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Genotypic Distribution of MNS Red Blood Cell Antigens Among Healthy Blood Donors in Northern Pakistan

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Abstract

Introduction: Blood transfusion is a life-saving measure but the body may react to the new blood, causing more harm than good. Matching donor red blood cells to recipient antigens prevents allo-immunization. In MNS, anti-M, anti-N antibodies are mostly clinically benign, anti-S and anti-s antibodies cause transfusion reactions. Our objective was to establish a genotypic profile of the MNS blood group in Pakistani population.

Methodology: This study was conducted at Armed Forces Institute of Transfusion, Rawalpindi from March - August 2022, on 385 blood donors after approval by Institutional Review Board. Written informed consent was taken, ABO and Rh phenotyping was performed with commercially available antisera, Genotyping was done using PCR-SSP for different antigens, along with positive and negative controls.

Results: Mean age of participants was 29.4 years with σ 7.11; 98.4%(379) males,1.6%(6) females. ABO and RhD phenotyping revealed: A = 23.4% (90), B = 40.3% (155), AB = 6.2% (24), O = 30.1% (116), RhD +ve = 88.1% (339) and RhD -ve = 11.9% (46). The genotypic prevalence of MNS antigens was M = 75.6%(291), N = 43.6%(168), S = 60.5%(233) and s = 75.6%(291) respectively. The most common genotypes were N/N (n=41, 73.2%) and S/s was (n= 193, 82.8%).

Conclusion: This research showed high genetic prevalence of N/N and S/s antigens in donors. Therefore, blood banks should routinely start antigen typing for MNS blood groups in addition to ABO and RhD. The outcomes of our study may assist them in establishing an extended phenotyping/ genotyping protocol to prevent any adverse transfusion event.

Introduction

Blood transfusions can be truthfully called a “life saving measure”. They are commonly administered in many injuries, surgeries and medical conditions. However, it is also possible for the body to react to the foreign blood. In order to prevent any unwanted reaction to occur, we match the donor red blood cells to the recipient antigens. Knowing the blood group genotypes of the donors in any population is important to improve health care services and to better serve patients.

After ABO blood group system discovery, Landsteiner and Levine identified the first two antigens of the MNS blood group system, the M and N antigens[1]. The MNS blood group system is one of the most complex blood group systems, second only after the RH system. It comprises of up to 50 antigens [2]. It is also the only system that is genetically represented by three homologous genes, *GYPA, GYPB*, and *GYP* [3]. The MNS antigens are fully expressed at birth. They are carried on two carrier molecules, glycophorin A (GPA) and glycophorin B (GPB). GPA carries the M and N antigens, whereas GPB carries the S and s antigens[4]. These glycophorin molecules serve as entry points into the red cells for various parasites, bacteria and viruses. They also contribute to red blood cell membrane stability as well as play an important role in the migration of leukocytes.

The anti-M and anti-N antibodies are generally harmless since they are naturally occurring IgM antibodies. These antibodies can be disregarded if they do not react at 37°C. Although rare instances of anti-M and anti-N antibodies causing hemolytic transfusion reactions (HTR) and hemolytic disease of the fetus and newborn (HDFN) have been reported, Anti-S and anti-s antibodies are IgG antibodies that have been associated with both immediate and delayed hemolytic transfusion reactions, as well as Hemolytic Disease of Fetus and Newborn[5]. HDFN is known to be caused by various low-prevalence MNS antibodies.

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The knowledge about the immunogenicity as well as genetic prevalence of red blood cell antigens can provide guidance during performing cross match of donor antigens to the recipient antibodies. This can help in making transfusion safer and optimizing the blood bank resources. To assess and minimize the risk of allo-immunization, it is essential to determine the frequency of both common and rare antigens. This is particularly important for patients who require frequent blood transfusions throughout their life, such as those with cancer, children with thalassemia major, thalassemia syndromes, and sickle cell anemia.

Therefore, in order to estimate and reduce the risk of allo-immunization, it is important to find out the frequency of common and less common antigens. This is especially crucial for such patients who receive multiple blood units all around the year such as oncology patients, children with thalassemia major, thalassemia syndromes and sickle cell anemias.

There is limited published research on this subject in Pakistan or South Asia. The main aim of this study was to create a genotypic profile of the MNS blood group in the population of Northern Pakistan.

Operational definitions:

Antigen: Any substance that can bind to a specific antibody[6].

Antibody: Antibodies are naturally produced by plasma cells within the human body to mediate an adaptive immune response against invading pathogens.

Alloimmunization: Induction of immunity in response to foreign antigen(s) encountered through exposure to cells or tissues from a genetically different member of the same species

Phenotype: The outward appearance of the individual. It is the product of interactions between genes, and between the genotype and the environment.

Methodology

This cross-sectional study was conducted at the Armed Forces Institute of Transfusion, Rawalpindi, from March to August 2022, following approval from the Institutional Review Board. A non-probability convenience sampling technique was used. Written informed consent was obtained from all participants before enrollment.

Blood samples were collected from healthy individuals residing in Northern Pakistan who either voluntarily donated blood or served as replacement donors at the blood bank of the Armed Forces Institute of Transfusion. All donors provided written informed consent in accordance with the Declaration of Helsinki and completed the standard blood donation questionnaire before donation. Donor eligibility was assessed according to the World Health Organization (WHO) criteria for healthy blood donation to ensure that only eligible and healthy individuals were included. Both male and female donors were enrolled.

Individuals who had donated blood within the preceding three months or had a hemoglobin (Hb) level below 12.5 g/dL were excluded from the study.

The required sample size was calculated using the OpenEpi sample size calculator, assuming a 95% confidence level, 5% absolute precision, and a reported prevalence of the S antigen of 49.4% in a Pakistani population.[7] The calculated sample size was 384; therefore, 385 blood samples were collected.

Venous blood samples (n=385) were collected in ethylenediaminetetraacetic acid (EDTA) tubes following standard venipuncture procedures. The venipuncture site was disinfected using povidone-iodine followed by an isopropyl alcohol swab. Each sample was appropriately labelled and processed for blood group analysis. ABO and Rh blood grouping was performed using forward and reverse tube techniques with commercially available antisera. Following testing, the samples were stored at 4°C.

Data were analyzed using Statistical Package for the Social Sciences (SPSS), version 20.0. Descriptive statistics, including frequencies, percentages, and means, were used to summarize the study population and estimate the prevalence of blood group antigens and genotypes.

The chi-square test was used to compare genotype frequencies within the study population and, where appropriate, with frequencies reported in other populations. A p-value of <0.05 was considered statistically significant.

Genomic DNA was extracted from lymphocytes using chelex method[8]. For RBC washing, 300ul of white blood cells were taken from the buffy coat layer through the

help of a micropipette. These WBCs were added to 900ul of distill water in pre-labelled Eppendorf tubes, in order to lyse any remaining red blood cells. The tubes were shaken and centrifuged at 5000rpm for 2 minutes. Supernatant was discarded, another 900ul distill water was added to cell pellet settled at the bottom of tube. This process of RBC washing was repeated three times until the supernatant became pale pink. Once all supernatant was discarded, 50uL chelex was added to each tube, vortexed and put into hot plate at 95 degrees centigrade for 20 minutes. The tubes were then placed into centrifuge at 10,000rpm for 2 minutes. The supernatant was transferred to another pre labelled Eppendorf tube and previous one was discarded. Then, according to the following temperature program, PCR steps were performed: 1 cycle at 95 ℃ for 10 min and 32 cycles (at 94 ℃ for 30 s, at 60 ℃ for 30 s and 72 ℃ for 30 s) and 1 cycle at 72 ℃ for 5 min.

Genotyping of the common antigens was carried out by using polymerase-chain-reaction-sequence-specific-primer (PCR-SSP)[9] for different antigens (M, N, S, s).and amplified products were separated on 2% polyacrylamide gel electrophoresis PCR technology. Known positive and negative controls for different antigens were used for validation of results. Primer sequences are presented in Table I.

Results

In full analysis dataset, the mean age of participants in our study came out to be 29.4 years with standard deviation of 7.11. There were 98.4%(379) male participants and 1.6%(6) female participants. Our study depicted the phenotypic prevalence of ABO blood group antigens as follows: A = 23.4% (90), B = 40.3% (155), AB = 6.2% (24), O = 30.1% (116). These results were similar to previous studies done on Pakistani populations: B > O > A > AB.[7] For Rh blood group antigens, our study indicated the phenotypic prevalence of RhD +ve donors = 88.1% (339) whereas RhD -ve donors = 11.9% (46). [7] The genotypic prevalence of MNS antigens in our population came out as M = 75.6%(291), N = 43.6%(168), S = 60.5%(233) and s = 75.6%(291) respectively.

The frequencies of ABO, Rh and MNS antigens in total observed samples in our study are shown in table 2 and figure S1.

The samples giving diffuse bands on electrophoresis were repeated using the PCR-SSP and gel electrophoresis technology and included back in our study, however the sample still giving diffuse bands on repeated run were reported as inconclusive.

Majority of participants of our population were from Punjab province 79% (304) while least number of

Table II: The frequencies of ABO, Rh and MNS antigens in total observed samples in our study

Antigen	Positive	Negative	Inconclusive
	% (n)	% (n)	% (n)
A	23.4 (90)	76.5 (294)	0 (0)
B	40.3 (155)	59.6 (229)	0 (0)
AB	6.20 (24)	93.7 (360)	0 (0)
O	30.1 (116)	69.7 (268)	0 (0)
Rh	88.1 (339)	11.7 (45)	0 (0)
M	75.6 (291)	14.5 (56)	9.9 (38)
N	43.6 (168)	43.1 (166)	13.2 (51)
S	60.5 (233)	28.3 (109)	11.2 (43)
s	75.6 (291)	10.9 (42)	13.5 (52)

Table I: MNS Primer Sequence Specific Primers.

Allele specificity	Product size (bp)	Primer name	Sequence 5' to 3'
M1	432	Mf2	AATTGTGAGCATATCAGCATC
		Mr2	GGGTCTGAGCTGAACTCAG
M2	262	Mf2	AATTGTGAGCATATCAGCATC
		NMr1	GCAAGAATTCCTCCATAGTAG
M3	250	Mf3	CAGCATCAAGTACCACTGGT
		NMr1	GCAAGAATTCCTCCATAGTAG
M4	109	NMf1	CAAAGCACAGAAATGATGCAC
		Mr2	GGGTCTGAGCTGAACTCAG
N1	262	Nf2	AATTGTGAGCATATCAGCATT
		NMr1	GCAAGAATTCCTCCATAGTAG
N2	250	Nf3	CAGCATTAAGTACCACTGAG
		NMr1	GCAAGAATTCCTCCATAGTAG
S1	128	gSas	ACGATGGACAAGTTGTCCCA
		SSf1	TGATTAAGAAAAGGAAACCCG
S2	128	Ksas	CGATGGACAAGTTGTCCCG

participants were from Gilgit Baltistan 0.5% (02) and Balochistan province 0.5% (02). The relative frequency of antigens across various participants of our study from different provinces of Pakistan is as shown as table III.

The most common genotypes were N/N (n=41, 73.2%) and S/s was (n= 193, 82.8%) in contrast to the least common genotype that was of the rare combination s-/s- (n=14, 12.8%).

Table IV: Genotypic comparison of our population and Irani population

Genotype	Our study	Irani population [11]
MN		
M+N+	42.3%	52%
M+N-	52.6%	38%
M-N+	73.2%	10%
Ss		
S+s-	11.2%	13%
S+s+	82.8%	45%
S-s+	81.7%	41%
S-s-	12.8%	

Discussion

In multi-transfused individuals, the development of the alloantibodies and/ or alloimmunization is a common complication. In majority of blood banking setups, only ABO and RhD grouping is performed in routine pre-transfusion testing. Hence, the incidence of alloimmunization from other RBC antigens is particularly high. This causes significant problems in cross matching donor blood to the recipient especially if recipient is undergoing chronic transfusion therapy.

DNA analysis is important to correctly identify the antigen profile in all patients who have received multiple transfusions. It is particularly significant in order to evaluate any genetic variations such as partial or weak antigens that could explain the presence of alloantibodies but give false positive phenotypes during standard blood typing procedures.

Our study results are similar to those derived in another study performed on Pakistani population in Islamabad[7]. The differences in antigenic composition of Pakistani population and the Indian, Caucasian, African and Chinese populations highlight certain important points. First one being, in most of our transfusion centers, the screening cell panels used to detect the presence of any/ all unexpected antibodies, are usually commercially prepared and they are procured from international companies and vendors. The intention of improving blood safety by performing antibody screening in all prospective patients is a great step towards reducing adverse transfusion reactions. However, the use of screening cells prepared from the blood of foreign donors still leaves a gap in research. It gives rise to the possibility that the antibodies especially ones against the minor antigens, in the local population may go undetected.

The M antigen was most prevalent antigen 89.26% observed in Saudi population while it came out to be 75.6% in our population, the prevalence of N antigen was 51.67% in Saudi population while 43.6% in our population. The frequencies of S and s were 61.07% and 82.55% in Saudis whereas 60.5% and 75.6% in Pakistani population respectively[10]. These similarities are

Table III: The relative frequency of antigens across various participants of different provinces of Pakistan

Ethnicity	% (n)	M		N		S		s	
		(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Punjab	79% (304)	75% (228)	15.8% (48)	48.0% (146)	38.5% (117)	62.2% (189)	27% (82)	74% (225)	12.8% (39)
Sindh	2.6% (10)	70% (7)	30% (3)	20.0% (02)	50.0% (05)	90% (9)	10% (1)	70% (7)	0% (0)
KPK	8.8% (34)	76.5% (26)	0% (0)	26.5% (09)	58.8% (20)	38.2% (13)	38.2% (13)	82.4% (28)	2.9% (1)
Islamabad	3.6% (14)	100 (14)	0 (0)	14.3 (02)	85.7 (12)	71.4 (10)	28.6 (4)	85.7 (12)	14.3% (2)
AJK	4.9% (19)	63.2 (12)	26.3 (5)	36.8 (07)	52.6 (10)	63.2 (12)	26.3 (5)	78.9 (15)	0% (0)
Gilgit Baltistan	0.5% (2)	100% (2)	0% (0)	100% (2)	0% (0)	0% (0)	100% (2)	100% (2)	0% (0)
Balochistan	0.5% (2)	100% (2)	0% (0)	0% (0)	100% (2)	0% (0)	100% (2)	100% (2)	0% (0)

noteworthy, just as how both Pakistani and Saudi population have high prevalence of the k antigen from the Kell blood group system.[15] Additionally K antigen is present at a lower frequency in both populations. Although there may be geographical variations and ethnic background differences between the individuals of Pakistani and Saudi population, many RBC antigen profiles are similar. However, the frequencies of some other genotypes were different.

Graphical Representation of Genotypic comparison of our population and Irani population is demonstrated in Figure S2. In the MN genotypic comparison of our population with that of the Irani people, M-N+ was the commonest genotype of our study, coming out to be 73.2% whereas it was reported in only 10% of Iranis. In the genotypic comparison of Ss antigens, S+s- was the most prevalent in our population; 82.8%, but this genotype was present in only 45% of Iranis[11]. Represented in table 4 & figure S2.

As there is little work published regarding MNS genotypes in the published data we researched, we drew a comparison of the recorded phenotypes of MNS antigen frequencies in the international data with our studies genotypes, repesented in table 5. In the MNS blood group system, the most common genotype in our study was M-N+, with the frequency of 73.2% which is much greater than observed phenotypes in Indians (11.3%)[12], Caucasians (22.0%), Africans (30.0%)[13], 20.3% in Chinese[14].

In case of Ss genotype, the highest prevalence in Pakistani population was found to be of S+s+ (82.8%), which was about two times more common than Indians 43.9%[12], Caucasians 44.0%, Africans 28.0%, [13], and Chinese 8.7%[14]. These findings suggest a unique distribution pattern of some blood groups among the Pakistani blood donors.

Although the sample size of this study was relatively small compared with the population of Pakistan, the findings provide a useful estimate of the frequencies of MNS blood group antigens in the study population. The study was conducted at the Armed Forces Institute of Transfusion, Rawalpindi, which is located in northern Pakistan. Although the blood bank receives patients and donors from various regions of the country, the majority of donors included in this study were from northern Pakistan. Therefore, the findings may not fully represent the genetic diversity of the Pakistani population, as differences in gene pools among geographical regions may influence the distribution of blood group antigens. Multicenter studies involving blood banks from different regions of Pakistan are therefore recommended to establish more representative estimates of MNS blood group antigen frequencies and to assess regional variations within the country.

Conclusion

This research has demonstrated high genetic prevalence of N/N and S/s antigens in donors that may lead to allo-immunization in recipients of transfusion. Therefore, it is important for all of Pakistan's blood banks to start routinely undertaking extended antigen typing for minor blood groups in addition to ABO and RhD groups. MNS antigen typing would be a small step in the right direction. Our study aimed at making transfusion safer through genotyping evidence. It presents a demographic snapshot of known ABO, Rh and MNS antigen frequencies that will be useful for providing antigen-matched blood units to all transfused individuals.

The outcomes of our study may assist the blood banks in Punjab province of Pakistan to establish an extended phenotyping/ genotyping protocol including the MNS antigens, in particular S and s antigens, to preclude any allo-immunization events as well as prevent hemolytic reactions secondary to these antigens.

Limitations: This study was conducted in Northern Pakistan. The MNS gene pool of our Southern counterpart may vary. The inconclusive/ missing samples run on PCR, in our study, may cause slight variation in the reported genotypic antigen frequencies. For more accurate analysis of the MNS gene pool, advanced DNA sequencing is needed.

References

1. Landsteiner K, Levine P. Further observations on individual differences of human blood. *Proc Soc Exp Biol Med*. 1927;24(9):941–942. doi:10.3181/00379727-24-3649
2. 2) Daniels GL, Fletcher A, Garratty G, et al. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. *Vox Sang*. 2004;87(4):304–316. doi:10.1111/j.1423-0410.2004.00564.x
3. 3) Lögdberg L, Reid ME, Lamont RE, Zelinski T. Human blood group genes 2004: chromosomal locations and cloning

strategies. *Transfus Med Rev*. 2005;19(1):45–57. doi:10.1016/j.tmrv.2004.09.007
4. 4) Daniels G. MNS blood group system. In: *Human Blood Groups*. 3rd ed. Wiley-Blackwell; 2013:96–161. doi:10.1002/9781118493595.ch3
5. 5) Reid ME. MNS blood group system: a review. *Immunohematology*. 2009;25(3):95–101. doi:10.21307/immunohematology-2019-240
6. 6) 2001. Immunobiology, 5th edition: The Immune System in Health and Disease. Garland Publishing
7. 7) Jabin F, Waheed U, Ahmed S, Arshad M, Arshad A, Zaheer HA. Red blood cell phenotyping of blood donors in Islamabad, Pakistan. Global Journal of Transfusion Medicine. 2018 Jan 1;3(1):26.
8. 8) S. A. (2012, July 5). Manual of PCR. http://grcpk.com/wp-content/uploads/2014/10/14.-PCR-in-Neoplastic-Disorders.pdf
9. 9) Heymann GA, Salama A. Sequence-specific primers for MNS blood group genotyping. Blood Transfus. 2010 Jul;8(3):159-62. doi: 10.2450/2009.0111-09. PMID: 20671875; PMCID: PMC2906194.
10. 10) Halawani AJ, Habibullah MM, Dobie G, Alhazmi A, Bantun F, Nahari MH, Dawmary I, Abu-Tawil HI. Frequencies of MNS Blood Group Antigens and Phenotypes in Southwestern Saudi Arabia. Int J Gen Med. 2021 Dec 3;14:9315-9319. doi: 10.2147/IJGM.S344826. PMID: 34887679; PMCID: PMC8650831.
11. 11) Gholamrezazade A, Amirizadeh N, Oodi A. Genotyping analysis of the MNS blood group system of thalassemia patients with alloantibodies in Iran. Transfus Apher Sci. 2021 Feb;60(1):103006. doi: 10.1016/j.transci.2020.103006. Epub 2020 Nov 4. PMID: 33229298.
12. 12) Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. Indian J Med Res. 2013 Mar;137(3):521-6. PMID: 23640559; PMCID: PMC3705660.
13. 13) Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. Academic press; 2012 Nov 7.
14. 14) Lin-Chu M, Broadberry RE, Chang FJ. The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. Transfusion. 1988 Jul 8;28(4):350-2
15. 15) Mehmood A, Alam M, Yazdani MS, Rathore MA. FREQUENCY OF KELL ANTIGENS (K & K) AMONG BLOOD DONORS OF NORTHERN PAKISTAN. Pak Armed Forces Med J [Internet]. 2019 Oct. 23 [cited 2025 May 26];69(5):977-80. Available from: https://www.pafmj.org/PAFMJ/article/view/3398