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"SILENT THREATS IN THE BLOOD SUPPLY: SERO-PREVALENCE OF HEPATITIS B, C, HIV, SYPHILIS AND MALARIA AMONG VOLUNTARY DONORS IN RBC MIRPUR, AJK"

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ABSTRACT

Transfusion transmissible infections (TTIs) remain a major threat to blood safety particularly in regions where hepatitis B and C are common. Screening of donors is the main safeguard against these infections. This study aimed to determine the prevalence and distribution of TTIs among blood donors according to gender, area of residence, age group, infection type and blood group.

This cross-sectional study was conducted at the Regional Blood Centre (RBC), Mirpur, Azad Jammu and Kashmir (AJK) from 01st January 2025 to 31st December 2025 all donors were screened for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis and malarial parasite (MP) using immunochromatographic (ICT) method. Positive results were reconfirmed using ELISA. Donors reactive for at least one marker were considered positive. Data on age, gender, area of residence and blood group were collected, end result were expressed as frequencies and percentages.

Of 9,434 donors screened, 88 (0.93%) were reactive for at least one TTI. All positive donors were male. Most of the donors (73) came from Mirpur (83.0%), while 15 (17.0 %) came from other areas. The 18-25 year group was the most affected (37; 42.0%), followed by 26-33 years (31; 35.2%), 34-41 years (15; 17.0%) and 42-50 years (5; 5.7%), so donors under 34 years made up 77.3% of positive cases. HBV was the most common infection (43; 48.9%), followed by HCV (23; 26.1%), syphilis (17; 19.3%), HIV (4; 4.5%) and MP (1; 1.1%). Relative to all donors' screened prevalence was 0.46% for HBV, 0.24% for HCV, 0.18% for syphilis, 0.04% for HIV (and 0.01%) for MP. No co-infections were found. Among positive donors, blood group O+ was the most frequent (25;28.4%), followed by B+ (24; 27.3%) and A+ (23; 26.1%) and 92.0% were Rh-positive.

The overall prevalence of Transfusion Transmissible Infections (TTIs) among blood donors was low (0.93%), but HBV and HCV together accounted for three quarters of all cases. Young male donors were the most affected group. These finding highlight the need for stronger donor selection, awareness program for young donors, promotion of voluntary donations and more sensitive screening methods.

INTRODUCTION:

Transfusion of blood and blood products have come to be indispensable in the treatment of many diseases, injuries, surgical interventions and conditions affecting the blood such as thalassemia and hemophilia etc. While transfusion-related risks include transmission of infection via transfused blood products, transfusion transmitted infections (TTI) pose significant threat to public health; the major concern being the blood borne pathogens such as the HBV and HCV which account for most transfusion transmitted infections. (Jan et al., 2023). The risk of contracting blood-borne infections in the developing world is higher due to high donor prevalence of transfusion transmitted infection and the general poor screening practice of blood. (Jamal et al., 2024). 9 million people are carriers of the hepatitis B virus (HBV) in a recent survey reported; however, donating your blood can be a lifesaving act that also poses a risk for TTIs, or Transfusion-Transmissible Infections. HBV, the hepatitis C virus (HCV), human immunodeficiency virus (HIV), Treponema pallidum (causing syphilis), and Plasmodium (malaria) are among the most infectious agents associated with this group of diseases. Among these infections HBV, HCV, and HIV are especially concerning because they can remain asymptomatic or latent for long periods, allowing infected individuals to donate blood unknowingly (Jamal et al., 2024).

Belonging to the genera Orthohepadnavirus and Hepacvirus, respectively, HBV and HCV and are associated with severe liver conditions that can persist to cirrhosis and hepatocellular carcinoma. Based on nucleotide sequence divergence HBV has been classified into 9 distinct genotypes (Umair et al., 2012). HBV present a major global health threat acting as a silent killer that persists in the body as a carrier, it affects approximately 350 million people worldwide (Zorob et al., 2023). HIV (Human immunodeficiency Virus), a lentivirus belongs to the

retroviridae family, it attacks CD4+ T cell and weaken the immune system potentially responsible for AIDS. HIV can spread through body fluids, infected blood, sharing needles contaminated with infected blood (Muhammad et al., 2020).

Besides viral infections, syphilis and malaria are also critical concerns for blood transfusion safety. Syphilis is an infectious disease triggered by the spirochete bacterium Treponema pallidum and can potentially spread via blood transfusion (Conti et al., 2024). Although it is mainly a sexually transmitted infection, transmission may also occur through vertical transmission and contaminated blood that is prevalent in developing and developed countries. People who are suffering from STDs like syphilis also have a chance of co-infection with HIV (Zameer et al., 2017). Therefore, screening for syphilis is an essential part of blood donor safety. Malaria is caused by parasites belonging to genus *Plasmodium*. In addition to vector-borne transmission, malaria can also be transmitted through blood transfusion, organ transplantation, needle-stick injuries, and congenital exposure. The likelihood of malaria spreading through blood is relatively low in non-endemic countries (Alwaseah et al., 2026). Globally, the prevalence of transfusion-transmitted infections (TTIs) in donated blood varies considerably (Iqbal et al., 2026)

Furthermore, the voluntary blood donation practice is already inadequate or below a level that is enough to meet transfusion need in Pakistan, and the country is mainly dependent on replacement donors (Javad et al., 2022). Systems based on replacement donors (RDs) pose high risks of transfusion transmissible infections (TTIs). Currently, only 10%–13% of blood donations are voluntary in Pakistan (Jamal et al., 2023).

Lack of awareness, suboptimal screening methods, barriers to accessing health services and ineffective surveillance system make difficult for us to ascertain exact number of TTIs present in the society. Despite of tremendous advancements globally, Pakistan is a long way from achieving “zero risk” blood supply considering the resource gap in the country and lack of awareness (Jamal et al., 2024). Consequently, WHO strongly highlights routine testing of all blood donations a practice now mandated and regularly implemented in most blood banks to minimize risks of transfusion transmitted infections. However, transfusion-transmitted infections (TTIs) still, pose a considerable threat, especially in low- and middle-income countries where healthcare resources and testing capabilities remain restricted (Iqbal et al., 2026).

Transfusion Transmissible Infections are still major health problems globally. These viruses cause serious illness and deaths, and they also create a heavy social and economic burden. Their impact is even greater in low- and middle-income countries, where access to screening and treatment is limited (Alharazi et al., 2022).

People which are infected with HBV are 2 billion, with HCV are 200 million and with HIV are 33.4 million reported by The World Health Organization (WHO). Centres for Disease Control (CDC) stated that HBV have 10 to 100 times more prevelance than HCV and HIV (Jamal et al., 2024). WHO reported that about 38.4 million people across the globe were infected with HIV and with HCV were 2-3%, by the end of 2021. U.S Centres for Disease Control and Prevention stated that about 3000 people die due to HBV yearly. Recent studies reported that out of 232 million population, 9 million were infected with HBV (Zorob et al., 2023).

Universally, people who are infected with HBV are 240 million and with HCV are about 170 million. 37.9 million people having HIV including 1.8 million children under 15 years of age reported by The World Health Organization (WHO). 36 million cases of syphilis and 1.5 million cases of malaria occur per year (Zeeshan et al., 2023). In Pakistan, higher rates of HBV are observed in rural areas, where socioeconomic factors are responsible for these high rates. Pakistan being the second highest burden of HCV internationally, with prevelance of 4.5-8.2%. People infected with HIV are elevated from 150,000 in 2017 to 300,000 in 2020. Larkana, Sargodha and Gujranwala have major outbreaks of HIV. Some of the factors contributing to the high incidence of hepatitis in Pakistan include unsafe blood transfusion (15%), hospitalization (14%), dental treatments (13%), nonsterile injections (12%), surgery (9%), sexual transmission, mother-to child transmission, lack of facilities for screening, old sanitation systems, and poor hygiene in barbershops (Sajjad et al., 2025; Zeeshan et al., 2023).

Studies from Pakistan reported varying prevalence of TTIs among blood donors. Recent studies have reported an overall TTI prevalence of 6.5% among 400 blood donors, with 26 testing positive at Sialkot medical college. Among positive tests, HCV was 34.61%, HBV 19.23%, HIV 3.84%, malaria 30.76% and Syphilis 11.53% (Bhatti et al., 2021). Studies substantiated 2.04% overall TTI prevalence, HCV 0.49%, HBV 1.12% and HIV 0.07%, Syphilis 0.36%. Studies from Regional Blood Centre in Peshawar, Khyber Pakhtunkhwa, Pakistan indicated 4.61% overall TTI prevalence, prevalence of HBV 1.95%, HCV 1.38%, HIV 0.23%, Syphilis 0.91% and malaria 0.14% (Iqbal et al., 2025; Saba et al., 2021). Studies from Red Crescent Lahore, Pakistan presented 2.19% overall TTIs prevalence. Among 6,380 blood donors, people who were infected with HBV was 0.83%, with HCV was 1.12%, with HIV was 0.03%, with Syphilis was 0.2% and no cases of malaria was found. Male donors were 61.34% and female donors were 38.65%. Studies from Armed Forces Institute of Transfusion (AFIT), Rawalpindi, Pakistan showed 2.10% of overall TTI prevalence 1.06% HBV, 0.54% HCV, 0.19% HIV, 0.31% Syphilis and no cases of malaria. Total blood donors were 15,405 (Aslam et al., 2023; Zorob et al., 2023). Studies from “Regional blood centre Mirpur Azad Jammu and Kashmir, Pakistan” reported low prevalence rate of TTIs. Among 8927 blood donors, voluntary donors were 72.02% and replacement donors were 27.97%. It showed 2.5% prevelance of HCV, 1.68% HBV, malaria 0.06% and no positive cases of HIV and Syphilis (Umair et al., 2012). Studies from Al-Khidmat Lahore, Pakistan reported 3.3% overall TTIs prevalence, with HCV 1.3%, HBV 0.6%, HIV 0.4%, Syphilis 1.0% and no cases of malaria. Total donors were 2,236. Studies from Peshawar Khyber Pakhtunkhwa, Pakistan indicated 2.15% overall TTIs prevelance. Among 6,378 donors, prevalence of HBV was 1.3%, HCV

0.7%, HIV 0.1%, Traponema Pallidum 0.03% and Plasmodium Malaria 0.0% (Amjed et al., 2023; Zeeshan et al., 2023).
Previous studies in AJK show high rates of hepatitis in different groups. HBV and HCV prevalence were studied among refugees from Indian Occupied Kashmir living in Muzaffarabad camps from 2014-2019 (Kazmi et al., 2022). Studies reported that HCV in blood donors is still a big issue due to unsafe injections, blood transfusions and lack of training among paramedics (Saleem et al., 2022; Waheed et al., 2024). Additionally, 5.36% HBV and 28.1% HCV were found in 224 thalassemia patients from Rawalakot, Palandari and Muzaffarabad, with the highest rate reported in Muzaffarabad (Mahmood et al., 2022).

This study will adopt a descriptive cross-sectional design to determine the seroprevalence of transfusion-transmissible infections (TTIs) specifically Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Syphilis, and Malaria among voluntary blood donors. Cross-sectional studies are well-suited for seroprevalence research as they enable an accurate estimation of disease burden within a specific population during a defined timeframe. The investigation will be conducted over a year, at the Regional Blood Centre, Mirpur, Azad Jammu and Kashmir (AJK). Serving as a critical regional facility, this facility routinely manages donor recruitment, blood collection, mandatory screening, storage, and distribution services across the region, maintaining an adequate and safe blood supply for patients requiring transfusions due to accidents, surgeries, obstetric emergencies, severe anemia, and other medical conditions. Because blood transfusions represent a major potential vector for viral and parasitic transmission, maintaining strict donor selection standards and robust screening protocols is paramount to ensuring blood safety. Furthermore, the RBC's comprehensive donor records offer a valuable dataset for evaluating local epidemiological patterns, making it an ideal setting to investigate the occurrence of these infections and inform regional transfusion safety practices.
The study population will consist of voluntary blood donors presenting to the Regional Blood Centre (RBC), Mirpur, Azad Jammu and Kashmir (AJK) during the study timeframe. Voluntary donors who are often relatives or friends of patients are preferred over unvetted replacement or commercial donors due to their established association with a lower prevalence of TTIs. To be included, prospective donors must be aged between 18 and 50 years, weigh more than 50 kg, exhibit acceptable hemoglobin levels, appear physically healthy, reside within the Mirpur division, and voluntarily grant written informed consent. Conversely, individuals will be excluded if they are under 18 or over 50 years of age, or if they present with a clinical history or evidence of jaundice, liver disease, severe anemia, or chronic illnesses. Prospective donors who have received a recent blood transfusion, have a prior diagnosis of HBV, HCV, HIV, Syphilis, or Malaria, are taking medications that compromise donation safety, or report high-risk behaviors—such as unsafe sexual practices or intravenous drug use—will also be excluded according to standard guidelines.
To ensure sample integrity and reduce pre-analytical and analytical errors, all medical laboratory processing and phlebotomy procedures will adhere strictly to standardized aseptic protocols. Blood collection will take place in a well-ventilated, properly illuminated, and climate-controlled environment, performed by trained phlebotomists under appropriate clinical supervision. Donor blood will be collected into sterile, closed plastic blood-bag systems (single, double, or triple bags depending on intended component separation) containing Citrate Phosphate Dextrose Adenine-1 (CPDA-1) as an anticoagulant-preservative solution, which maintains cellular metabolism and prevents coagulation for up to 35 days at 2–6°C. Prior to collection, the bag is labeled with a unique donor identification number and date. The donor is placed in a comfortable reclined position, an antecubital vein is selected and thoroughly disinfected with an approved antiseptic, and venipuncture is performed under tourniquet application. The blood bag is placed on a collection scale below arm level to allow gravity flow and volume monitoring (typically 450–500 mL), while the donor gently squeezes a soft ball to maintain steady blood flow. The procedure is kept within a target duration of approximately 8 minutes, with regular mixing of blood and CPDA-1 solution. Once completed, the tubing is clamped, the needle is withdrawn and safely discarded into a biohazard sharps container, direct pressure is applied to the venipuncture site with sterile gauze, and the blood bag is gently inverted before being transferred to refrigerated storage at 2–6°C. Donors are monitored during donation, allowed an 8-to-10-minute rest period post-phlebotomy, provided with light refreshments, and is guided with post-donation care instructions.
Laboratory analysis of serum and plasma specimens will utilize commercially available rapid lateral flow immunochromatographic technique (ICT) devices for qualitative screening of antibodies against HIV-1, HIV-2, HCV, Syphilis, and Malaria, as well as Hepatitis B surface Antigen (HBsAg). ICT rapid screening is widely favored in RBC operations for its simplicity, cost-effectiveness, rapid turnaround, and independence from complex instrumentation. The lateral flow principle relies on membrane-immobilized target antigens or antibodies and colloidal gold-conjugated reagents; as the sample migrates up the strip via capillary action, target analytes bind to the conjugates to produce visible colored bands at the test line, while a control line confirms test validity. For HBsAg testing, test cassettes, assay buffers, and specimens are brought to room temperature (15–30°C) before opening the sealed foil pouch. One drop of serum or plasma (30 µL) is added vertically into the sample well using a disposable dropper, immediately followed by one drop of assay buffer (40 µL), taking care to avoid air bubbles. Results are visually read for exactly 15 minutes, where the presence of both control and test lines indicates a reactive (positive) result, a single control line signifies a non-reactive (negative) result, and any line appearing after 15 minutes is deemed invalid. Analogous, standardized manufacturer protocols following the same lateral

flow principle will be conducted concurrently to evaluate anti-HCV, anti-HIV-1/2, Treponema pallidum, and Plasmodium antigen reactivity.
Moreover, the samples that show positive results on ICT will be isolated and sent for further confirmation on ELISA. If the result is again positive on ELISA, patients will be called in the RBC and will be counseled for the situation. The early treatment will be encouraged.

During the study, a total of 9,434 blood donors were screened for HBV, HCV, HIV, syphilis and malarial parasite. Of these, 88 donors were reactive for at least one transfusion transmissible infection, giving an overall prevalence of 0.93%. No donor had more than one infection so there were no coinfections.

Seropositivity was highest in 18-25 years age group (42%) followed by the 26-33 year group(35.2%). The 34-41 year group accounted for 15 cases (17%), while the lowest number was recorded in 42-50 year age group (5.7%). Overall, seropositivity decline steadily with increasing age, with younger individuals, particularly those aged 18-

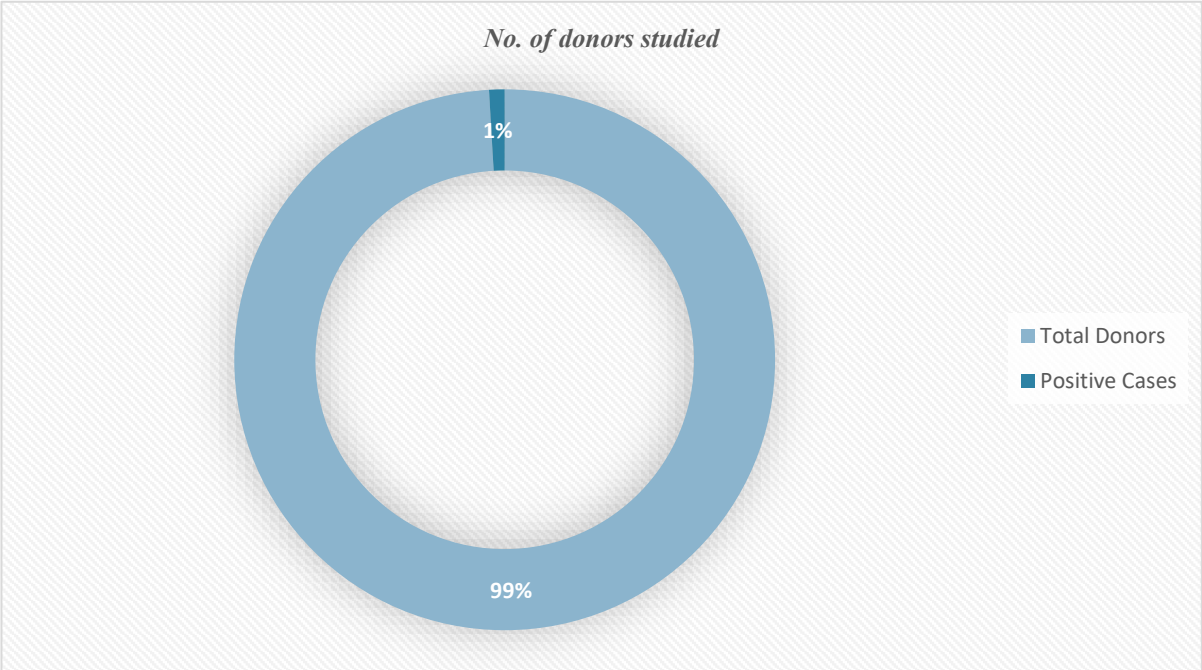


Figure 1: Frequency of Positive vs Negative Donors

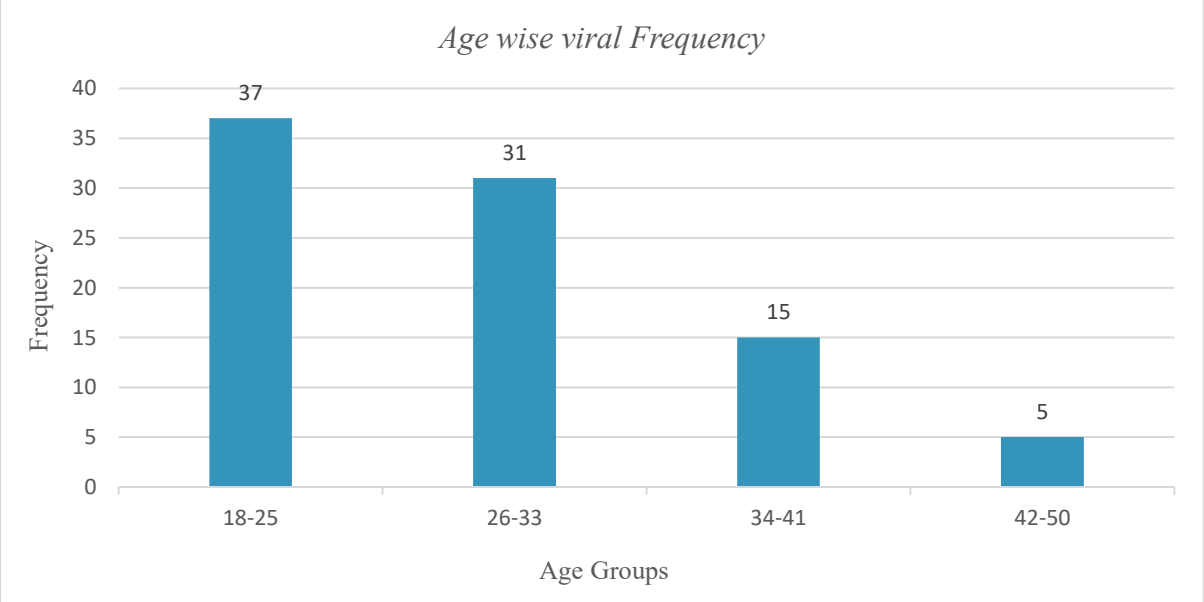


Figure 2: Age wise Viral Frequency

33 years, comprising most positive cases (77.3%). This age-related pattern may reflect higher exposure risk among young adults, although this should be interpreted cautiously.
HBV was the most common transmissible infection (48.9%), followed by HCV (26.1%), syphilis (19.3%), HIV (4.5%), and MP (1.1%). When calculated against all donors screened, the overall prevalence was 0.46% for HBV,

0.24%for HCV, 0.18% for Syphilis, 0.04% for HIV and 0.01% for MP. HBV and HCV together accounted for 75% of all infections detected highlighting them as the principal threat to transfusion safety in this donor population. In contrast, HIV and malaria were uncommon, together contributing only 5.7% of total seropositive cases.

All seropositive donors (88; 100%) were male, and no female donor tested positive for any of the screened infections. Geographically, the majority of positive cases originated from Mirpur (73; 83%), whereas the remaining 15 donors (17%) came from other areas. This indicates a marked male predominance among seropositive donors, which may largely reflect the male dominated nature of donor pool. Similarly, the concentration of cases in Mirpur likely reflects the catchment area of the RBC.

Among the positive donors' blood group O+ was the most frequent (25; 28.4%), followed by B+ (24; 27.3%), A+ into (23; 26.1%) and AB+ (9; 10.2%). The Rh-negative groups were uncommon: B – (3; 3.4%), A– (2; 2.3%) percent, AB – (1; 1.1%) and O – (1; 1.1%). Overall, 92.0% of positive donors were Rh positive.

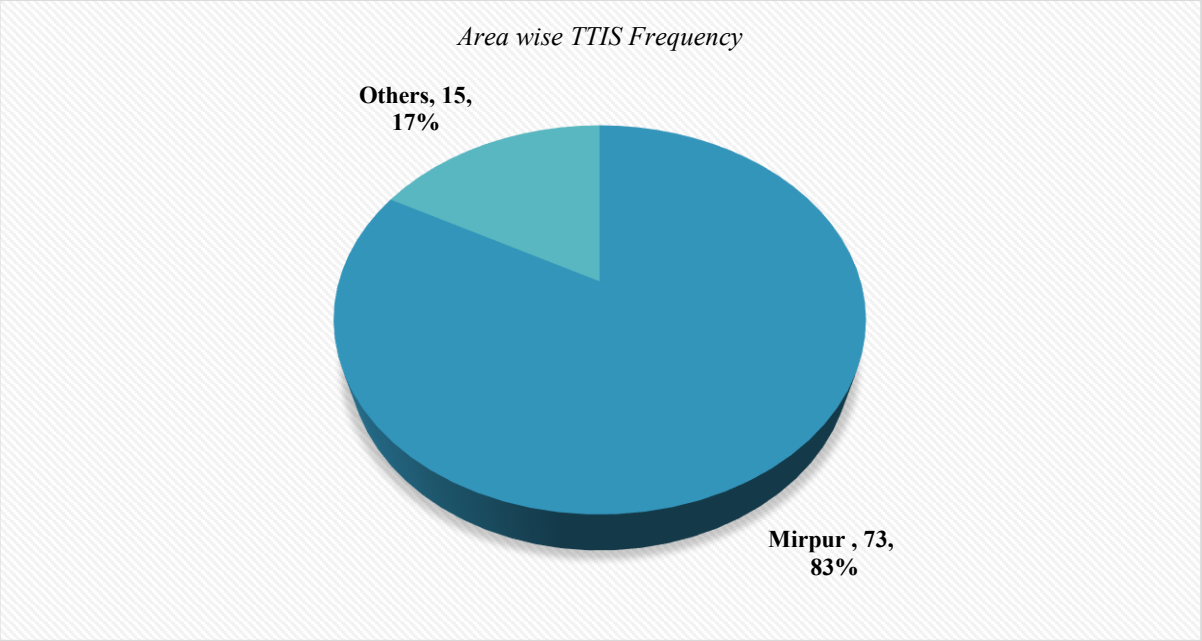


Figure 3: Area wise TTIS Frequency

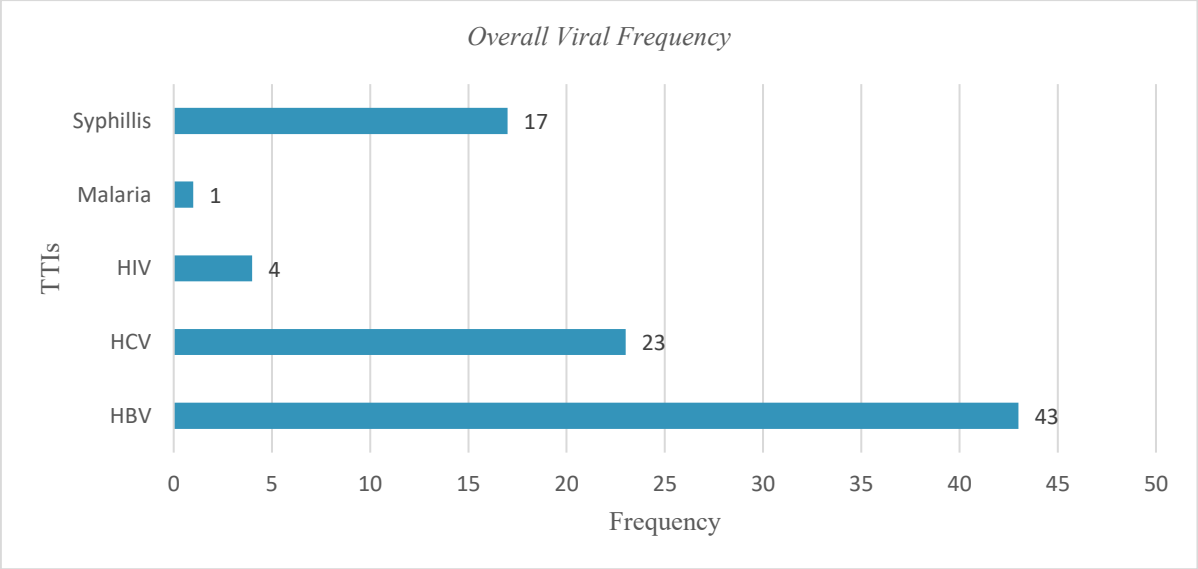


Figure 4: Overall Viral Frequency

Blood safety depends on identifying infected donors before their blood reaches a recipient. In this study of 9,434 donors, 88 (0.93%) were reactive for at least one transfusion transmissible infection (TTI) meaning that roughly one in every 107 donors represented to the regional blood Centre carried an infection that could have been passed on through transfusion. Every infection was detected in a single donor with no co-infections, so each positive result represents a separate donor who differed and kept out of donor pool.

Viral hepatitis dominated the findings. HBV and HCV together accounted for 66 of 88 cases (75.0%), With HBV alone responsible for almost half (48.9%). Adding syphilis raises the share to 94.3%, which shows the small group

of infections account for nearly all the burden. HIV (0.04%) and Malarial parasite (0.01%) were rare. The fact that hepatitis B and C remain the leading infections among otherwise apparently healthy donors indicate that these infections circulate silently in the community and are often unrecognized until screening. Because both can persist without symptoms, the donor's own sense of good health cannot be relied upon as a safeguard, and laboratory screening remains essential.

The demographic pattern points to a clear high-risk group. All positive donors were male, but this should be interpreted with caution. Female donors usually make up only a small share of donors, so the absence of positive females more likely reflects who donates then who is infected. Likewise, the predominance of donors from (83.0%) probably reflects the regional blood centre's catchment population rather than a higher local risk. Age showed the most meaningful trend: positivity was highest in the 18-25 year group (42.0%) and declined steadily with each older group, down to 5.7 % in those aged 42-50 years. Donors under 34 years made up 77.3% positive cases. Since young adults also form the bulk of doner pool, this finding suggests that targeted pre donation counselling and risk assessment for young donors could give the greatest benefit.

Among positive donors, O+ was the most common blood group (28.4%), followed by B+ (27.3%) and A+ (26.1%), and 92.0% were Rh-positive. These figures describe the composition of positive groups only. They shouldn't be taken as evidence that any blood group carries a higher or lower risk of infection since that would require comparison with blood group distribution of all donors. This distribution of infections within blood group is therefore reported as descriptive information. The prominence of Rh-positive groups is expected in a population

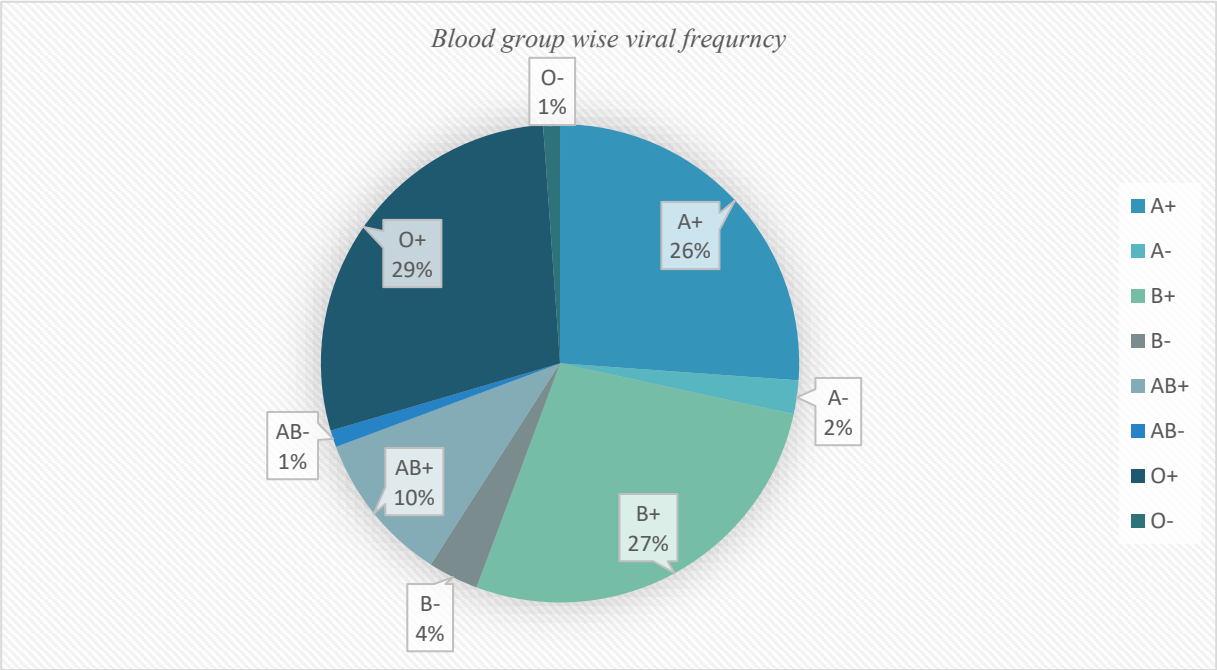


Figure 5: Blood group wise viral frequrncy

where these groups are common among donors in general.

Future studies should be multi-centre and multi-year across AJK and neighbouring regions to give representative estimates and track trends. Upgrading to ELISA/CLIA and introducing NAT would close window period and occult HBV, and local cost effectiveness studies would support this. Stronger analysis includes group specific prevalence along with comparison of voluntary, replacement and repeat donors is needed to identify independent risk factors. Risk Factor Questionnaire, genotyping and interventions studies on pre donation counselling and youth awareness will help the prevention. Strict donor selection and quality control screening must be maintained. The concentration of infections in a few types suggests that resources should focus on hepatitis B and C prevention, including donor education and encouragement of vaccination against HBV.

This single centre, one-year cross-sectional study reflects the donor population at one point in time, so the 0.93% prevalence may not generalize to the rest of AJK or Pakistan. Screening used ICT rapid tests which are less sensitive than ELIZA, CLIA or NAT and may miss window-period and occult HBV infections; because only ICT reactive samples were confirmed, false negative went undetected and prevalence is probably underestimated. The analysis was descriptive only without dominators for each group. Data on specific risk factors such as injections, dental procedures and HBV vaccination were also not collected.

CONCLUSION

Of 9434 donors ignorant at the Regional Blood Central, Mirpur, 88 (0.93%) were reactive for at least one TTI, with no co-infections. HBV (0.46%) and HCV (0.24%) accounted for 75% of the cases, while syphilis (0.18%), HIV (0.04%) and Malaria (0.01%) were less common. All positive donors were male, and 73% were under 34 years. Although prevalence was low, the dominance of hepatitis B and C shows these infections still circulate silently among healthy-appearing donors. These findings support stronger donor selection, awareness programs for young donors, promotion of voluntary donation, HBV vaccination, and more sensitive screening methods such as ELISA, CLIA and NAT.

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