10	10
Pakistan Institute of Medical	10	10

Sciences Hospital Islamabad		
Total	**40**	**40**

The study findings demonstrated that Helicobacter pylori seropositivity was higher among HCV-positive individuals than among non-HCV controls. Among the 40 HCV-positive participants, 20 individuals, representing 50%, were found positive for Helicobacter pylori infection. In contrast, only 10 out of 40 individuals, representing 25%, in the non-HCV control group showed seropositivity for Helicobacter pylori. This difference indicates that Helicobacter pylori infection was more common among patients infected with HCV than among those without HCV infection. The comparative frequency observed in the present study suggests a possible association between chronic HCV infection and increased prevalence of Helicobacter pylori [21]. This relationship may be of clinical importance because both infections are chronic in nature and may contribute to inflammatory burden, poor health status, and complex disease presentation when they occur together. A more detailed examination of the HCV-positive group further revealed an interesting gender-wise distribution pattern. Among the 40 HCV-positive individuals, 30 were males and 10 were females. Out of the 30 male participants, 13, representing 43.33%, were positive for Helicobacter pylori. In comparison, 7 out of 10 female participants, representing 70%, showed seropositivity. These findings indicate that although males constituted the greater proportion of the HCV-positive group, the percentage of Helicobacter pylori positivity was higher among females. The gender-wise distribution of Helicobacter pylori seropositivity in the HCV-positive population is presented in Table 7.

Table 7: Gender-wise distribution of Helicobacter pylori among HCV-positive participants

Characteristics	**Total HCV-positive participants**	**Helicobacter pylori positive**
Male	30/40 (75%)	13/30 (43.33%)
Female	10/40 (25%)	7/10 (70%)
Total	**40/40 (100%)**	**20/40 (50%)**

In the non-HCV control group, Helicobacter pylori infection was also detected, but at a lower frequency than in the HCV-positive group. Among the 40 non-HCV participants, 10 individuals, corresponding to 25%, were found positive for Helicobacter pylori. The gender composition of the control group was balanced, with 20 males and 20 females. Among the male controls, 3 out of 20, representing 15%, were positive for Helicobacter pylori, whereas 7 out of 20 females, representing 35%, showed seropositivity. Similar to the HCV-positive group, the control group also demonstrated a higher percentage of Helicobacter pylori positivity among females than males. These findings are summarized in Table 8.

Table 8: Gender-wise distribution of Helicobacter pylori among non-HCV participants

Characteristics	**Total non-HCV participants**	**Helicobacter pylori positive**
Male	20/40 (50%)	3/20 (15%)
Female	20/40 (50%)	7/20 (35%)
Total	**40/40 (100%)**	**10/40 (25%)**

When the results of both groups are viewed together, the comparative pattern becomes more evident. The HCV-positive group showed double the prevalence of Helicobacter pylori seropositivity compared with the non-HCV control group. Specifically, the seropositivity rate was 50% in HCV-infected individuals, whereas it was 25% in non-HCV participants. This overall comparison is shown in Table 9. The findings strongly

indicate that Helicobacter pylori infection was more frequent in individuals with HCV infection, thereby suggesting a possible epidemiological and serological relationship between the two conditions.

Table 9: Comparative prevalence of Helicobacter pylori in HCV-positive and non-HCV groups

Study group	Total participants	Helicobacter pylori positive	Percentage positive
HCV-positive group	40	20	50%
Non-HCV group	40	10	25%

The observed difference between the two groups may be explained by several biological and clinical factors. HCV infection is characterized by chronic hepatic inflammation, altered immune regulation, and long-term physiological stress. Persistent viral infection may impair normal host defense mechanisms and increase susceptibility to other chronic infections. In such a state, Helicobacter pylori may colonize more easily or persist for longer durations, thereby increasing the likelihood of seropositivity in HCV-positive individuals. The coexistence of both infections may therefore reflect a combination of immune dysregulation, inflammatory imbalance, and reduced resistance to secondary or concurrent microbial exposure. These mechanisms may help explain the higher frequency of Helicobacter pylori antibodies observed among HCV-infected participants in the present study. Another important point emerging from the results is the relatively high burden of Helicobacter pylori infection in the overall study population. Even in the non-HCV control group, one-quarter of the participants were positive for Helicobacter pylori. This finding is not unexpected in a developing-country setting, where poor sanitation, overcrowding, low socioeconomic conditions, and limited access to clean water can increase exposure to Helicobacter pylori [22]. However, despite this background prevalence, the HCV-positive group still showed a markedly higher rate of seropositivity. This strengthens the argument that the greater frequency observed in HCV-infected individuals is not merely due to general population exposure alone, but may also reflect the influence of HCV-related clinical status on susceptibility to Helicobacter pylori infection. The gender-wise findings also deserve consideration. In both HCV-positive and non-HCV groups, female participants showed a higher percentage of Helicobacter pylori seropositivity than male participants. In the HCV-positive group, 70% of females were positive compared with 43.33% of males, while in the non-HCV group, 35% of females were positive compared with 15% of males. Although this pattern appears notable, it should be interpreted with caution. The number of females in the HCV-positive group was relatively small, and therefore the observed difference may not be sufficient to establish a definite gender-based conclusion. Nevertheless, the trend suggests that gender-related differences in infection prevalence may exist and could be further explored in larger studies with more balanced participant distribution. A combined overview of gender-wise positivity in both groups is provided in Table 10.

Table 10: Comparative gender-wise Helicobacter pylori positivity in study groups

Group	Male positivity	Female positivity
HCV-positive	13/30 (43.33%)	7/10 (70%)
Non-HCV	3/20 (15%)	7/20 (35%)

The present findings are in line with the broader scientific view that coinfection may increase disease burden and complicate patient management. Since HCV is a chronic viral infection associated with liver dysfunction and immune changes, and Helicobacter

pylori is a chronic bacterial infection associated with persistent gastric inflammation, the coexistence of these two pathogens may contribute to more complex clinical outcomes. HCV-positive patients may experience additional physiological stress in the presence of Helicobacter pylori infection, and this may have implications for diagnosis, monitoring, and treatment. From a clinical perspective, the findings suggest that screening for Helicobacter pylori in HCV-infected individuals may be beneficial, particularly in populations where both infections are common. Despite the significance of these results, certain limitations should be acknowledged. The total sample size of the study was relatively small, which may affect the generalizability of the findings to larger populations. In addition, the detection of Helicobacter pylori was based on serological methods, which identify antibody response rather than direct active infection. Therefore, positive serology may represent either current infection or previous exposure. Furthermore, local environmental and socioeconomic factors may also have influenced the frequency of Helicobacter pylori in both groups [23]. Even with these limitations, the study provides useful preliminary evidence that Helicobacter pylori seropositivity is more frequent among HCV-positive individuals than among non-HCV controls. Overall, the combined results and discussion of the present study indicate that Helicobacter pylori infection was more prevalent in HCV-positive participants than in the non-HCV population. The findings also showed a relatively higher proportion of positivity among females in both groups, although this observation requires cautious interpretation. The data presented in Tables 6 to 10 collectively support the possibility of an association between HCV infection and Helicobacter pylori serology. These findings highlight the need for further large-scale investigations to better understand the epidemiological, immunological, and clinical relationship between the two infections. A clearer understanding of this association may contribute to better screening strategies, improved patient management, and a more comprehensive approach to coinfection in clinical practice.

4 Future Work:

Although the present study provides useful insight into the serological relationship between Helicobacter pylori infection and Hepatitis C virus infection, further research is needed to develop a more comprehensive understanding of this association. One important direction for future work is the inclusion of a larger sample size drawn from multiple regions and healthcare centers. A broader study population would improve the generalizability of the findings and provide stronger epidemiological evidence regarding the prevalence of Helicobacter pylori among HCV-infected individuals [24]. Future studies should also incorporate more advanced diagnostic approaches in addition to serological testing. Since antibody-based methods may not clearly distinguish between current and past infection, the use of stool antigen tests, urea breath tests, molecular techniques, or biopsy-based methods could provide more accurate confirmation of active Helicobacter pylori infection. Similarly, more detailed virological assessment of HCV infection, including viral load and disease stage, may help clarify whether the severity of HCV is associated with the frequency of Helicobacter pylori infection [25]. Another important area for future investigation is the exploration of clinical and demographic risk factors that may influence coinfection. Variables such as age, gender, socioeconomic status, sanitation, dietary habits, and underlying liver function should be studied in greater detail to better understand their contribution to infection patterns. In addition, longitudinal studies may help determine whether Helicobacter pylori infection affects the progression, complications, or clinical management of HCV-related liver disease over time. Further research is also needed to examine the possible biological mechanisms underlying the association between these two infections. Studies focusing on immune response, inflammatory pathways, and host-pathogen interactions may help explain why Helicobacter pylori seropositivity appears to be higher in HCV-positive individuals [26]. Such investigations could

provide a stronger scientific basis for understanding coinfection and its pathological significance. Future work should aim to expand the scope of the present findings through larger, more detailed, and methodologically advanced studies. This would contribute to a clearer understanding of the epidemiological, immunological, and clinical relationship between Helicobacter pylori and HCV infection and may ultimately support improved screening, diagnosis, and management strategies for affected patients.

5 Conclusion:

The present study was conducted to assess Helicobacter pylori serology in relation to Hepatitis C virus (HCV) infection and to compare its prevalence between HCV-positive individuals and non-HCV controls. The findings showed that Helicobacter pylori seropositivity was higher among HCV-infected participants than among the control group. Out of 40 HCV-positive individuals, 20 were found positive for Helicobacter pylori, representing 50% of the group, whereas only 10 out of 40 non-HCV individuals, representing 25%, showed seropositivity. These results indicate a possible association between HCV infection and increased prevalence of Helicobacter pylori infection. The study also revealed a gender-wise variation in infection frequency, with female participants showing a higher percentage of Helicobacter pylori positivity than males in both HCV-positive and non-HCV groups. Although this pattern is noteworthy, it should be interpreted with caution because of the limited sample size. Nevertheless, the overall findings suggest that Helicobacter pylori infection may be more frequent among individuals suffering from HCV infection and that coinfection may have clinical importance.

REFERENCES

Abbas, Z., Jafri, W., Khan, A. H., & Shah, M. A. (1998). Prevalence of Helicobacter pylori antibodies in endoscopy personnel and non-medical volunteers of Karachi. *Journal-Pakistan Medical Association*, *48*, 201-202.

Afzal MS. Hepatitis C virus and interferon-free antiviral therapeutics revolution: implications for Pakistan. Viral Immunol 2017;30:252–257.

Bardhan PK. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis.* 1997;25:973–8.

Bashir T, Asim M, Ahsan M, et al. A case report: patient with the history of hepatitis C virus. J Antivir Antiretrovir 2017;9:055–057

Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis 2010; 28: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

Bhimani DR. Design and development of stomach site specific drug system against helicobacter pylori infections. Ganpat University. 2013.

Buzás GM. Metabolic consequences of Helicobacter pylori infection and eradication. World J Gastroenterol 2014; 18:5226–5234

De Martel C, Forman D, plummer M (2013) Gastric cancer epidemiology and risk factors. Gastroenterol clin North Am 42(2): 219-240.

E. C. Skoog, V. A. Morikis, M. E. Martin et al., "CagY-dependent regulation of type IV secretion in Helicobacter pylori is associated with alterations in integrin binding," mBio, vol. 9, no. 3, 2018.

El-Masry, S., El-Shahat, M., Badra, G., Aboel-Nour, M. F., & Lotfy, M. (2010). Helicobacter pylori and hepatitis C virus coinfection in Egyptian patients. *Journal of Global Infectious Diseases*, *2*(1), 4.

El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature.* 2000;404:398–402.

Graham DY. *Helicobacter pylori* infection in the pathogenesis of duodenal ulcer and gastric cancer. *Gastroenterology.* 1997;113:1983–6.

Gray RR, Strickland SL, Veras NM, et al. Unexpected maintenance of hepatitis C viral diversity following liver transplantation. J Virol 2012;86:8432–8439.

Heim MH, Thimme R. Innate and adaptive immune responses in HCV infections. J Hepatol. 2014;61(1S):S14–25. doi:10.1016/j.jhep.2014.06.035.

Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology 2017;153:420-9.

Hopkins RJ, Vial PA, Ferreccio C, Ovalle J, Prado P, Sotomayor V, et al. Seroprevalence of *Helicobacter pylori* in Chile: Vegetables may serve as one route of transmission. *J Infect Dis.* 1993;168:222–4.

Hu Y, Wan JH, Li XY, Zhu Y, Graham DY, Lu NH. Systematic review with metaanalysis: the global recurrence rate of Helicobacter pylori. Aliment Pharmacol Ther 2017;46:773-9

Jing C, Huang ZJ, Duan YQ, et al (2012). Glulathione-stransferases gene polymorphism in prediction of gastric cancer risk by smoking and helicobacter pylori infection status. Asian Pac J Cancer Prev, 13, 3325-8.

Jyh-Chin Yang, Chien-Wei Lu, Chun-Jung Lin, (2014). *Treatment ofHelicobacter pyloriinfection: Current status and future concepts.* World J Gastroenterol 2014 May 14; 20(18): 5283-5293

Kaito M, Watanabe S, Tsukiyama-Kohara K, Yamaguchi K, Kobayashi Y, Konishi M, Yokoi M, Ishida S, Suzuki S, Kohara M. Hepatitis C virus particle detected by immunoelectron microscopic study. J Gen Virol 1994; 75 (Pt 7): 1755-1760 [PMID: 7517432 DOI: 10.1099/0022-1317-75-7-1755]

Khan AW, Nawab S, Nasim Z, et al. Pattern of hepatitis C virus genotypes and subtypes circulating in war-stricken Khyber Pakhtunkhwa, Pakistan: review of published literature. Asian Pac J Trop Med 2017;10:1037–1042

Krüttgen A, Horz HP, Weber-Heynemann J, Vucur M, Trautwein C, et al. (2012) Study on the association of Helicobacter species with viral hepatitis-induced hepatocellular carcinoma. Gut Microbes 3(3): 228- 233.

Kumar T, Ahmad N, Hayat MK, et al. Prevalence and genotypic distribution of hepatitis C virus in Peshawar KPK, Pakistan. Hayati J Biosci 2017;24:22–25.

L. S. Tran, M. Chonwerawong, and R. L. Ferrero, "Regulation and functions of inflammasome-mediated cytokines in _Helicobacter pylori_ infection," Microbes and Infection, vol. 19, no. 9-10, pp. 449–458, 2017.

Lange CM, Jacobson IM, Rice CM, Zeuzem S: Emerging therapies for the treatment of hepatitis C. EMBO Mol Med 2014; 6: 4–15.

Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. Clinical Microbiology and