

Association of Chronic Viral Hepatitis B and C With ABO Blood Groups and Rhesus (Rh) Factor

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ABSTRACT

Background: Chronic viral hepatitis B (CVHB) and hepatitis C (CVHC) remain major public health challenges worldwide, particularly in developing countries such as Pakistan. While several risk factors for disease susceptibility have been identified, the role of ABO and Rhesus (Rh) blood groups in influencing infection risk is underexplored and not well defined in local populations.

Objective: To evaluate the prevalence of CVHB and CVHC in relation to ABO and Rh blood group distribution among Pakistani patients.

Methods: A retrospective study was conducted on 200 serologically confirmed CVHB and CVHC patients. Blood group distribution and Rh status were determined, and statistical analyses were performed to assess associations between blood group types and hepatitis infection.

Results: Among CVHB patients, blood group distribution showed higher frequencies of group O, followed by B, A, and AB. Rh-positive status predominated significantly. Notably, Rh-positive individuals with blood group O demonstrated disproportionately higher prevalence compared to previous literature. These findings suggest population-specific differences in susceptibility patterns.

Conclusion: This locational research highlights a potential genetic or immunological correlation between specific blood groups and susceptibility to Chronic viral hepatitis. The observed peculiarities of the Pakistani population warrant further investigation through large-scale, multicenter studies to better clarify these associations.

Keywords: Hepatitis B, Hepatitis C, ABO blood groups, Rh factor, Blood donors, Immunoassay.



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INTRODUCTION

The second leading global health issue is chronic viral hepatitis, especially that due to hepatitis B virus (HBV) and the hepatitis C virus (HCV). These infections cause serious liver disorders including fibrosis, cirrhosis and hepatocellular cancer [1]. Recent research studies have revealed that host determinants, specifically ABO blood types, and Rhesus (Rh) factor are genetic variables, which could predispose one to developing chronic infections. This relationship is presumed to be connected with the role of blood type antigens in the regulation of immune

response and permitting the intercourse of viruses with cells [2]. The ABO blood group distribution was investigated using a comprehensive national cross-sectional survey in China, and basic data on frequency of blood types in the order O, A, B, and AB was obtained [3]. Researchers in a Pakistani study have found a strong connection between HBV positive and the blood type B, however, individuals in the blood group O were not as susceptible [4].

ABO blood type is already associated with natural immunity and pathogen adhesion, and it possibly has an

effect on viral entry and immunological clearance. An analysis of a Gabon blood donor population revealed that BLOOD type O individuals were least susceptible to infection by HBV, but BLOOD type B individuals were nearly twice as susceptible to contract the disease [5]. To this effect, a study conducted in Nigeria with a focus on adult patients revealed that HBV infection was higher in persons with the blood type B, particularly those with Rh positive status, which implies that the Rh factor can predispose persons to the infection [6].

Other evidence on Egypt investigated prevalence of HBV, HCV and COVID-19 seropositivity in patients in Assiut and found that prevalence of HBV was significantly higher in patients with blood group O but those with blood group A along with Rh positivity were also not rare among infected individuals [7].

Equally, in a hospital-based study in Anbar Province, Iraq, it was shown that HBV infection was more common among individuals with blood type A and B [8]. Other than the risk of infection, blood group type has been associated with the development and prognosis of illness. A study of hepatocellular carcinoma (HCC) patients found that those with blood group A and Rh-positive status had a worse prognosis and were more likely to be diagnosed at advanced stages of liver disease, implying that blood antigens may play a role in cancer development and progression [9]. To back up these findings, a major systematic review and meta-analysis of 38 research found that those with blood categories B and AB were at a greater risk of both HBV and HCV, but those with blood group O consistently showed a protective effect [10].

Although a small number of studies, particularly those with small sample sizes, have found weak or no association between HCV and ABO blood groups, the majority of literature suggests the existence of a recurring pattern in which non-O blood groups are associated with a higher risk of viral infection. Despite major ethnic and geographical disparities, similar results have been reported in large population-based studies from China, Africa, and the Middle East [11]. Furthermore, research in Gabon revealed that virtually all infected people were Rh-positive, supporting the role of the Rh antigen as an additional risk-sensitive factor [12].

To summarize, the concept that ABO and Rh blood types may alter susceptibility to persistent HBV or HCV infections is well supported by the current research. Blood types B and AB, as well as Rh-positive status, are consistently linked to an increased risk of infection, but blood group O has been shown to provide some protection. These relationships have clinical implications for blood donor screening programs, immunization prioritization, and public health intervention planning in endemic locations. They also stress the need of conducting more large-scale, multicenter, and molecular-level research to elucidate the biological processes underlying these

relationships and enable the translation of this information into therapeutic and preventative treatment [13-16].

MATERIALS AND METHODS

This cross-sectional research was carried out at Jinnah Hospital and Shareef Medical Complex City Hospital in Lahore, Pakistan, from March 7, 2021, to March 7, 2024. 200 patients with either chronic viral hepatitis B (CVHB) or chronic viral hepatitis C (CVHC) and 506 healthy blood donors who acted as controls made up the 706 participants in total. Adults between the ages of 18 and 80 who had anti-HCV antibody positive with confirmatory testing or serologically proven hepatitis B surface antigen (HBsAg) positivity for more than six months without signs of acute infection were eligible patients. Individuals who had insufficient clinical or demographic information, co-infection with HIV, or other chronic liver illnesses such as alcoholic liver disease, autoimmune hepatitis, or drug-induced hepatitis were not included. Healthy controls were volunteer blood donors who were seronegative at the time of donation with HIV, HBV and HCV and who did not report a recent transfusion nor had a history of chronic disease.

A stratified random sample procedure was applied to ensure that cases and controls were represented fairly in both sexes and across all age groups.

Demographic and clinical information including age, sex, a history of transfusion, and previous donor status were collected using a systematic and pre-tested questionnaire administered by professional staff. A venous blood sample of all the participants was aseptically taken. The presence of co-infections was excluded by screening serums with commercial enzyme-linked immunosorbent assay (ELISA) kits: hepatitis B surface antigen (Hepanostika HCV Ultra, Beijing United Biomedicine Company), HIV antigen-antibody (Vironostika HIV Ag-Ab, bioMerieux), and anti-HCV antibodies. To ensure the accuracy of the diagnosis, the samples that yielded when subjected to the Western blotting and recombinant immunoblot assay (RIBA) confirmation test were positive.

The classic forward typing method was performed with anti-A, anti-B, and anti-D antisera in order to perform blood grouping and Rh typing. Blood groups and Rh status were determined by the presence or absence of agglutination and then registered and compared between the patients and controls. The data were analyzed with the help of SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Frequencies and percentages were used to summarize categorical variables. After controlling for possible confounders, logistic regression analysis was used to quantify the strength of relationships between ABO/Rh blood types and hepatitis infection. Chi-square testing was used to assess the connection. Statistical significance was defined as a p value of less than 0.05.

A study of pertinent comparative literature was conducted in order to contextualize the results. A study in

Lagos found that patients with hepatitis B were more likely to have blood group O (48.1%) and Rh-positive status (94.4%), which may indicate a role for these antigens in disease susceptibility. A study in Karachi that used anti-HBc testing and Roche Diagnostics blood grouping kits verified methodological comparability with the current study.

Prior to the study's start, consent was acquired from the participating hospitals and the University of Lahore's Institutional Review Board approved the protocol (ERC/18D/12/2021). Prior to enrollment, all participants provided written informed permission, and personal information confidentiality was rigorously maintained. Every process was carried out in compliance with local legal requirements as well as the Declaration of Helsinki's ethical standards.

RESULTS

A total of 200 patients with Chronic viral hepatitis were included in the study cohort. Of these, 103 individuals (51.5%) were diagnosed with Chronic viral hepatitis C (CVHC), while 97 (48.5%) had Chronic viral hepatitis B (CVHB), demonstrating that both infections were almost equally represented in the patient population (Table 1). Regarding sex distribution, males constituted a large majority of the sample with 151 cases (75.5%), whereas females accounted for only 49 cases (24.5%). When disease type was stratified by gender, it was observed that among males, 77 (50.9%) were positive for CVHC and 74 (49.0%) for CVHB. Among females, 26 (53.1%) were affected by CVHC and 23 (46.9%) by CVHB (Table 2). These figures highlight that, although males were more frequently infected overall, the proportional distribution between hepatitis B and C remained consistent across both sexes.

Age distribution analysis revealed that most CVHB cases occurred among individuals aged 26–45 years (46.7%), followed by those aged 46–80 years (38.5%), with the lowest prevalence in the 1–25 years age group (16.7%). A similar pattern was noted in CVHC patients, where 26–45 years accounted for the largest group (53.2%), followed by 46–80 years (23.3%) and 1–25 years (20.4%). Statistical testing using the chi-square method yielded a value of 4.27 with a p value of 0.118, indicating that there was no significant difference in the age group distribution between CVHC and CVHB ($p > 0.05$). These findings are visually represented in Figure 1, which depicts the age-wise distribution of both types of Chronic viral hepatitis with error bars demonstrating the standard error. The figure reinforces the observation that both HBV and HCV predominantly affect adults in the most socio-economically active age group, 26–45 years.

The distribution of ABO blood groups and Rh factor was then evaluated among hepatitis patients and compared

with 600 healthy blood donors. In CVHB patients, the frequencies of blood groups were as follows: group A in 55.8%, group B in 47.7%, group O in 46.4%, and group AB in 38.5% (Table 2). In contrast, CVHC patients exhibited group A in 44.1%, group B in 52.2%, group O in 53.5%, and group AB in 61.5% (Table 3). When these distributions were compared with the control group of healthy donors, the proportions were markedly lower, with group A (27.1%), group B (26.6%), group O (24.8%), and group AB (21.3%). These differences are clearly illustrated in Figure 2 and Figure 3, which show higher prevalence of all ABO blood groups among both CVHB and CVHC patients compared to the control population. Statistical analysis confirmed that these variations were highly significant. For CVHB patients compared with healthy donors, the chi-square test value was 19.74 ($p = 0.00019$), and for CVHC patients compared with donors, the chi-square test value was 21.29 ($p = 0.00009$). These values demonstrate a strong and statistically significant association between ABO blood group distribution and susceptibility to both hepatitis B and hepatitis C infections.

The Rh factor was also assessed and showed even more striking results. Among CVHB patients, 84 (86.6%) were Rh positive and 13 (13.4%) were Rh negative. In the CVHC group, 94 (91.2%) were Rh positive and only 9 (8.7%) were Rh negative. In contrast, the control group of healthy donors had a substantially lower Rh positivity rate of 57.8%, with 42.1% being Rh negative. The chi-square test values for Rh distribution further highlighted this significant difference: for CVHB vs. healthy donors, $\chi^2 = 28.07$ ($p = 1.17 \times 10^{-7}$), and for CVHC vs. healthy donors, $\chi^2 = 40.60$ ($p = 1.87 \times 10^{-10}$). These findings demonstrate a highly significant association between Rh positivity and Chronic viral hepatitis infection. Both Figure 2 and Figure 3 underscore this difference, as the blue bars representing patient groups show disproportionately higher Rh positivity compared with the red bars representing healthy controls.

In summary, the results of this study reveal several important trends. Both hepatitis B and C infections were almost equally distributed in the study cohort, with a clear male predominance. The age distribution showed that the majority of cases occurred among individuals aged 26–45 years, though no statistically significant differences were observed between age groups. Most strikingly, ABO blood group analysis revealed significantly higher frequencies of all blood groups among infected patients compared with healthy donors, and Rh positivity emerged as a consistent and strong marker associated with infection. These findings collectively suggest that blood group antigens and Rh factor may play a role in modulating host susceptibility to Chronic viral hepatitis in the studied population.

Table-1: Age-wise Distribution of Hepatitis B and C Patients

Age Group	Total Patients (n=200)	CVHC (n=103)	CVHB (n=97)
1–25 years	52 (26.0%)	21 (40.3%)	31 (16.7%)
26–45 years	109 (54.5%)	58 (53.2%)	51 (46.7%)
46–80 years	39 (19.5%)	24 (61.5%)	15 (38.5%)

Table 2: Distribution of Blood Groups and Rh Factor in CVHB Patients vs. Healthy Donors

Blood Group / Rh	CVHB (n=97)	% in CVHB	Healthy Donors (n=600)	% in Donors
A	24	55.8%	163	27.1%
B	42	47.7%	160	26.6%
O	26	46.4%	149	24.8%
AB	5	38.5%	128	21.3%
Rh Positive	84	86.6%	347	57.8%
Rh Negative	13	13.4%	253	42.1%

Table 3: Distribution of Blood Groups and Rh Factor in CVHC Patients vs. Healthy Donors

Blood Group / Rh	CVHC (n=103)	% in CVHC	Healthy Donors (n=600)	% in Donors
A	19	44.1%	163	27.1%
B	46	52.2%	160	26.6%
O	30	53.5%	149	24.8%
AB	8	61.5%	128	21.3%
Rh Positive	94	91.2%	347	57.8%
Rh Negative	9	8.7%	253	42.1%

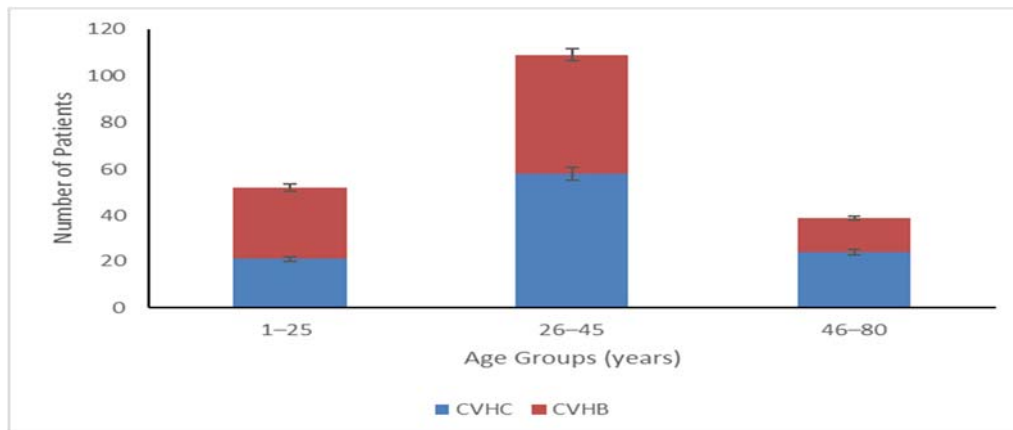


Figure 1: Age-wise distribution of patients with Chronic viral hepatitis C (CVHC) and Chronic viral hepatitis B (CVHB) with standard error bars.

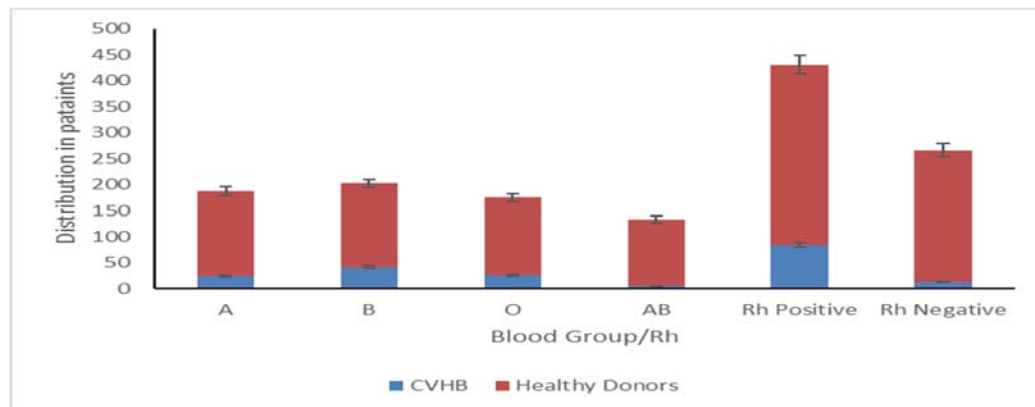


Figure 2: Distribution of Chronic viral hepatitis B (CVHB) patients and healthy donors according to ABO blood groups and Rh factor. Blue bars represent CVHB patients, and red bars represent healthy donors. Error bars indicate standard error of the mean.

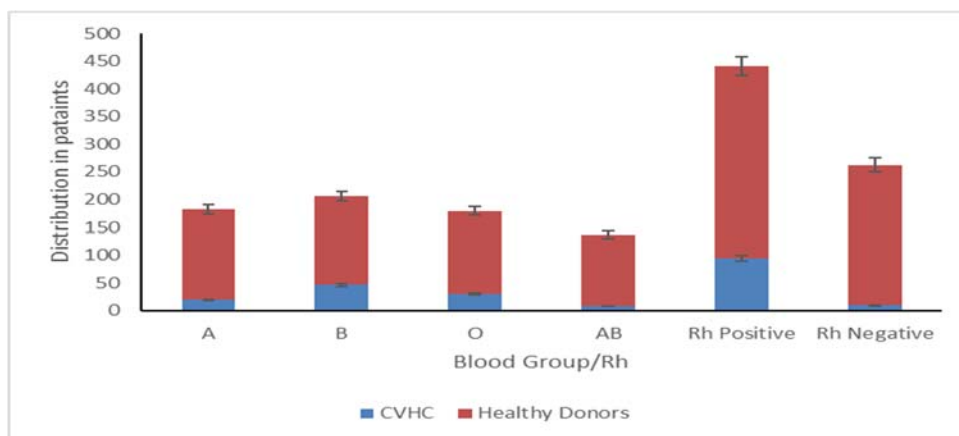


Figure 3: Distribution of CVHC patients and healthy donors by ABO blood groups and Rh factor, with error bars showing standard error.

DISCUSSION

This study aimed at investigating the correlation between ABO/Rh blood group systems and the prevalence of chronic viral hepatitis B and C. In our study population, most infected patients were of blood group O and Rh positive. This observation is consistent with previous reports that have established a possible predisposition linked to blood group O in terms of hepatitis susceptibility. Indicatively, a study of thalassemia patients showed that blood group O antigens were strongly associated with the risk of HCV infection, implying the possibility of blood group antigens playing a predisposing role in chronic viral hepatitis [17].

We also found that the rate of infection was higher in males than in females, especially in the 20-40 years age category. The same pattern of gender distribution has been already noted in previous research works that revealed that male patients are more prone to blood-borne diseases as a result of occupational exposure risk, and behavioral patterns [18,19]. In addition, this younger adult age group is more socially and economically active; this also could potentially contribute to an increased risk of exposure to the routes of viral transmission, i.e., unsafe medical procedures or transfusion [20]. Our data shows that the proportion of hepatitis cases among persons with blood group O is high, in agreement with the results of Saravanan et al. and Lemu et al. who reported significant associations between blood group O and seropositivity to HBsAg and anti-HCV [21,22]. Likewise, Indian research also reported the prevalence of Rh-positive individuals to viral hepatitis, which also supports the possible value of Rh factor as a susceptibility factor [23].

Interestingly, while much of the international literature emphasizes an association between blood group B and hepatitis infection, especially in relation to HCV, our findings deviated from this pattern. In our cohort, blood group O accounted for the highest proportion of infections, whereas blood group B did not show any remarkable trend. This contrasts with data from other regions [24,25] and suggests that population-specific factors, including genetic

variation, geography, and demographic composition, may influence the observed differences.

The relationship between ABO blood groups and viral hepatitis has also been explored in oncological contexts. In hepatocellular carcinoma (HCC), patients with blood group A and Rh-positive status were more likely to present with advanced-stage disease and poorer prognosis, highlighting a possible role of blood group antigens not only in infection susceptibility but also in cancer development [26]. Similar patterns have been observed in COVID-19 research, where blood group A individuals demonstrated increased susceptibility to infection, while blood group O appeared protective. These findings strengthen the hypothesis that antigen-antibody interactions mediated by ABO systems contribute to viral entry and host immune responses across multiple viral diseases [27].

Our findings are further supported by large-scale epidemiological data. A national cross-sectional study in China reported significant differences in ABO distribution among HBV-infected patients, confirming that these associations extend to wider Asian populations [28]. Data from transfusion-transmitted infections (TTIs) also reinforce this pattern, with blood group B donors found to have a significantly higher risk of HCV infection [14]. Additionally, a retrospective study from Nigeria revealed that blood group O was the most frequently infected group among blood donors, and intriguingly, it also carried the highest incidence of HIV infection, whereas no HIV cases were recorded among individuals with blood groups B and AB. This supports the concept of group-specific vulnerability and the inherent resistance that may exist in some blood groups [30].

Overall, our findings demonstrate that chronic hepatitis B and C are more common among individuals with blood group O and Rh positivity, though the precise biological mechanisms underlying these associations remain unclear. Previous studies suggest that ABO and Rh antigens may influence immune modulation, viral attachment, or clearance rates, but these hypotheses require

further validation [17,21,23,27]. Future research should aim to address these questions using large, multicenter cohorts while integrating genetic, immunological, and behavioral data to produce more conclusive evidence.

There are, however, several limitations to be acknowledged. The retrospective design relied on existing records, which may contain inaccuracies due to incomplete documentation, thereby limiting the reliability of certain variables [18,20]. Critical information such as vaccination history, intravenous drug use, transfusion exposure, and other risk factors was unavailable, reducing the ability to control for confounding influences [19]. Furthermore, this was a single-center study with a relatively small sample size, which may limit the generalizability of the findings to other populations [23]. Another limitation was the absence of molecular confirmation such as polymerase chain reaction (PCR) testing, as diagnoses were based solely on serological markers, which carry the risk of false positives or negatives [26]. Similarly, the lack of genotypic analysis prevented exploration of possible host-virus genetic interactions that may mediate susceptibility [27]. Lastly, given the cross-sectional nature of the study, causal relationships cannot be established, and the results should be interpreted as associations rather than definitive cause-effect links [14,30].

CONCLUSION

In this study it comes out that there is a significant correlation between ABO and Rh blood groups and prevalence of Chronic viral hepatitis B and C and incidence of ABO blood group O, rh positive individuals on incidence of the virus is higher. These results indicate that there is possibly an incidence of biological predisposition or that there is a greater exposure risk in these categories. The findings are also congruent with past researches but point to regional differences, a phenomenon that demonstrates the necessity of conducting studies with large scale and multicenter. The awareness of these associations can be used to improve screening practices and to create awareness to the high-risk groups. Nonetheless, there are some limitations to the study, which should be addressed in the future research: the sample size and the absence of molecular analysis.

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