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Twenty-Year Trends in HBsAg Seroprevalence and Associated Risk Factors Among Blood Donors in Sistan and Baluchestan, Iran (2005 - 2024)

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Abstract

Background: Long-term surveillance of transfusion-transmitted infections is essential, particularly in border regions.

Objectives: This study analyzed 20-year trends in hepatitis B virus (HBV) seroprevalence and associated risk factors among blood donors in Sistan and Baluchestan Province, Iran, from 2005 to 2024.

Methods: This retrospective analysis included 935,864 blood donors. Screening methods evolved from manual enzyme-linked immunosorbent assay (ELISA; 2005 - 2009) to automated ELISA beginning in 2009, chemiluminescence immunoassay (CLIA) beginning in 2019, and nucleic acid testing (NAT) beginning in 2024. A nested case-control study of 300 HBsAg-positive and 300 HBsAg-negative donors was analyzed using multivariable logistic regression.

Results: The overall HBsAg seroprevalence was 0.634% (5,930/935,864). Annual prevalence declined from 2.039% in 2005 to 0.189% in 2024 (relative reduction > 90%; $P < 0.001$), reaching a minimum of 0.019% in 2019. First-time donors had a higher prevalence than repeat donors (1.861% vs 0.021%; $P < 0.001$). Independent risk factors included household contact with an HBV-infected person (adjusted odds ratio [aOR] = 54.5), drug abuse (aOR = 19.4), a history of jaundice (aOR = 8.1), and knife wounds (aOR = 7.3). Higher education was a protective factor.

Conclusions: HBsAg seroprevalence decreased by more than 90% over two decades, reflecting the success of national vaccination and donor-selection strategies. Expanding the repeat-donor pool and maintaining NAT are essential for further improving blood safety.

Keywords: Hepatitis B Virus, HBsAg, Blood Donors, Seroprevalence, Blood Safety

1. Background

Hepatitis B virus (HBV) infection remains a major global public health threat. The World Health Organization estimated 254 million chronic infections in 2022, with nearly one million annual deaths (1). Blood transfusion is a primary route of transmission of blood-borne pathogens, making rigorous donor selection and advanced screening essential (2). Global strategies, including universal infant vaccination and nucleic acid testing (NAT), have substantially reduced HBV prevalence among blood donors (3, 4).

Nevertheless, epidemiological disparities persist between developed and developing countries and across regions within countries. In Iran, HBV epidemiology has changed substantially. The 1993 national mandatory infant vaccination program and the transition to 100% voluntary non-remunerated blood donation by 2007 shifted the country from intermediate to low endemicity (5, 6). National estimates indicate an

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HBsAg prevalence below 1.5% in the general population, with significantly lower rates among blood donors (7, 8).

However, Sistan and Baluchestan Province, located in southeastern Iran, faces unique challenges, including low socioeconomic indices and extensive borders with high-endemicity countries such as Pakistan and Afghanistan. Cross-border movement and localized risk behaviors make this province critical for blood-safety monitoring (9, 10).

2. Objectives

Long-term, comprehensive epidemiological investigations spanning two decades are scarce. This study aimed to conduct a 20-year retrospective analysis of HBsAg seroprevalence and secular trends among blood donors in Sistan and Baluchestan Province from 2005 to 2024, evaluate donor selection and screening protocols, and identify independent risk factors for HBsAg positivity in this geopolitically sensitive border region.

3. Methods

3.1. Study Design and Population

This retrospective epidemiological study combined a 20-year trend analysis with a nested case-control design and was conducted from January 2005 to December 2024 in Sistan and Baluchestan Province, southeastern Iran. The target population comprised all individuals who met the Iranian Blood Transfusion Organization (IBTO) donor criteria. In total, 935,864 blood donors were included in the final trend analysis. Deferred individuals were excluded.

3.2. Blood Donor Classification

Donors were categorized as first-time donors (first donation ever), lapsed donors (prior donation but > 12 months since the last donation), and repeat donors (≥2 donations within the past 12 months).

3.3. Data Collection and Nested Case-Control Design

Data were retrieved from Negareh, the IBTO national electronic registry. For each donation, age, sex, marital status, education, occupation, donation history, and residence were recorded. Using a nested case-control approach, 300 HBsAg-positive donors were selected as cases and 300 HBsAg-negative donors as controls, matched on age (± 2 years) and sex. A structured questionnaire administered by trained specialists collected data on risk exposures, including transfusions, surgeries, dental work, tattooing, cupping, drug abuse, needle-stick injuries, and household HBV contact.

3.4. Laboratory Screening and Confirmation

Screening methods evolved over the study period: manual ELISA (2005 - 2009), automated ELISA from October 28, 2009, CLIA from November 23, 2019, and NAT from August 21, 2024. Initially reactive samples underwent duplicate repeat testing. Repeatedly reactive samples were confirmed using HBsAg neutralization, anti-HBc testing, and NAT in the later period. Only confirmed positive samples were classified as true HBsAg-positive cases.

3.5. Statistical Analysis

Analyses were performed using SPSS version 26.0. The Cochran-Armitage test was used to evaluate linear trends in seroprevalence. Univariable logistic regression was used to identify variables with $P < 0.10$ for entry into multivariable models. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were calculated. Two-tailed $P < 0.05$ indicated statistical significance.

3.6. Ethical Considerations

The study followed the Declaration of Helsinki. Data were anonymized. The protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1404.392).

4. Results

4.1. Baseline Demographics

Among 935,864 donors, 5,930 were HBsAg-positive, corresponding to an overall crude seroprevalence of 0.634%. HBsAg seroprevalence was significantly associated with all sociodemographic variables ($P < 0.001$; Table 1). Males constituted 91.1% of donors and had a significantly higher prevalence than females (0.642% vs 0.544%; $P < 0.001$). Widowed individuals had the highest prevalence (0.96%). A strong inverse gradient was observed between education level and infection, ranging from 2.82% among illiterate individuals to 0.19% among those with a university degree. Unemployed (1.06%) and self-employed (0.96%) individuals had the highest prevalence, whereas healthcare workers had the lowest prevalence (0.11%) (Table 1).

4.2. Twenty-Year Trends

HBsAg seroprevalence declined from 2.039% in 2005 to 0.189% in 2024, corresponding to a 90.7% relative

Table 1. Sociodemographic Characteristics and HBsAg Seroprevalence Among Blood Donors (2005 - 2024) [a]

Variables and Subgroups	Total Donor (No.)	HBsAg Positive (No.)	HBsAg Positive (%)	P-Value
Gender				
Male	852458	5476	0.64	< 0.001
Female	83406	454	0.54	< 0.001
Marital Status				
Married	749028	4737	0.63	< 0.001
Single	184343	1179	0.64	< 0.001
Divorced	1871	8	0.43	< 0.001
Widowed	622	6	0.96	< 0.001
Education				
Illiterate	47706	1347	2.82	< 0.001
Primary/Middle School	298630	2835	0.95	< 0.001
High School (Diploma)	279457	1104	0.40	< 0.001
University degree	300203	569	0.19	< 0.001
Student	9868	75	0.76	< 0.001
Occupation				
Office (Administrative)	227450	708	0.31	< 0.001
Healthcare worker	18532	21	0.11	< 0.001
Self-employed	345771	3306	0.96	< 0.001
Homemaker	49378	394	0.80	< 0.001
Retired	22548	93	0.41	< 0.001
Unemployed	24007	254	1.06	< 0.001
Student	55319	265	0.48	< 0.001
Driver	87357	538	0.62	< 0.001
Others	105503	351	0.33	< 0.001

[a] Statistical significance was evaluated using the chi-square test.

reduction (Cochran-Armitage P < 0.001). Prevalence declined steadily during the first decade and then decreased sharply after 2015, from 0.438% in 2015 to 0.129% in 2016. The lowest rates were observed in 2019 (0.019%) and 2018 (0.022%), remaining below 0.07% from 2017 to 2020. A modest fluctuation occurred from 2021 to 2024, ranging from 0.112% to 0.189%, while rates remained substantially lower than those in the first decade (Table 2).

4.3. Prevalence by Donation Status

A profound disparity was observed across donor strata (P < 0.001). First-time donors accounted for 96.2% of HBsAg-positive cases, whereas lapsed and repeat donors accounted for 2.5% and 1.3%, respectively. Crude prevalence was several-fold higher among first-time donors (1.861%) than among lapsed donors (0.059%) and repeat donors (0.021%). From 2016 onward, annual prevalence among repeat donors remained between 0.00% and 0.005%, with zero positive cases in multiple years (Table 3).

4.4. Geographic Distribution

Seroprevalence varied across districts (P < 0.001). The highest overall prevalence was observed in Iranshahr (0.709%) and Chabahar (0.696%), followed by Zahedan (0.642%) and Zabol (0.593%); Saravan had the lowest prevalence (0.157%). All districts showed consistent downward trends, with annual rates below 0.4% by 2024 (Table 4).

4.5. Age Distribution

Age-specific analysis showed significant downward trends across all cohorts (P = 0.0001). From 2005 to 2010, the highest prevalence was observed in older age groups (55 - 61 years: 5.06% in 2005). By 2024, prevalence in all age brackets had fallen below 0.4%, with the lowest rates among donors younger than 30 years (0.04%–0.05%). Donors aged 21 to 25 years constituted the largest proportion of positive cases (21.13%).

4.6. Multivariable Risk Factor Analysis

Table 2. Annual Trend of HBsAg Seroprevalence Among Blood Donors in Sistan and Baluchistan Province (2005 - 2024) [a]

Year	Total Donation	Total Donor	HBsAg positive(n)	Prevalence (%)
2005	45928	39144	798	2.039
2006	58957	49990	972	1.944
2007	57528	48200	666	1.382
2008	65893	54826	662	1.207
2009	66912	55030	455	0.827
2010	60203	46594	411	0.882
2011	63151	49088	407	0.829
2012	68043	52191	383	0.734
2013	69607	52803	324	0.614
2014	72870	53506	281	0.525
2015	70375	49552	217	0.438
2016	69299	45743	59	0.129
2017	71358	46743	23	0.049
2018	68371	44683	10	0.022
2019	63337	42034	8	0.019
2020	58679	40165	25	0.062
2021	58797	41015	46	0.112
2022	57548	41601	55	0.132
2023	53242	38601	44	0.114
2024	61827	44355	84	0.189
Total	1261925	935864	5930	0.634

[a] Statistical significance was evaluated using the chi-square test.

Univariable analysis identified significant associations with household HBV contact, drug abuse, jaundice, knife wounds, dental procedures, imprisonment, blood transfusion, and tattooing ($P < 0.05$). Cupping, alcohol consumption, surgery, and endoscopy were not significant.

The final multivariable model identified four independent risk factors: household contact with an HBV-positive individual, which was the strongest predictor (aOR = 54.51; 95% CI, 15.86 - 187.32; $P < 0.001$); a history of drug abuse (aOR = 19.40; 95% CI, 2.22 - 169.78; $P = 0.007$); a history of jaundice/icterus (aOR = 8.12; 95% CI, 2.32 - 28.43; $P = 0.001$); and a history of knife wounds (aOR = 7.35; 95% CI, 1.85 - 29.23; $P = 0.005$). Dental procedures were protective (aOR = 0.35; 95% CI, 0.14 - 0.89; $P = 0.028$). Imprisonment (aOR = 2.24; $P = 0.085$) and blood transfusion (aOR = 3.08; $P = 0.195$) lost significance after adjustment (Table 5).

5. Discussion

This 20-year analysis of 935,864 blood donors in Sistan and Baluchestan Province found an overall HBsAg seroprevalence of 0.634%, with a marked decline from 2.039% in 2005 to 0.189% in 2024 (> 90% reduction; $P < 0.001$). This decline reflects the success of Iran's 1993 universal infant HBV vaccination program (11) and the 2007 transition to voluntary non-remunerated donation (6).

5.1. Comparison With National and Regional Data

The observed seroprevalence of 0.634% exceeds the national pooled estimate of 0.53% (95% CI, 0.37%–0.72%) for Iranian blood donors (12), which is consistent with expectations for a border province. The general population prevalence in Sistan and Baluchestan has historically ranged from 2.5% to 3.38% (6, 13). The donor prevalence observed in this study (0.634%) is substantially lower, confirming the healthy donor effect and indicating that many community infections remain undiagnosed.

Nationwide data estimated a prevalence of 0.053% among Iranian blood donors in 2018 (14), compared with 0.022% in this study for the same year. Studies from Fars Province reported declines from 0.36% to 0.024% (12), similar to the trajectory observed here. These findings contrast sharply with data from eastern neighbors; Attaullah et al. reported an HBsAg prevalence of 2.68% among blood donors in Peshawar, Pakistan (2), reflecting later vaccine introduction and differences in donor-safety infrastructure. Despite cross-border

Table 3. Annual HBsAg Seroprevalence by Donation Status (2005 - 2024)

Year	First-Time Donors			Lapsed Donors			Repeat Donors		
	N-total	Positive (No.)	Prev (%)	total	Positive (No.)	Prev (%)	Total	Positive (No.)	Prev (%)
2005	27091	766	2.828	3400	13	0.382	8653	19	0.220
2006	31803	936	2.943	5740	20	0.348	12447	16	0.129
2007	27234	644	2.365	7801	15	0.192	13165	7	0.053
2008	29972	635	2.119	10660	16	0.150	14194	11	0.077
2009	28357	445	1.569	11485	8	0.070	15188	2	0.013
2010	17779	384	2.160	12621	22	0.174	16194	5	0.031
2011	17832	392	2.198	13441	12	0.089	17815	3	0.017
2012	17397	370	2.127	15687	10	0.064	19107	3	0.016
2013	16151	315	1.950	16475	8	0.049	20177	1	0.005
2014	15235	271	1.779	16080	6	0.037	22191	4	0.018
2015	12366	210	1.698	14494	3	0.021	22692	4	0.018
2016	8472	58	0.685	13337	0	0.000	23934	1	0.004
2017	6752	21	0.311	13945	2	0.014	26046	0	0.000
2018	5895	6	0.102	13444	4	0.030	25344	0	0.000
2019	5373	6	0.112	12822	1	0.008	23839	1	0.004
2020	6546	24	0.367	13002	0	0.000	20617	1	0.005
2021	6962	43	0.618	13450	2	0.015	20603	1	0.005
2022	8296	55	0.663	13927	0	0.000	19378	0	0.000
2023	7466	41	0.549	13395	3	0.022	17740	0	0.000
2024	9549	82	0.859	15144	2	0.013	19662	0	0.000
Total	306528	5704	1.861	250350	147	0.059	378986	79	0.021

movement, Iran's relatively low rate of 0.634% indicates effective prevention efforts.

5.2. Sharp Decline After 2015

The striking decline after 2015, culminating in a nadir of 0.019% in 2019, was driven by two factors. First, the IBTO enforced stringent deferral criteria for first-time donors, who accounted for 96.2% of positive cases, while expanding the recall of repeat donors. This strategic transition to a secure repeat-donor pool explains the near-zero prevalence observed in 2017 and 2018. Second, the switch from automated ELISA to CLIA in November 2019 enhanced diagnostic accuracy.

The modest post-pandemic increase from 2021 to 2024 (0.112% to 0.189%) mirrors global trends. US data confirmed a demographic shift toward repeat donors during the pandemic, with HBV odds remaining resiliently higher (15). In this setting, the minor resurgence reflects post-pandemic normalization of collections and the return of first-time donors, in addition to NAT implementation in August 2024, which detected occult HBV infections previously missed by serological screening.

5.3. Age-Specific Trends and Vaccination Impact

In the early study years (2005 - 2010), prevalence was highest among older donors (55 - 61 years: 5.06%), reflecting the pre-vaccination baseline. By 2024, prevalence in all cohorts had dropped below 0.4%, with the most profound reductions among donors younger than 30 years (0.04%–0.05%). This finding underscores the efficacy of Iran's 1993 universal infant vaccination program (11). Although 21.13% of positive cases were among donors aged 21 to 25 years, this reflects the large number of young first-time donors rather than a higher risk of infection.

5.4. Independent Risk Factors

Household contact with an HBV-infected person was the strongest predictor (aOR = 54.51), highlighting intrafamilial transmission in southeastern Iran, where large family size and collective living may facilitate spread. This finding aligns with evidence on familial clustering of HBV in low-income populations (16) and historical data from Zahedan (17). Other risk factors included drug abuse (aOR = 19.40), a history of jaundice (aOR = 8.12), and knife wounds (aOR = 7.35). The association with knife injury suggests cultural practices or nonsterile environments that require targeted education. Imprisonment lost significance after

Table 4. Geographic Distribution of HBsAg Seroprevalence by City (2005 - 2024) [a]

Year	Zahedan			Zabol			Iranshahr			Chabahar			Saravan		
	N	n	Prevalence (%)	N	n	Prevalence (%)	N	n	Prevalence (%)	N	n	Prevalence (%)	N	n	Prevalence (%)
2005	30376	623	2.051	5049	112	2.218	NA	NA	NA	3719	63	1.694	NA	NA	NA
2006	34037	656	1.927	6244	65	1.041	6260	181	2.891	3449	70	2.030	NA	NA	NA
2007	33846	452	1.335	4950	67	1.354	5784	83	1.435	3620	64	1.768	NA	NA	NA
2008	39091	420	1.074	5378	95	1.766	6020	94	1.561	4337	53	1.222	NA	NA	NA
2009	38078	251	0.659	5500	68	1.236	5780	71	1.228	5672	65	1.146	NA	NA	NA
2010	27899	192	0.688	5747	76	1.322	7204	79	1.097	5744	64	1.114	NA	NA	NA
2011	29377	190	0.647	6182	76	1.229	8006	79	0.987	5523	62	1.123	NA	NA	NA
2012	31670	204	0.644	5705	41	0.719	9057	91	1.005	5759	47	0.816	NA	NA	NA
2013	32228	190	0.590	6711	40	0.596	8507	59	0.694	5357	35	0.653	NA	NA	NA
2014	32195	171	0.531	6487	33	0.509	8125	45	0.554	6699	32	0.478	NA	NA	NA
2015	28602	107	0.374	6494	24	0.370	7977	49	0.614	5080	29	0.571	1399	8	0.572
2016	26232	24	0.091	6274	10	0.159	6657	9	0.135	4352	11	0.253	2228	5	0.224
2017	25991	8	0.031	6493	6	0.092	7140	4	0.056	4151	3	0.072	2968	2	0.067
2018	25136	4	0.016	6865	1	0.015	6510	3	0.046	3993	2	0.050	2179	0	0.000
2019	22863	3	0.013	7185	0	0.000	5940	2	0.034	3521	3	0.085	2525	0	0.000
2020	21202	6	0.028	6378	2	0.031	5386	8	0.149	3633	2	0.055	3566	7	0.196
2021	21376	22	0.103	6342	5	0.079	5881	9	0.153	3977	10	0.251	3439	0	0.000
2022	20666	22	0.106	6907	5	0.072	5962	18	0.302	3940	5	0.127	4126	5	0.121
2023	18249	15	0.082	6656	7	0.105	5936	9	0.152	3743	3	0.080	4017	10	0.249
2024	21000	34	0.162	7702	10	0.130	7134	24	0.336	3756	4	0.106	4763	12	0.252
Total	560114	3594	0.642	125249	743	0.593	129266	917	0.709	90025	627	0.696	31210	49	0.157

[a] Abbreviation: NA, Data not available (Saravan center not active or data not recorded in early years).

Table 5. Univariable and Multivariable Logistic Regression Analysis of Potential Risk Factors for HBsAg Positivity [a]

Risk Factors	Case (HBsAg+) | n = 300	Control (HBsAg-) n = 300	Univariable Analysis | OR (95% CI)	P-Value	Multivariable Analysis | aOR (95% CI) | [b]	P-Value
Household contact with HBV+	93 (31.0)	3 (1.0)	44.48 | (13.897 - 142.353)	0.000002	54.509 | (15.862 - 187.322)	0.00000002
Drug Abuse History	23 (7.3)	1 (0.3)	23.66 | (3.168 - 176.712)	0.00028	19.396 | (2.216 - 169.782)	0.007
History of Jaundice (Icterus)	23 (7.7)	3 (1.0)	8.22 | (2.44 - 27.68)	0.00006	8.12 | (2.32 - 28.43)	0.001
Blood Transfusion History	16 (5.3)	3 (1.0)	5.57 | (1.608 - 19.347)	0.007	3.077 | (0.561 - 16.870)	0.195
Knife Wound History	20 (6.7)	7 (2.3)	3.01 | (1.249 - 7.207)	0.014	7.345 | (1.846 - 29.231)	0.005
History of Imprisonment	32 (10.7)	14 (4.7)	2.44 | (1.274 - 4.671)	0.007	2.244 | (0.893 - 5.639)	0.085
Unsafe Sexual contact	20 (6.7)	10 (3.3)	2.07 | (0.953 - 4.504)	0.06	1.145 | (0.370 - 3.540)	0.814
Tattooing	49 (16.4)	31 (10.3)	1.70 | (1.051 - 2.753)	0.03	0.935 | (0.446 - 1.959)	0.858
Alcohol consumption	11 (3.7)	7 (2.3)	1.56 | (0.611 - 4.182)	0.34	1.506 | (0.362 - 6.264)	0.574
Surgical History	94 (31.3)	86 (28.7)	1.14 | (0.801 - 1.610)	0.48	0.848 | (0.464 - 1.551)	0.593
Endoscopy History	18 (6.0)	19 (6.3)	0.94 | (0.485 - 1.837)	0.87	1.341 | (0.500 - 3.593)	0.56
Dental procedure	159 (53.7)	214 (71.3)	0.47 | (0.332 - 0.655)	0.0001	0.354 | (0.140 - 0.894)	0.028
cupping	69 (23.0)	62 (20.7)	1.152 | (0.781 - 1.697)	0.47	1.575 | (0.859 - 2.887)	0.142

[a] Values are expressed as percentage unless otherwise indicated. P values < 0.05 indicate statistical significance. Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

[b] The multivariable model was adjusted for baseline matching and socioeconomic variables, including age, sex, educational attainment, and occupational status.

adjustment, likely because of collinearity with drug abuse (18). Dental procedures were protective (aOR =

0.35), potentially serving as a proxy for higher socioeconomic status and a greater likelihood of HBV vaccination, consistent with the protective effect of higher education.

5.5. Geopolitical and Operational Implications

Sistan and Baluchestan shares porous borders with high-endemicity Pakistan and Afghanistan (19). Persistent hotspots, including Iranshahr (0.709%) and Chabahar (0.696%), indicate that standard deferral is insufficient. The disparity between first-time donors (1.861%) and repeat donors (0.021%) provides a clear roadmap: expanding the repeat donor pool and strengthening pre-donation screening are essential. This aligns with domestic models in which pre-donation viral screening for first-time donors reduced confirmed transfusion-transmitted infection markers to zero (20). NAT implementation in August 2024 is a critical milestone for closing the diagnostic window and detecting occult HBV infection (21, 22).

5.6. Study Limitations

This study has several limitations. First, the findings are limited to blood donors, who are healthier than the general population and may not be generalizable. Second, self-reported risk factors are subject to recall bias. Third, Saravan data were unavailable in the early years. Fourth, NAT was implemented only at the end of the study period; therefore, pre-2024 occult infections could not be assessed. Fifth, changes in screening technology and donor policies over time may have influenced trends. Sixth, small case numbers in some risk categories produced wide confidence intervals, although the associations remained significant.

5.7. Conclusions

This 20-year retrospective analysis demonstrates a sustained decline of more than 90% in HBsAg seroprevalence among blood donors in Sistan and Baluchestan Province, shifting the region to low endemicity. This success reflects Iran's universal infant vaccination program and voluntary blood donation system. However, persistent local hotspots and cross-border population movement require continued vigilance. Strategies to further secure the blood supply should focus on expanding the repeat donor pool, maintaining rigorous pre-donation screening, and using NAT to detect occult infections.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: Acquisition of data: S. K. Analysis and interpretation of data: A. A. M. and S. K. Drafting of the manuscript: S. K. and M. Z. Critical revision of the manuscript for important intellectual content: A. A. M. Statistical analysis: A. A. M. Administrative, technical, and material support: S. K. Study supervision: A. S. and S. K.

Conflict of Interests Statement: The authors do not declare any conflicts of interests for this study.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data will be provided in the form of information tables exported from the Negareh software. The data are not publicly available due to privacy and confidentiality restrictions regarding blood donors' personal and medical information.

Ethical Approval: This study was approved under the ethical approval code of IR.ZAUMS.REC.1404.392 by the Research Ethics Committee of Zahedan University of Medical Sciences (webpage of ethical approval code is: https://ethics.research.ac.ir/ProposalCertificateEn.php?id=IR.ZAUMS.REC.1404.392)

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Informed Consent: For this study, institutional informed consent was obtained from all blood donors at the time of donation. Between 2005 and 2024, every blood donor signed a standardized Donor Certification Form during each donation visit. This mandatory administrative form includes a specific clause explicitly granting permission for their de-identified demographic and clinical data to be utilized for scientific research and medical studies. Because this study is a retrospective analysis of routinely collected, anonymized data from these blood donors, individual study-specific consent was not required, as broad consent had already been legally obtained at the time of donation. All patient data were fully anonymized prior to analysis.

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