Original Article

Serological markers of transfusion transmissible infections and ABO blood groups in Najran, Saudi Arabia

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ABSTRACT

الأهداف : التعرف على معدل انتشار العدوى المنقولة عن طريق نقل الدم « تي تي آي» (TTIs) بين المتبرعين في منطقة نجران . بالإضافة إلى التعرف على الارتباط المحتمل بين تطور « TTT » ونوع فصيلة الدم حسب نظام التصنيف (ABO) / (ABO) .

المنهجية: تمت المراجعة بائر رجعي لبيانات 4120 من المتبرعين خلال فترة امتدت من شهر يناير إلى ديسمبر 2020. تم مسح دم المتبرعين بحثاً عن العلامات المصلية بما في ذلك المستضد السطحي لفيروس الكبد الوبائي ب (HBs Ag)، الجسم المضاد لفيروس من دالوبائي ب (anti-HBc)، الجسم المضاد لفيروس الكبد الوبائي سي (-anti anti-HIV)، الجسم المضاد لفيروس نقص المناعة البشري نوع 1 و 2 (& syphilis 2)، الجسم المضاد لفيروس تي اللمغاوي البشري نوع 1 و 2 ومستضد الزهري syphilis).

النتائج: تم تحديد علامات (TTI) بشكل إيجابي بين المتبرعين بنسبة 10.9% (العدد=449). يعتبر الجسم المضاد لفيروس الكبد الوبائي ب الإيجابي الاكثر تحديداً ((8.9% العدد= 366) يليه المستضد السطحي لفيروس الكبد الوبائي ب (%0.7) العدد=29). العلامات المصلية الاخرى تم تحديدها في أقل من %1 من المتبرعين. الجسم المضاد لفيروس الكبد الوبائي ب الإيجابي كان مرتفع بشكل ملحوظ بين غير السعوديين. يوجد ارتباط بين الفتات العمرية وإيجابية الجسم المضاد لفيروس الكبد الوبائي سي (6.002 م)، الجسم المضاد لفيروس تي اللمفاوي البشري (دلالة إحصائية = (0.004 و مستضد الزهري (دلالة إحصائية = 0.00). أظهرت فصيلة الدم AB+ أكثر و مراكل مالي مائيل مائل مائل مائل من (دلالة إحصائية = إيجابية لعلامات TTT، يليها فصيلة الدم O+. بشكل مشابه، يوجد ارتباط بين فصائل الدم ABA وإيجابية المستضد السطحي لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) ، الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) ، الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) ، الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) ، الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) ، الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية = 10.00) . الجسم المضاد لفيروس الكبد الوبائي ب (دلالة إحصائية =

الخلاصة : التركيز على تنفيذ تدابير مسح فاعلة للدم المتبرع به تم التأكيد عليه في هذه الدراسة . هناك حاجة إلى دراسة مستقبلية لتقييم العدوى المنقولة عير الدم (TTI) على نطاق واسع لتعزيز فهمنا لاتجاهات TTI .

Objectives: To ascertain the prevalence of transfusion transmissible infections (TTIs) across diverse donor groups in the Najran province. Additionally, to establish a potential association between the development of TTI and the donors' blood group, as determined by the ABO/Rh blood grouping system.

Methods: Blood donation data of 4120 donors, spanning from January to December 2020, were retrospectively reviewed. The blood were screened for TTI markers, including hepatitis B surface antigen (HBsAg), anti-hepatitis B core (anti-HBc), anti-hepatitis C virus (anti-HCV), anti-human immunodeficiency

viruses 1 and 2 (anti-HIV1&2), anti-human T-lymphotropic virus types 1 and 2 (anti-HTLV-1&2), and syphilis antigen.

Results: Positive TTI markers were detected in 10.9% of the donors. The most detected TTI marker was anti-HBc (8.9%), followed by HBsAg (0.7%). Other markers were individually detected in <1% of the donors. Anti-HBc-positive was significantly elevated among non-Saudi blood donors. There was an association between age groups and anti-HCV (p=0.002), anti-HTLV (p=0.004) and syphilis antigen (p=0.02) markers positivity. The AB positive blood group exhibited the most positivity for TTI markers, followed by O positive blood group. Similarly, association was found between ABO group and HBsAg (p=0.01), anti-HBc (p=0.001), and anti-HCV (p<0.001) markers positivity.

Conclusion: Emphasis on implementing robust screening measures for donated blood is underscored by this study. There is the need for future study to extensively evaluate TTI status to enhance our understanding of the trend in TTI.

Keywords: transfusion transmissible infection, TTI, blood donor, blood group, marker, seroprevalence

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Blood transfusion, being a crucial component of healthcare services saves millions of lives across the globe. It is often used to support complex medical and surgical procedures, such as transplants and cardiovascular surgery in developed nations, whereas in developing nations, it is used in cases of trauma, obstetric emergencies in women, and malnourished and anemic children.^{1,2} Hence, scarcity of qualitative safe blood or its components may impede necessary health care services.

Transfusion transmissible infections (TTI) are major public health concerns, particularly in developing countries.³ Importantly, the frequency of TTI among blood donors varies across different nations of the world, as well as within Saudi Arabia.⁴⁻¹¹ There is a threat of developing life-threatening illnesses, including TTI, due to unsafe blood transfusion. Human immunodeficiency virus (HIV) types 1 and 2, and hepatitis B and C viruses have all been implicated in TTI.¹²⁻¹⁶ In clinical practice, the ABO blood grouping is widely used. These blood groups are associated with susceptibility towards a number of chronic diseases and viral infections.¹⁷

In the Kingdom of Saudi Arabia (KSA), Ministry of Health (MOH) actively oversees donor blood collection and its related services. All donated blood is screened for various immunological markers including anti-HBc, anti-HBsAg, anti-HCV, anti-HIV1&2, anti-HTLV-1&2, HBsAg, the TPHA and malaria antigen test. However, it is not clear whether any of the TTI has specific prevalence among a peculiar group of donors, such as specific age, gender, or nationality within KSA. In fact, only few studies have attempted to address these issues.² In addition, it is not clear weather incidences of infection due to transfusion is linked to the recipient's ABO/Rh blood group. However, previous studies suggest that ABO/Rh blood groups may influence the susceptibility of a subject to a specific TTI development.¹⁸ In this study, our aim was to evaluate the occurrence of TTI in various donor groups in Najran province and establish a potential correlation between TTI development and the donors' blood group, basing on the ABO/Rh blood grouping.

Methods. Prior to commencing data collection, ethical approval for this study was obtained from the

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relevant local authority. The study received approval from the Health Affairs' General Directorate in Najran city (IRB No. 2022-39 E). Past studies related to this work were reviewed after searching well-known publicly available databases such as PubMed and Google Scholar, among others. Further, this study was conducted in agreement with Helsinki declaration.

Archived data of directed blood donations received at King Khalid Hospital (KKH), the main hospital in Najran province of the Kingdom of Saudi Arabia, were retrospectively reviewed and analyzed. The data consists of 4187 blood donations from January 2020 to December 2020. Subjects with missing demographic data (n=67) were excluded from the study. Inclusion criteria for the actual study subjects (n=4120) were age between 17-65 years, weight not less than 50 kg, being in a good health, no infectious diseases record and hemoglobin level above 13 grams/dl for men and 12 grams/dl for women. The criteria were in line with the national guidelines for blood donation. All participants were screened using appropriate screening tests. Demographic characteristics (age, gender, and nationality), ABO/Rh blood group, and serological markers for TTI were extracted from donor databases.

Blood grouping. Blood group tests including ABO and Rh typing were performed in the hospital laboratory utilizing the IH-500 fully automated blood typing system (Bio-Rad Laboratories Inc., headquartered in California, USA). This cutting-edge system conducts in vitro serological analysis to determine blood grouping and detect antibodies in blood samples, leveraging data from gel card images to deliver accurate results.

Blood screening for TTI. Every blood donation underwent screening via enzyme-linked immunosorbent assay test kits obtained from Abbot Laboratories, Chicago, US, for HBsAg, anti-HBc, anti-HCV, anti-HIV1&2 and anti-HTLV-1&2, rapid plasma reagin card (RPR) test kit (Thermo Fisher Scientific, Waltham, US) for syphilis antigen and the Care Start Malaria PF (HRP-2) Ag RDT (Access Bio, Somerset, New Jersey, USA) for malaria. Each test was performed as per the instructions of the manufacturer. All positive samples underwent additional confirmation by the regional laboratory to validate the results further.

Statistical analysis. Demographic and ABO/Rh blood group data were statistically evaluated utilizing the sixth version of Graph Pad Prism (San Diego, California, USA) to determine the relationship of age, nationality, and blood group with TTI. Pearson correlation coefficient test was performed for the association of different age groups with TTI serological markers, while Spearman's rank correlation coefficient

test was performed for the association of ABO groups with TTI serological markers. Furthermore, tests including Chi-square and ANOVA were done on each of the outcome evaluated by grouping the demographic factors of the donor. A *p*-value of < 0.05 was used to indicate significance.

Results. The current research involved 4120 volunteer blood donors from Najran to determine the prevalence of TTI among the subjects, and their correlation with ABO/Rh blood grouping. A total of 4020 subjects were selected for the study while 67 subjects were excluded as there were missing data. All samples were collected between January 2020 to December 2020 (Figure 1).

The majority of subjects were males, representing 98.7% (n=4067), compared to female subjects (1.3%). The subjects were within 70 years old, and virtually all age groups were represented as depicted in Table 1. Saudi citizens constituted 70.9% (n=2922) of subjects while non-Saudi citizens constituted 29.1% (n=1198). A total of 449 (10.9%) samples had a positive TTI markers. The anti-HBc is recorded as the highest serological marker (8.9%). This was followed by HBsAg (0.7%) and other serological markers which were all individually found to be less than 1% of the entire subjects. A co-infection pattern was observed among 12 subjects as shown in Table 1. The anti-HBc was found to be the dominant marker among the subjects with co-

infection. Further, the distribution of ABO/Rh blood types among the subjects revealed that O positive (+ve) was the most dominant blood group (50%) followed by A+ve (26.8%), B+ve (9.9%), and O negative (-ve) 6.5%.

The association between different age groups and TTI markers positivity was analyzed using Pearson correlation coefficient test. Unlike anti-HBc, anti-HIV I/II p24 and HBsAg, the statistical analysis showed a significant association between anti-HCV, anti-HTIV and RPR with age group. The association between ABO group and TTI markers positivity is also represented in Table 2.

The correlation between age and serological markers of TTI was analyzed and showed a significant increase in anti-HBc with age (Figure 2A). The anti-HCV serological marker showed a significant result in 51-60 years age group as compared to 21-30 years (Figure 2B). Although a higher positivity rate was recorded in the age group 51-60 years, there was no significant difference in anti-HIV-I/II p24, anti-HTLV-I/II and RPR serological markers.

Transfusion transmissible infections test positivity was significantly associated with nationality for anti-HBc, with non-Saudi subjects significantly higher than Saudi subjects. The data of HBsAg, anti-HIV-I/ II, anti-HTLV-I/II and RPR revealed that Saudi and non-Saudi subjects have fairly similar positivity rate (Figure 3).



Figure 1 - A flowchart showing the study design with participant selection criteria.

Table 1 - Characteristics of subjects within this study.

Demographic characteristics of subjects						
Demographic characteristics	n	(%)				
Age						
≤ 20 years old	249	6.0				
21-30 years old	1663	40.3				
31-40 years old	1418	34.4				
41-50years old	613	14.8				
51-60 years old	166	4.0				
61-70 years old	11	0.3				
Gender						
Male	4067	98.7				
Female	53	1.3				
Nationality						
Saudi	2922	70.9				
Non-Saudi	1198	29.1				
Total	n= 4120	100 %				
Prevalence of serological marker	s of TTI among	study subjects				
Serological Marker		• /				
HBsAg	29	0.70				
Anti-HBc	366	8.88				
Anti-HCV	23	0.56				
Anti-HIV-I/II p24	4	0.10				
Anti-HTLV-I/II	16	0.39				
RPR	11	0.27				
Prevalence of multiple markers of	TTI among sero	positive samples				
Co-infection pattern	0	•				
Anti-HBc + Anti-HCV	3	0.07				
Anti-HBc + Anti-HTLV-I/II	3	0.07				
Anti-HBc + RPR	5	0.12				
Anti-HCV + Anti-HIV-I/	1	0.02				
II p24	1	0.02				
Positivity rate of all conventional TTI markers with respectiveABO/						
Rh blood groups*						
ABO/Rh		Seropositive				
		samples, n (%)				
AB+	107 (2.6)	18 (16.8)				
A+	1105 (26.8)	122 (11.0)				
B+	409 (9.9)	39 (9.5)				
O+	2063 (50.1)	239 (11.6)				
AB-	10 (0.2)	1 (10.0)				
A-	120 (2.9)	12 (10.0)				
В-	37 (0.9)	1 (2.7)				
O-	269 (6.5)	16 (5.9)				
Total	4120 (100)	448 (10.9)				
*One sample positive for a TTI marker (anti-HTLV-I/II) did not have						
the blood group record and was thus excluded from the analysis of						
1 11 markers based on blood groups. 1 11: transfusion transmissible infection HBsAg benatitis B surface antigen Anti-HBC anti-benatitis						
B core, Anti-HCV: anti-hepatitis C virus. Anti-HIV-I/II: anti-human						
immunodeficiency viruses 1 and 2, Anti-HTLV-I/II: anti-human						
T-lymphotropic viruses types 1 and 2, RPR rapid plasma reagin test for						
syphilis antigen.						

The TTI serological tests for anti-HBc among blood grouping showed a significant association between AB+ve and O-ve, as well as between O+ve and O-ve. A-ve blood group demonstrated high positivity towards the anti-HCV test. The O-ve blood group showed a

Fable	2 -	Association	between	transfusion	transmissible	infections
(TTIs) markers positivity and different age and ABO groups.						O groups.

TTI markers	P-value			
Association with age groups				
HBsAg	0.19			
Anti-HBc	0.34			
Anti-HCV	0.002			
Anti-HIV	0.40			
Anti-HTLV	0.004			
RPR	0.02			
Association with ABO group				
HBsAg	0.01			
Anti-HBc	0.001			
Anti-HCV	< 0.001			
Anti-HIV	0.15			
Anti-HTLV	0.09			
RPR	0.06			
HBsAg: hepatitis B surface antigen, Anti-HBc: Anti-hepatitis B				
core, Anti-HCV: Anti-hepatitis C virus, Anti-HIV: anti-human				
immunodeficiency virus, Anti-HTLV: anti-human T-lymphotropic				
virus, RPR: rapid plasma reagin test for syphilis antigen				

significant rise in anti-HIV-I/II p24 serological marker compared to O+ve blood group. In the anti-HTLV-I/II serological test data, the A-ve and AB+ve blood groups showed the highest positivity among other blood groups. The O+ blood group had the highest positivity in PRP serological marker test (Figure 4).

Discussion. The identification of TTI antibodies in donor blood is crucial for ensuring the integrity and safety of the blood supply. It protects both donors and recipients by preventing the transmission of infectious agents during blood transfusions and other medical procedures. These have made rigorous screening protocols and compliance with regulatory standards essential components of blood safety programs worldwide. Thus, our study aims to estimate the occurrence of TTI markers among donors and their correlation with ABO/Rh blood grouping.

The donors in this study were found to be predominantly male (98.7%). This disparity in gender composition was not surprising as women tend to have higher deferral rates. Moreover, the finding is comparable to other previous reports where majority of donors were males.^{15,19} Few studies on TTI seroprevalence among blood donors in Saudi Arabia was conducted previously.¹⁵ The study conducted by Alshahrani et al¹⁹ in Abha, revealed that the overall prevalence of TTI serological markers was 11%, which is similar to the finding in our study (10.9%). However, our finding is higher than the overall prevalence rate reported in Qatar (2.7%), as well as in Riyadh, Saudi Arabia (0.7%).^{4,20} These results highlight the need for



Figure 2 - Age groups versus markers of transfusion transmissible infections (TTI) from positive subjects. Comparison of different age groups yielded different statistical results for (A) Anti-hepatitis B core (anti-HBc): ≤20 versus 21-30 (*p*=0.14), 21-30 versus 31-40 (*p*<0.001), 31-40 versus 41-50 (*p*<0.001), 41-50 versus 51-60 (*p*<0.009), 51-60 versus 61-70 (*p*=0.76), 21-30 versus 41-50 (*p*<0.001) (B) Anti-hepatitis C virus (anti-HCV): ≤20 versus 21-30 (*p*=0.86), 21-30 versus 31-40 (*p*=0.92), 31-40 versus 41-50 (*p*=0.83), 41-50 versus 51-60 (*p*=0.08), 31-40 versus 51-60 (*p*=0.06), 21-30 versus 51-60 (*p*=0.03), ≤20 versus 51-60 (*p*=0.16), 31-40 versus 51-60 (*p*=0.17) (C) Anti-human immunodeficiency viruses 1 and 2 (Anti-HIV-I/II p24): ≤20 versus 21-30 (*p*=0.12), ≤20 versus 31-40 (*p*=0.16), 31-40 versus 51-60 (*p*=0.86), 21-30 versus 31-40 (*p*=0.45), 31-40 versus 51-60 (*p*=0.46) and (E) Rapid plasma reagin (RPR): ≤20 versus 21-30 (*p*=0.79), ≤20 versus 31-40 (*p*=0.37), 41-50 versus 51-60 (*p*=0.61), 31-40 versus 51-60 (*p*=0.79), ≤20 versus 31-40 (*p*=0.37), 41-50 versus 51-60 (*p*=0.61), 31-40 versus 51-60 (*p*=0.79), ≤20 versus 31-40 (*p*=0.45), 31-40 versus 51-60 (*p*=0.61), 31-40 versus 51-60 (*p*=0.79), ≤20 versus 31-40 (*p*=0.40), 31-40 versus 51-60 (*p*=0.79), ≤20 versus 31-40 (*p*=0.45), 31-40 versus 51-60 (*p*=0.79), ≤20 versus 51-60 (*p*=0.79).

further awareness campaigns, especially in regions with higher prevalence rate of TTI.

In line with the study of Alshahrani et al¹⁹ who identified anti-HBc as dominant among other serological markers, the serological marker was the most dominant in our study with the highest rate of 8.9%. However, this rate is higher than that previously reported in Abha (5.9%) and the Eastern region of Saudi Arabia (2.9%).¹⁹ Additionally, lower rates have been reported in Croatia (1.3%) and Lebanon (7.7%). were reported.^{21,22} Variable rates of anti-HBc among blood donors have been reported in other parts of the world including much higher rates of 41% in Lao and 48.5% in Nigeria.^{23,24} Additionally, we found a significantly higher rates of anti-HBc positivity among the non-Saudi donors compared to the Saudis in this present study. The observation was, however, not startling, as anti-HBc positivity rates that hovers around 10% have been documented among some blood donor groups in neighboring Yemen which shares border with Saudi Arabia.^{25,26} Indeed, approximately 64% of donors with anti-HBc positivity in this study were Yemenis (data not shown). Anti-HBc serves as a valuable indicator of exposure to HBV, regardless of the individual's current infectious status. This is particularly significant at the end of a resolving infection when HBsAg and HBV nucleic acid testing might not yield positive results.²⁷ Notably, our study also demonstrates that anti-HBc positivity increased with age. TTI markers and ABO blood groups ... Alshehri et al



Figure 3 - Nationality versus serological markers of transfusion transmissible infections (TTI) from positive subjects. Comparison of Saudi and non-Saudi participants yielded different statistical results for hepatitis B surface antigen (HBsAg) (p>0.05), anti-hepatitis B core (anti-HBc) (p<0.001), anti-hepatitis C virus (anti-HCV) (p>0.05), Anti-human immunodeficiency viruses 1 and 2 (Anti-HIV-I/II p24) (p>0.05), Anti-human T-lymphotropic virus types 1 and 2 (anti-HTLV-I/II) (p>0.05), and rapid plasma reagin (PRP) (p>0.05).



Figure 4 - All blood groups versus serological markers of transfusion transmissible infections (TTI) from positive subjects. Comparison of different blood groups yielded different statistical results for (A) anti-hepatitis B core (anti-HBc): A+ve versus A-ve (p=0.55), B+ve versus B-ve (p=0.25), AB+ve versus AB-ve (p=0.67), O+ve versus O-ve (p<0.004) (B) Anti-hepatitis C virus (Anti-HCV): A+ve versus A-ve (p=0.14), A-ve versus B+ve (p=0.01), O+ve versus O-ve (p=0.57) (C) Anti-human immunodeficiency viruses 1 and 2 (Anti-HIV-I/II p24): B+ve versus O+ve (p=0.20), O+ve versus O-ve (p=0.03), B+ve versus O-ve (p>0.05) (D) Anti-human T-lymphotropic virus types 1 and 2 (Anti-HTLV-I/II): A+ve versus A-ve (p=0.57), AB+ versus O-ve (p=0.39) and (E) Rapid plasma reagin (PRP): B+ve versus O+ve (p=0.57), and O+ve versus O-ve (p=0.87).

Unlike anti-HBc, we observed a low prevalence of anti-HBsAg (0.7%) among the donors. This rate is lower than the 5.8% reported in Asir, but comparable to the 1.1% in Makkah 29 and 0.3% in Eastern Saudi Arabia.^{28,30} Our finding is, however, not surprising as the prevalence of HBV chronic disease in Saudi Arabia is estimated at <2%, making the country a low-risk area for international travellers.³¹ The other serological markers assessed in this study were found in less than 1% of the study population, suggesting the relative safety of the donated blood. Although possible cases of co-infection were identified, the cases represented an infinitesimal fraction of the donors assessed in this study. Overall, although not accounted for in this study, the differences in the reported TTI markers occurrence could be attributed to the study participant's economic status and lifestyle.

In our study, donors with blood group O were the most dominant, accounting for more than 50% of the donors. This is not surprising as blood group O is considered the most common blood type among people.^{18,32} Moreover, in a previous report in Saudi Arabia, about 47% of blood donors were identified as O+ve.¹⁵ In a study conducted in India, the highest TTI sero-reactivity was found in blood group B+ve blood donors (1.8%) followed by O+ve (1.54%).³³ Another study similarly reported the highest sero-reactivity for TTIs among donors with blood group B (2.2%) and blood group A (2.2%).³⁴ In contrast, our study detected the highest TTI sero-reactivity among AB+ve blood group donors, followed by those with O+ve blood group.

We further assessed the potential association of the donors' blood groups and TTI positivity given that the ABO blood group has been associated with certain infectious diseases in the past. For example, a meta-analysis study found an association between blood groups and infection with SARS-CoV-2. It was hypothesized that blood group A has the highest risk, whereas the lowest risk is associated with the O blood group.³⁵However, a recent retrospective study in Ethiopia investigating TTI prevalence among 27027 blood donors did not find any significant association between the donors' ABO/Rh blood group and the TTI markers tested (HIV, HBV, HCV, and syphilis).³² Interestingly, our study found an association between ABO blood group and HBsAg, anti-HBc, and anti-HCV positivity. While these associations were observed in Najran, it is crucial to evaluate the phenomena in donors from other regions of Saudi Arabia.

Study limitations. Although expected, the number of female donors were low. This hindered us from

performing an extensive gender-based analysis. Further, the study is a single-center study even though it was conducted at the main hospital in Najran province. Inclusion of donors from multiple locations within Najran may provide a broader overview of the status of TTI among blood donors in Najran province.

In conclusion, TTI antibody screening plays a central role in maintaining the safety and integrity of the blood supply. It is a critical practice that helps check infectious diseases transmission, protect recipients' health, and ensure the overall safety of blood transfusions and related medical interventions. This study investigated several TTI markers among blood donors in Najran, Saudi Arabia, and found an overall TTI rate of 10.9%, with anti-HBc being the most dominant. The findings from this study underscore the need for effective and robust screening of donor blood.

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