Systematic Review

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The National Burden of Hepatitis C Among Blood Donors in Pakistan: A Systematic Review and Meta-Analysis (1996-2024)

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Author`s Contribution

[1,3]Substantial contribution to the conception or design of the work; or the acquisition; [2,4]Active participation in methodology and literature review; [2,3]Analysis, or interpretation of data for the work, [1,5]Drafting the work and revising it critically for important intellectual content

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ABSTRACT

Objective: Hepatitis C virus (HCV) is a major public health risk globally, particularly in low- and middle-income countries like Pakistan. Blood donors represent an important group for monitoring HCV prevalence, as they can unknowingly harbor and transmit the virus. This systematic review and meta-analysis aim to assess the burden of HCV among blood donors in Pakistan from 1996 to 2024, offering insight into the epidemiology and regional disparities in the prevalence of the virus.

Methods: The review followed PRISMA guidelines, incorporating studies from databases including PubMed, Scopus, Embase, Google Scholar, Web of Science, PakMediNet, and Cochrane Library. Eligible studies (n=122) reported primary data on HCV prevalence in blood donors across Pakistan. The pooled prevalence of HCV was estimated using a random-effects model, and heterogeneity between studies was evaluated through I^2 statistics. Sensitivity analyses were performed to ensure the robustness of findings, and time-series analyses identified trends in HCV prevalence across years.

Results: The meta-analysis revealed an overall HCV prevalence of 2.71% among blood donors in Pakistan, based on 8.88 million donations screened. Significant regional variations were observed, with Punjab showing the highest prevalence (3.94%) and Balochistan the lowest (0.87%). Screening practices varied across provinces, contributing to these differences. A time-series analysis indicated fluctuating trends over the years, with notable spikes in certain years.

Conclusion: HCV prevalence among blood donors highlights ongoing transmission risks, the need for enhanced screening protocols and implementation of blood safety regulations in Pakistan.

Key words: Hepatitis C Virus, Prevalence, Pakistan, Blood Donors

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Introduction

Infection with Hepatitis C virus (HCV) is an increasing global concern because of its significant impact on morbidity and mortality.[1] The disease imposes a substantial health and economic burden on countries, especially developing ones due to its hepatic and extrahepatic effects.[2] The WHO (World Health Organization) recently obtained officially authenticated data from 130 countries and supplemented it with partner-provided data from 70 countries. In 2019, it was estimated that 15.2 million people living with HCV had been diagnosed between 2015 and 2019, and 9.4 million received direct-acting antiviral (DAA) treatment during that period.[3] Globally, about 58 million people were living with chronic HCV, with 1.5 million new infections

reported in 2019. In 2022, approximately 242,000 deaths were attributed to HCV, primarily due to cirrhosis and hepatocellular carcinoma.[4] In 2016, at the 69th World Health Assembly (WHA), all 194 member states endorsed the Global Health Sector Strategy aimed at eradicating hepatitis infections by 2030.[5] The disease has seen a downward trend in many countries due to the introduction of DAA, a ground-breaking treatment that offers prospects to reduce HCV infection and disease burden.[3] Despite these advances in treatment, hepatitis C remains a pressing concern, particularly in low- and middle-income countries like Pakistan, where both the prevalence of infection and the associated risks are exceptionally high.[6] Hepatitis C is a blood-borne virus, primarily transmitted through exposure to infected blood. The routes of transmission include the use of unsterilized medical instruments, unsafe blood transfusions, and injection drug use.[7]

Globally, the prevalence of HCV varies significantly across regions, with lower-income countries generally exhibiting higher rates of infection. According to WHO, the global seroprevalence of HCV is around 1%, but this figure obscures significant regional variations.[8] Countries in North Africa, the Middle East, and South Asia have some of the highest prevalence rates, with Egypt and Pakistan being notable examples.

In South Asia, Pakistan has emerged as a country with alarmingly high HCV infection rates, with estimates suggesting that around 5-6% of the adult population is infected with HCV, translating to approximately 9-10 million individuals.[9] These estimates vary widely across studies and are influenced by regional disparities in healthcare access and quality, as well as socio-economic and cultural factors. The rural population appears to be disproportionately affected due to inadequate access to healthcare services and poor awareness regarding safe medical practices.[10]

In Pakistan, the socio-economic factors, inadequate healthcare infrastructure, and poor blood screening assays significantly contribute to the transmission of HCV, placing specific groups, including blood recipients, at high risk of infection.[11] Among these key risk factors, unscreened blood transfusions have been consistently identified as a major contributor to the spread of HCV in Pakistan. This makes the population of blood donors an important group for monitoring the prevalence of hepatitis C. Blood donation is a critical component of healthcare systems, particularly in developing countries like Pakistan, where blood is frequently needed for medical emergencies, surgeries, and maternal care. The importance of ensuring the safety of blood transfusions is paramount, as it directly impacts patient outcomes. However, in Pakistan, the blood donation process is often marred by insufficient screening for infectious diseases, including hepatitis C, particularly in rural and under-resourced areas. This has led to a situation where blood donors, rather than being a safe source of blood, have sometimes become vectors of disease transmission, particularly when screening protocols are not rigorously followed.

Gaining insight into the epidemiology of HCV in Pakistan is essential for designing and implementing cost-effective prevention and treatment strategies, aligning with global efforts to eliminate HCV. As stated above, blood donors are a vital population for the surveillance of infectious diseases like hepatitis C, as they represent a subgroup of individuals who are often asymptomatic and presumed to be healthy. However, because HCV can remain asymptomatic for years, blood donors can unknowingly harbor and transmit the virus, posing significant risks to recipients of blood transfusions. The prevalence of hepatitis C among blood donors is, therefore, a crucial metric, as it not only reflects the level of virus circulation in the general population but also points to potential gaps in the safety of blood transfusion services and the overall healthcare system. The screening of HCV has been introduced in Pakistan since August 1993 while the first study assessing the prevalence of HCV in blood donors was published in 1996. However, there is no systematic overview of literature on the prevalence of HCV in blood donors. The objective of this systematic review is to ascertain the burden of HCV among blood donors in Pakistan between 1996 and 2024.

Methodology

We searched the PROSPERO database (International Prospective Register of Systematic Reviews) and the Database of Abstracts of Reviews of Effects (http://www.library.UCSF.edu) to identify relevant published or ongoing projects. The protocol for this systematic review was registered on PROSPERO (ID CRD42024605145).

The review was conducted following the pre-defined methodological standards of the Centre for Evidence-Based Practice and reported in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[13]

The eligibility criteria was established based on the PICOS (Population, Intervention, Comparison, Outcome, Study design) framework, i.e. population (blood donors);

intervention/exposure (presence of HCV in blood donors); comparison (no specific comparison groups, but studies that reported prevalence rates of HCV in different regions or populations (urban vs. rural) were included); outcome (prevalence of HCV among blood donors, reported as a percentage or proportion); and study design (cross-sectional studies, observational studies, cohort studies, and annual reports, were included while case reports, commentaries, editorials, and studies with incomplete data were excluded).

A comprehensive and systematic literature search was conducted across multiple electronic databases, including PubMed, Scopus, Web of Science, Embase, Google Scholar, PakMediNet, and the Cochrane Library (both the Cochrane Database of Systematic Reviews and the Cochrane Controlled Register of Controlled Trials).

The search was conducted for studies published from 1996 (when the first article was published) to December 2024. The search strategy involved a combination of Medical Subject Headings (MeSH) terms and keywords related to Hepatitis C, blood donors, and Pakistan. The search terms used included "Hepatitis C" OR "HCV", "Blood donors", "Prevalence", “Epidemiology”, "Pakistan".

Boolean operators such as “AND” and “OR” were used to refine and focus the search results. No language restrictions were applied, although only studies published in English were included. A hand-comb search of the reference lists from included studies and relevant reviews was also conducted to identify any additional relevant studies. Only articles reporting HCV incidence or prevalence based on primary data were included in this review. Exclusions applied to case reports, case series, editorials, letters to the editor, commentaries, reviews, studies referring to HCV as non-A non-B hepatitis, those with duplicate information, self-reported HCV prevalence, or studies involving Pakistani nationals living abroad.

The study selection process was conducted in two stages. First, the titles and abstracts were screened. All articles identified through the database search were imported into reference management software (EndNote™X8.2)[14] for the removal of duplicates. Titles and abstracts of the remaining studies were then screened independently by two researchers (UW, SS) for relevance to the research question. This was followed by retrieval of full-text studies that met the initial screening criteria. These articles were independently reviewed by two researchers (UW, NS) based on the eligibility criteria. Any disagreements between researchers were resolved by discussion or consultation with a third researcher. The study selection process was documented in a PRISMA flow diagram to visually represent the number of records identified, included, and excluded at each stage of the review.

A standardized data extraction form was developed to ensure consistency in the data collection process. Data were extracted independently by two researchers (UW, JB) and cross-checked for accuracy. The information extracted from each article included: study characteristics [author(s), year of publication, study location, and study design]; population characteristics [number of blood donors, age, gender distribution, and setting (urban or rural)]; outcome [prevalence of Hepatitis C virus among blood donors, reported as a percentage or proportion]; and screening methods [type of diagnostic test used for HCV screening, e.g., RDT, ELISA, CLIA, PCR]. To maintain transparency and reduce the risk of bias, any discrepancies in the extracted data were resolved through discussion between the researchers.

The methodological quality of the included studies was evaluated using the Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence data, chosen for its validity in assessing prevalence studies.[15] Alternative tools, such as the Cochrane Risk of Bias tool, were considered unsuitable for this study design. Each publication was rated on nine JBI items, receiving scores of '1' (poor), '2' (good), or '3' (excellent), resulting in an aggregate quality score ranging from 9 to 27. Two reviewers (NS, UA) independently assessed the studies, resolving disagreements through discussion. Studies with a total score below 50% of the maximum (<13.5) were classified as poor quality.

The data were synthesized quantitatively through a meta-analysis using STATA v.14.2 (StataCorp, College Station, TX, USA). A random-effects model was used to check for the potential heterogeneity between studies. Heterogeneity was evaluated using the Cochran Q test and quantified by the I^2 index. Heterogeneity was deemed significant if the *p-value* from the Cochran Q test was less than 0.05. Publication bias was assessed using a Funnel plot, which plotted transformed proportions against sample size. The plot’s symmetry was assessed with the Egger test, with a threshold of $p < 0.1$ indicating potential bias.

The mean, median, and range of HCV prevalence across different studies were calculated to understand the central tendency and spread of the prevalence data. A meta-analysis was performed through a random-effects model to pool the prevalence estimates from different studies.

Forest plot was used to visually display the individual prevalence estimates and their confidence intervals, alongside the overall pooled prevalence estimate.

A time series analysis was performed to see how the prevalence of HCV has changed over time (1996-2024) to identify patterns, spikes, or declines in HCV prevalence over the years. Regression models were used to explore the association between time (years) and HCV prevalence. Further, a sensitivity analysis (Leave-One-Out Analysis) was conducted by systematically excluding each study from the meta-analysis to assess the impact of individual studies on the overall prevalence estimate.

The studies included in this systematic review had their ethical approval for their respective study settings and design. Therefore, as this review depends on data from previously published studies, no ethical approval was needed. Although, the ethical guidelines outlined by the PRISMA statement were followed to ensure transparency and reproducibility in reporting the findings.

Results

Figure 1 presents the process (flow diagram) through which articles were selected based on PRISMA guidelines. The initial search identified 3,816 records, with an additional 11 records found through hand searching. After removing duplicates, 3,423 records remained for title and abstract screening. Following full-text screening and consensus on disagreements, 121 records representing 122 unique studies were included in the review.[16-136]

Detailed information on the studies included and their characteristics is shown in Table 1. Among the 122 studies, 115 (94.26%) screened for anti-HCV antibodies while 7 (5.74%) screened for HCV RNA. The total number of blood donations screened for anti-HCV antibodies was 8,346,153 (94.04%) while 529,133 (5.96%) were screened for HCV RNA.

The overall prevalence of HCV among blood donors in different provinces and regions of Pakistan, based on the data from 1996 to 2024, was 2.71%. This calculation is based on a total sample size of 8,875,286 donors.

Regarding the screening assays used, 30.32% (n=37) studies utilized rapid diagnostic test (RDT), 38.52% (n=47) enzyme-linked immunosorbent assay (ELISA), 25.40% (n=31) chemiluminescence immunoassay (CLIA), and 5.74% (n=7) nucleic acid testing (NAT)/polymerase chain reaction (PCR).

Regional distribution of studies revealed that majority of the studies were conducted in the province of Punjab 40.16% (n=49), followed by Sindh 22.13% (n=27), Khyber Pakhtunkhwa 20.49% (n=25), Islamabad Capital Territory 6.55% (n=8), Balochistan 4.09% (n=5), Azad Jammu & Kashmir 4.09% (n=5), and Gilgit-Baltistan 2.45% (n=3).

From the 122 records studied in this systematic review, the overall sample size was 8,875,286 that tested the prevalence of HCV in blood donors. The sample size across the studies ranged from 80 (Khyber Pakhtunkhwa)[23] to 4.19 million (Sindh).[134] There was a wide variation in HCV prevalence among individual studies, e.g. it ranged from 0% (in Khyber Pakhtunkhwa)[23], 0.07% (Punjab)[42], and 0.07% (AJK)[133] to 28% in Punjab Province.[54]

Table 2: Province-wise pooled prevalence of HCV

Province/Region	Studies (n)	Sample size	HCV Positive (%)
Azad Jammu and Kashmir	5	45,842	476 (1.03%)
Balochistan	5	48,959	427 (0.87%)
Gilgit Baltistan	3	13,585	139 (1.02%)
Islamabad Capital Territory	8	571,730	13,748 (2.40%)
Khyber Pakhtunkhwa	25	981,432	23,795 (2.42%)
Punjab	49	2,055,717	81,180 (3.94%)
Sindh	27	5,158,021	121,541 (2.35%)
Total	**122**	**8,875,286**	**241,306 (2.71%)**

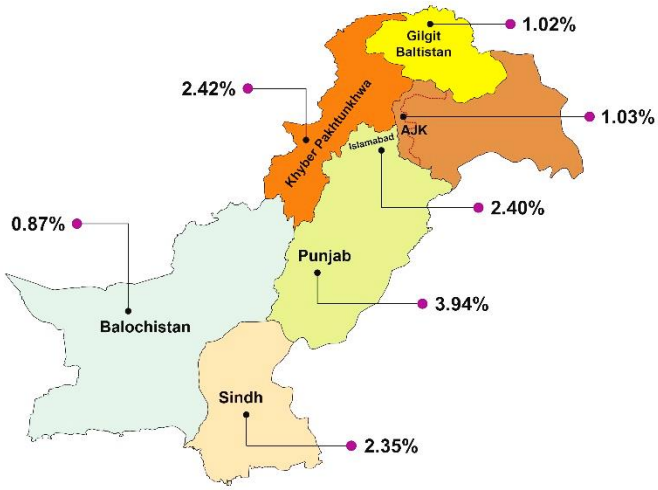

Fig. 2: Geographical distribution of HCV prevalence across Pakistan

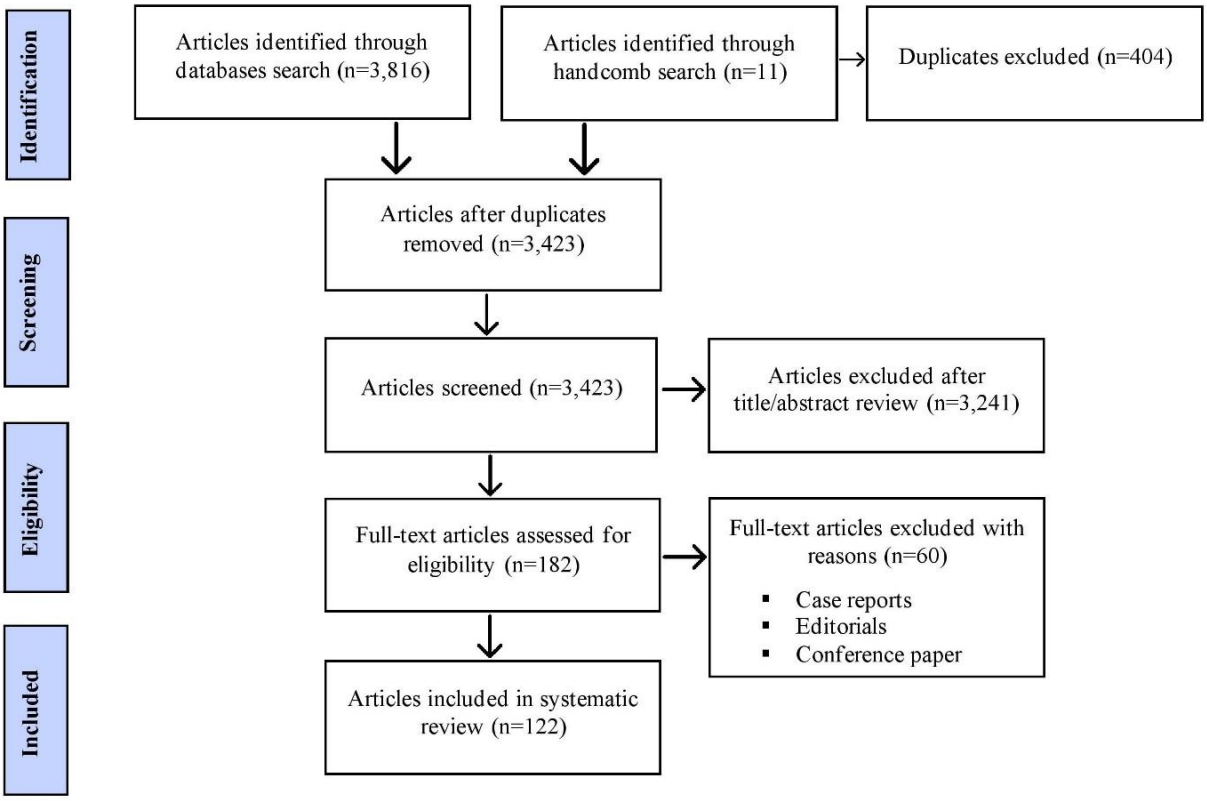

Fig. 1: Flowchart of the process through which articles were selected based on PRISMA

Table 1: Studies included in the systematic review that assessed the prevalence of HCV among blood donors across Pakistan

S. No.	Author	Year	Province/Region	Sample size	HCV Positive (%)	Technique
1.	Mujeeb *et al.*,[16]	1996	Sindh	839	20 (2.38%)	ELISA
2.	Bhatti *et al.*,[17]	1996	Punjab	750	36 (4.80%)	ELISA
3.	Kakepto *et al.*,[18]	1996	Sindh	16,705	198 (1.18%)	ELISA
4.	Rehman *et al.*,[19]	1996	Punjab	91	13 (14.28%)	ELISA
5.	Rehman *et al.*,[20]	1996	Sindh	2,557	15 (0.58%)	RDT
6.	Lone *et al.*,[21]	1999	Punjab	186	8 (4.30%)	RDT
7.	Hashmi *et al.*,[22]	1999	Punjab	426	89 (20.89%)	RDT
8.	Jadoon *et al.*, [23]	1999	Khyber Pakhtunkhwa	80	0 (0%)	RDT
9.	Mujeeb *et al.*,[24]	2000	Sindh	612	3 (0.49%)	ELISA
10.	Ahmed *et al.*,[25]	2001	Sindh	1,410	96 (6.80%)	ELISA
11.	Ryas *et al.*,[26]	2001	Punjab	1,885	88 (4.66%)	ELISA
12.	Ahmad *et al.*,[27]	2002	Punjab	5,789	284 (4.90%)	RDT
13.	Khattak *et al.*,[28]	2002	Punjab	103,858	4,164 (4.00%)	ELISA
14.	Mumtaz *et al.*,[29]	2002	Punjab	553	34 (6.14%)	RDT
15.	Rahman *et al.*,[30]	2002	Punjab	166,183	7,312 (4.39%)	RDT
16.	Ali *et al.*,[31]	2003	Balochistan	1,500	28 (1.86%)	ELISA
17.	Ahmad *et al.*,[32]	2004	Khyber Pakhtunkhwa	4,000	88 (2.20%)	ELISA
18.	Akhtar *et al.*,[33]	2004	Sindh	351,309	6,349 (1.80%)	ELISA
19.	Asif *et al.*,[34]	2004	ICT	3,430	164 (4.78%)	ELISA
20.	Mahmood *et al.*,[35]	2004	Punjab	6,000	16 (0.26%)	RDT
21.	Zaidi *et al.*,[36]	2004	Khyber Pakhtunkhwa	4,000	88 (2.20%)	ELISA
22.	Sirhindi *et al.*,[37]	2005	Punjab	18,216	758 (4.16%)	RDT

23.	Chaudry *et al.*,[38]	2005	Punjab	890	54 (6.06%)	ELISA
24.	Ujjan *et al.*,[39]	2006	Punjab	3,677	246 (6.69%)	ELISA
25.	Mujeeb *et al.*,[40]	2006	Sindh	7,325	264 (3.60%)	ELISA
26.	Fayyaz *et al.*,[41]	2006	Punjab	27,938	704 (2.51%)	RDT
27.	Khan *et al.*,[42]	2006	Punjab	1,426	1 (0.07%)	RDT
28.	Aziz *et al.*,[43]	2006	Gilgit-Baltistan	850	9 (1.05%)	ELISA
29.	Ahmad *et al.*,[44]	2006	Khyber Pakhtunkhwa	41,613	936 (2.24%)	RDT
30.	Sultan *et al.*,[45]	2007	Punjab	41,498	1,529 (3.68%)	ELISA
31.	Masood *et al.*,[46]	2007	Punjab	1,428	36 (2.52%)	ELISA
32.	Khan *et al.*,[47]	2007	Balochistan	1,474	26 (1.76%)	RDT
33.	Ishaq *et al.*,[48]	2007	Sindh	310	4 (1.29%)	RDT
34.	Ijaz *et al.*,[49]	2007	Punjab	7,431	397 (5.34%)	RDT
35.	Bhatti *et al.*,[50]	2007	Sindh	94,177	3,918 (4.16%)	CLIA
36.	Chaudhary *et al.*,[51]	2007	Punjab	1,428	36 (2.52%)	ELISA
37.	Azam *et al.*,[52]	2007	Sindh	688	30 (4.36%)	RDT
38.	Alam *et al.*,[53]	2007	Gilgit-Baltistan	8,949	121 (1.35%)	RDT
39.	Ahmad *et al.*,[54]	2007	Punjab	300	84 (28%)	NAT
40.	Farooqi *et al.*,[55]	2007	Khyber Pakhtunkhwa	166,189	5,312 (3.19%)	RDT
41.	Nazar *et al.*,[56]	2008	Sindh	11,459	237 (2.068%)	RDT
42.	Mujeeb *et al.*,[57]	2008	Sindh	5,345	401 (7.50%)	ELISA
43.	Khattak *et al.*,[58]	2008	Khyber Pakhtunkhwa	1,131	46 (4.06%)	ELISA
44.	Waheed *et al.*,[59]	2009	ICT	18,202	602 (3.30%)	ELISA
45.	Manzoor *et al.*,[60]	2009	Punjab	6,659	512 (7.68%)	ELISA
46.	Abbas *et al.*,[61]	2009	Sindh	804	121 (15%)	ELISA
47.	Shah *et al.*,[62]	2010	Khyber Pakhtunkhwa	32,042	502 (1.56%)	ELISA
48.	Safi *et al.*,[63]	2011	Khyber Pakhtunkhwa	62,251	1,622 (2.6%)	ELISA
49.	Bangash *et al.*,[64]	2011	Khyber Pakhtunkhwa	1,300	15 (1.1%)	RDT
50.	Khan *et al.*,[65]	2011	Khyber Pakhtunkhwa	7,148	477 (6.67%)	ELISA
51.	Borhany *et al.*,[66]	2011	Sindh	5,717	109 (1.90%)	ELISA
52.	Waheed *et al.*,[67]	2012	ICT	10,145	846 (8.33%)	ELISA
53.	Bhutta *et al.*,[68]	2012	Punjab	100	12 (12%)	ELISA
54.	Attaullah *et al.*,[69]	2012	Khyber Pakhtunkhwa	127,828	3,147 (2.46%)	ELISA
55.	Umair *et al.*,[70]	2012	AJK	8,927	224 (2.5%)	ELISA
56.	Arshad *et al.*,[71]	2012	Khyber Pakhtunkhwa	6,538	793 (12.12%)	NAT
57.	Tunio *et al.*,[72]	2013	Sindh	2,696	93 (3.44%)	CLIA
58.	Khan *et al.*,[73]	2013	Balochistan	356	74 (20.78%)	ELISA
59.	Irfan *et al.*,[74]	2013	Sindh	108,393	2,832 (2.61%)	CLIA
60.	Akhtar *et al.*,[75]	2013	Punjab	245	37 (17.78%)	ELISA
61.	Moiz *et al.*,[76]	2014	Sindh	42,830	708 (1.65%)	CLIA
62.	Iqbal *et al.*,[77]	2014	Punjab	217,847	6,400 (2.9%)	RDT
63.	Tabassum *et al.*,[78]	2014	Punjab	15,898	249 (1.56%)	ELISA
64.	Pervaiz *et al.*,[79]	2015	Punjab	5,894	857 (14.5%)	RDT
65.	Niazi *et al.*,[80]	2015	Punjab	56,772	1,046 (1.84%)	NAT
66.	Wazeer *et al.*,[81]	2015	AJK	4,622	129 (2.79%)	RDT
67.	Hussain *et al.*,[82]	2015	Punjab	48,020	1,652 (3.44%)	RDT
68.	Sial *et al.*,[83]	2016	Punjab	29,522	743 (2.51%)	CLIA
69.	Karim *et al.*,[84]	2016	Khyber Pakhtunkhwa	5,318	56 (1.05%)	NAT
70.	Saeed *et al.*,[85]	2016	Punjab	18,274	479 (2.62%)	RDT
71.	Arshad *et al.*,[86]	2016	Sindh	16,602	282 (1.69%)	CLIA
72.	Ahmed *et al.*,[87]	2016	Khyber Pakhtunkhwa	8,439	42 (0.5%)	CLIA
73.	Zameer *et al.*,[88]	2017	Punjab	10,048	377 (3.75%)	RDT
74.	Wadood *et al.*,[89]	2017	Sindh	536	16 (2.98%)	ELISA
75.	Sultan *et al.*,[90]	2017	Sindh	16,957	361 (2.12%)	CLIA
76.	Saeed *et al.*,[91]	2017	Punjab	18,274	480 (2.62%)	RDT
77.	Butt *et al.*,[92]	2017	Punjab	10,516	287 (2.72%)	CLIA
78.	Batool *et al.*,[93]	2017	Khyber Pakhtunkhwa	41,033	566 (1.37%)	CLIA
79.	Ahmed *et al.*,[94]	2017	AJK	10,519	53 (0.50%)	RDT
80.	Saba *et al.*,[95]	2018	Khyber Pakhtunkhwa	3,211	35 (1.09%)	CLIA

81.	Naz *et al.*,[96]	2018	Sindh	14,652	226 (1.54%)	CLIA
82.	Billah *et al.*,[97]	2018	Punjab	1,195	27(2.25%)	ELISA
83.	Rehman *et al.*,[98]	2018	Khyber Pakhtunkhwa	1,400	16 (1.14%)	ELISA
84.	Awan *et al.*,[99]	2018	ICT	30,470	392 (1.28%)	NAT
85.	Akhter *et al.*,[100]	2018	Punjab	7,270	196 (2.69%)	RDT
86.	Azeem *et al.*,[101]	2018	Khyber Pakhtunkhwa	204,942	3189 (1.5%)	ELISA
87.	Wadood *et al.*,[102]	2019	Sindh	517	13 (2.51%)	ELISA
88.	Siddiqui *et al.*,[103]	2019	ICT	847	15 (1.7%)	CLIA
89.	Mengal *et al.*,[104]	2019	Balochistan	23,814	162 (0.68%)	CLIA
90.	Sadiq *et al.*,[105]	2019	Sindh	37,845	2208 (5.83%)	ELISA
91.	Zeeshan *et al.*,[106]	2019	Khyber Pakhtunkhwa	32,012	4,587 (14.32%)	CLIA
92.	Jiskani *et al.*,[107]	2019	Sindh	3,028	65 (2.14%)	ELSIA
93.	Hameed *et al.*,[108]	2019	Punjab	76,530	2,095 (2.73%)	RDT
94.	Latif *et al.*,[109]	2019	Gilgit-Baltistan	3,786	9 (0.23%)	CLIA
95.	Ahmed *et al.*,[110]	2019	AJK	10,519	62 (0.58%)	CLIA
96.	Zaheer *et al.*,[111]	2019	ICT	75,348	941 (1.24%)	CLIA
97.	Ahmad *et al.*,[112]	2019	Punjab	79,774	1,404 (1.75%)	RDT
98.	Rauf and Cheema[113]	2019	Punjab	6,594	214 (3.24%)	CLIA
99.	Saba *et al.*,[114]	2021	Khyber Pakhtunkhwa	41,817	577 (1.37%)	CLIA
100.	Khan *et al.*,[115]	2021	Khyber Pakhtunkhwa	119,263	908 (0.76%)	CLIA
101.	Qadir *et al.*,[116]	2021	Sindh	29,732	908 (3%)	CLIA
102.	Ghazanfer *et al.*,[117]	2021	Punjab	84,305	1,283 (1.52%)	RDT
103.	Saeed and Ullah[118]	2021	ICT	312,320	8,951 (2.86%)	CLIA
104.	Bhatti *et al.*,[119]	2021	Punjab	400	9 (2.25%)	RDT
105.	Arshad *et al.*,[120]	2021	Punjab	200	25 (12.5%)	RDT
106.	Ghazanfar *et al.*,[121]	2022	Punjab	223,242	3,105 (1.391%)	RDT
107.	Bhatti *et al.*,[122]	2022	ICT	120,968	1837 (1.5%)	NAT
108.	Ullah *et al.*,[123]	2022	Punjab	7,858	77 (0.97%)	ELISA
109.	Zorob *et al.*,[124]	2023	Punjab	15,405	83 (0.53%)	CLIA
110.	Shah *et al.*,[125]	2023	Khyber Pakhtunkhwa	6,311	20 (0.316%)	ELISA
111.	Jamal *et al.*,[126] (a)	2023	Sindh	185,781	20,807 (11.19%)	CLIA
112.	Jamal *et al.*,[126] (b)	2023	Punjab	406,039	38,979 (9.59%)	CLIA
113.	Kashif *et al.*,[127]	2023	Punjab	1,500	106 (7.06%)	RDT
114.	Ali *et al.*,[128]	2023	Khyber Pakhtunkhwa	625	9 (1.44 %)	CLIA
115.	Sabir *et al.*,[129]	2023	Punjab	308,767	4,457 (1.4%)	NAT
116.	Aslam *et al.*,[130]	2023	Punjab	6,380	72 (1.12%)	ELISA
117.	Amjed *et al.*,[131]	2023	Punjab	2,236	28 (1.25%)	RDT
118.	Saba *et al.*,[132]	2024	Khyber Pakhtunkhwa	62,185	749 (1.20%)	CLIA
119.	Wazeer *et al.*,[133]	2024	AJK	11,255	8 (0.07%)	CLIA
120.	Jamal *et al.*,[134]	2024	Sindh	4,199,195	812,66 (1.93%)	CLIA
121.	Haleem *et al.*,[135]	2024	Balochistan	21,815	137 (0.62%)	CLIA
122.	Khan *et al.*,[136]	2024	Khyber Pakhtunkhwa	756	15 (1.98%)	ELISA
	Total			**8,875,286**	**241,306 (2.71%)**	

RDT: Rapid Diagnostic Test; ELISA: Enzyme Linked Immunosorbent Assay; CLIA: Chemiluminescence Immunoassay; NAT: Nucleic Acid Testing; AJK: Azad Jammu & Kashmir; ICT: Islamabad Capital Territory

Table 2 shows the pooled subgroup prevalence of HCV among blood donors, stratified by province. The Punjab province reported the highest prevalence at 3.94% (49 studies, 2,055,717 samples), followed by Khyber Pakhtunkhwa at 2.42% (25 studies, 981,432 samples), Sindh at 2.35% (27 studies, 5,158,021 samples), and Islamabad Capital Territory at 2.40% (8 studies, 571,730 samples). Lower prevalence rates were observed in Azad Jammu & Kashmir at 1.03% (5 studies, 45,842 samples), Gilgit-Baltistan at 1.02% (3 studies, 13,585 samples), and Balochistan at 0.87% (5 studies, 48,959 samples). Fig. 2 shows a map of Pakistan to visually represent the geographical distribution of HCV prevalence across Pakistan, highlighting regional variations. The central tendency and spread of HCV prevalence across different provinces/regions were as follows; mean prevalence, 2.07%; median prevalence, 2.35%; and range, 3.07% (from 0.87% in Balochistan to 3.94% in Punjab).

For assessing the potential for publication bias, a Funnel plot (Fig. 3) was constructed by plotting the standard error against log of the prevalence estimates from each study. Funnel plot asymmetry was evaluated visually, and Egger's test was conducted for Funnel plot asymmetry. According to Egger's test results (after adjusting for zero prevalence values), the coefficient for standard error was 88.46 ($p < 0.0001$), and the intercept (const) -4.01 ($p < 0.0001$). As the p-value for the coefficient of the standard error is < 0.0001, it indicated a statistically significant relationship between the standard error and the log prevalence estimates. Hence, suggesting potential publication bias. Likewise, the intercept (const) is negative and statistically significant, indicating asymmetry in the Funnel plot.

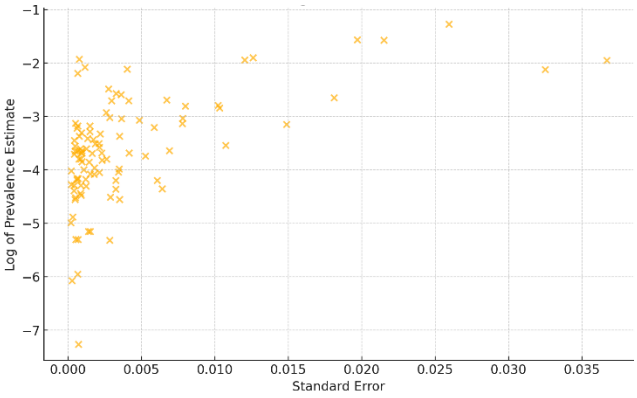

Fig. 3: Funnel plot: Assessing potential for publication bias

The Forest plot (Fig. 4 a & b) visually represents the individual HCV prevalence estimates with their assumed confidence intervals (±0.5%) for each year, alongside the overall pooled prevalence estimate. The relatively tight clustering of individual estimates around the overall mean suggests the stability of the prevalence trend, with no single year showing a significant deviation.

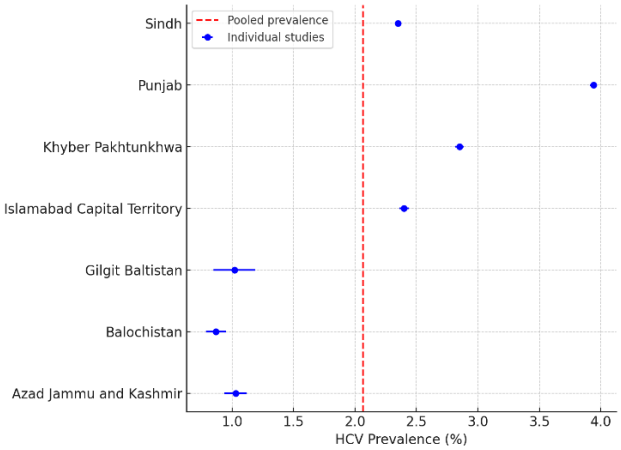

Fig. 4 (a): Forest plot: HCV prevalence across regions

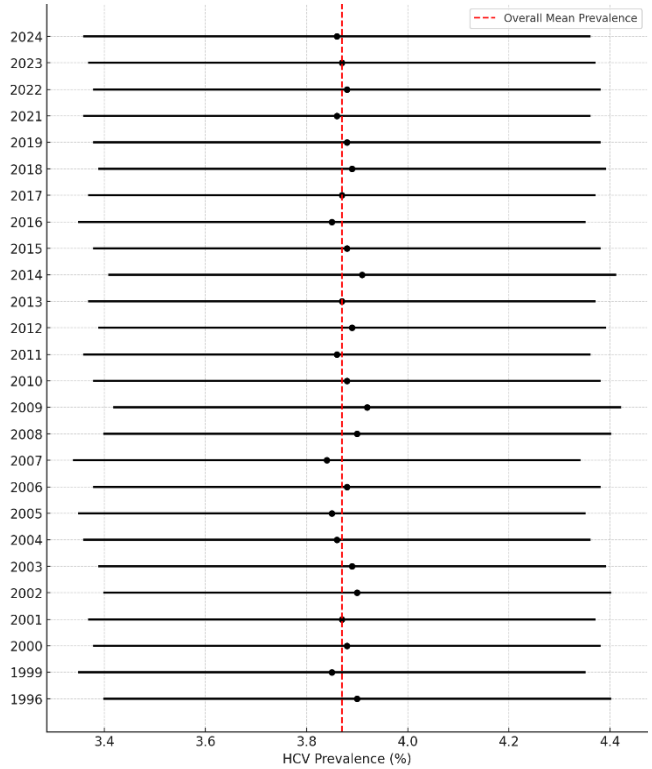

Fig. 4 (b): Forest plot: HCV prevalence estimates and confidence intervals

In Fig. 5, a time series plot is shown with the trend of HCV prevalence among blood donors across Pakistan from 1996

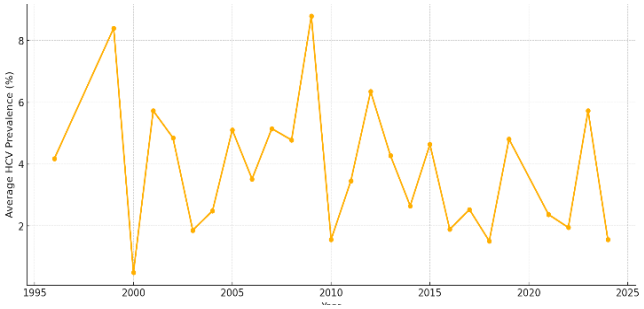

Fig. 5: HCV prevalence trend in blood donors

to 2024. The analysis showed three key phases; (1) initial fluctuations (1996–2005), during which prevalence varied, peaking in 1999 (8.4%) and 2005 (5.11%), with some outliers possibly due to testing limitations or localized outbreaks; (2) stabilization and moderate increase (2006–2015), during which prevalence averaged between 3.5% and 5%, with occasional spikes, reflecting consistent reporting across provinces; and (3) declining trend (2016–2024), when a steady decline began in 2016, stabilizing around 1.5%-2.5%, with the lowest point in 2024 (1.56%) indicating better screening and preventive measures.

The linear regression analysis (Fig. 6) suggested the slope (rate of change) is -0.0700, indicating a decreasing trend in HCV prevalence, with an average decline of 0.07% per year. The intercept is 144.66, suggesting that, based on the linear model, the estimated starting prevalence in 1996 would align with a theoretical intercept. The red regression line in the plot shows the declining trend (negative slope), confirming that HCV prevalence has generally decreased across the years.

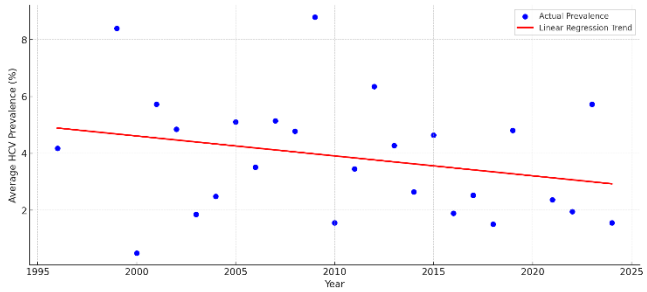

Fig. 6: Linear regression: HCV prevalence (%) over time

The Leave-One-Out (LOO) analysis plot shows (Fig. 7) how the overall mean HCV prevalence changes when each study (year) is excluded from the analysis. The overall mean HCV prevalence across all years is approximately 3.87%, suggesting that no individual year had a significant impact on the overall mean HCV prevalence, with deviations falling within the defined threshold of ±0.5%. This suggests that the overall mean estimate is stable and not heavily influenced by any specific year.

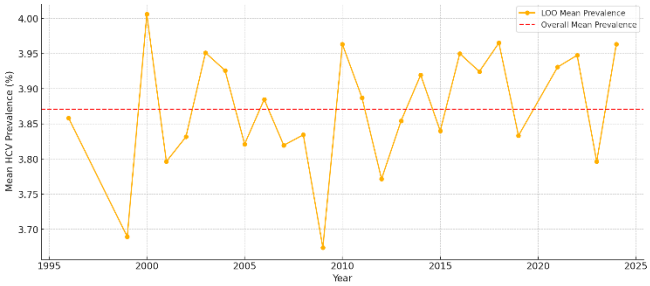

Fig. 7: Leave-One-Out analysis: Mean prevalence over time

Discussion

The majority of blood donations (~ 82%) in Pakistan are collected through family or replacement donations, rather than from voluntary non-remunerated donors,[137,138] which is the gold standard recommended by WHO and national blood safety legislations for ensuring blood safety.[139,140] Family or replacement donations occur when patients' relatives or friends donate blood in exchange for transfusions, a practice that increases the risk of transmitting infectious diseases like HCV, as these donors may feel pressured to donate even when they are not fully aware of their health status.[141] In 2019, Pakistan's blood banking network comprised 650 facilities across the public, private, and NGO sectors. These blood banks varied widely in size, with some collecting fewer than 500 units annually, while others exceeded 100,000 units per year. In the same year, around 2.7 million units of blood were collected nationwide.[142]

Additionally, the overall infrastructure for blood donation and transfusion services in Pakistan remains underdeveloped. Although some progress has been made through the German-funded national blood transfusion programme in establishing regional blood centres and improving the safety of blood transfusions, many facilities still lack the resources necessary for proper screening and storage.[143]

In rural areas, where healthcare access is particularly limited, blood donations are often handled by on-job trained technicians, which may not rigorously screen donations for HCV or other infectious agents. These gaps in the system allow contaminated blood to enter the blood supply chain, further contributing to the spread of hepatitis C.

The findings from this current systematic review and meta-analysis of HCV prevalence among blood donors in Pakistan indicate significant public health concerns. With an overall pooled prevalence of 2.71% from 1996 to 2024, the study underscores the constant challenge of HCV transmission in a resource-constrained healthcare setting.

The observed pooled prevalence of 2.71% among blood donors suggests a continued circulation of HCV within the general population, considering that blood donors are usually considered a healthy cohort. This prevalence aligns with previous studies indicating that HCV infection in Pakistan is disproportionately high when compared with other countries such as Turkey (0.07%),[144] Canada (0.07%),[145] Yemen (0.82%),[146] and China (0.53%).[147] However, in contrast when compared with certain developing countries, our findings were on the lower side, e.g. 8.8% in Ethiopia,[148] 5-25% in Egypt,[149] 3.21% in Kenya,[150] and 3.6% in Nigeria.[151]

Blood donors unknowingly carrying HCV present a significant transmission risk, especially in areas where screening mechanisms are inadequate. As asymptomatic carriers, these donors exemplify the hidden burden of the disease, further complicating efforts to eliminate HCV as a public health concern.

The current findings showed considerable regional variations in HCV prevalence among blood donors. The province of Punjab reported the highest prevalence (3.94%), followed by Khyber Pakhtunkhwa (2.42%) and Sindh (2.35%). These disparities reflect the uneven distribution of healthcare resources and differences in blood safety measures across the provinces. Punjab's high prevalence could be attributed to a combination of poor screening practices, both behavioral and serological. Conversely, regions such as Balochistan (0.87%) and Gilgit-Baltistan (1.02%) reported lower prevalence rates, though these figures could also reflect under-reporting or limited access to diagnostic services.

Unsafe blood transfusions remain a critical factor in HCV transmission in Pakistan. Although the government introduced HCV screening in 1993, inconsistencies in implementation and low-quality screening kits have undermined efforts to ensure safe blood donations.[152]

The time-series analysis indicated fluctuating trends in HCV prevalence over the study period, with notable spikes in 2007, 2013, and 2023. These fluctuations may reflect shifts in public health interventions, including targeted awareness campaigns and enhanced diagnostic capabilities during certain years. The slight downward trend in prevalence observed in the regression analysis suggests some progress in controlling HCV transmission. However, the continued presence of the virus in 2024 emphasizes that Pakistan remains far from achieving the WHO's goal of HCV elimination by 2030.

Pakistan must adopt a multi-pronged strategy to reduce the burden of HCV among blood donors and achieve elimination targets. This includes (but not limited to) strengthening of blood safety regulatory authorities, expanding access to blood transfusion services in rural areas, enhancing public awareness campaigns, integrating treatment with prevention programmes, and establishing a nationwide HCV surveillance programme focusing on high-risk populations, including blood donors, to enable better monitoring and control of the disease.

Conclusion

The prevalence of hepatitis C among blood donors in Pakistan represents a significant public health concern. As one of the highest HCV-burdened countries in the world, Pakistan must prioritize the development of robust screening and prevention protocols to safeguard both blood donors and recipients. Strengthening healthcare infrastructure, particularly in rural areas, improving public awareness, implementing blood safety legislations, and adhering to WHO-recommended guidelines for blood donation and screening practices are essential steps toward reducing the transmission of hepatitis C. The study offers a comprehensive overview of the epidemiological landscape of HCV in Pakistan, serving as a foundation for future policy development and research efforts. Addressing the gaps in blood safety practices and healthcare delivery will be pivotal in controlling the spread of HCV and safeguarding the health of both donors and recipients.

Limitations

The potential limitations of this review include differences in study settings (e.g., urban vs. rural blood donors) and variations in sample sizes may impact the generalizability of the findings. Likewise, the heterogeneity between studies in terms of screening tests used for HCV screening (RDT vs. ELISA vs. CLIA vs. PCR/NAT) could introduce bias as all have different levels of sensitivity and specificity.

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