Frequency of neutrophil alloantigens by polymerase chain reaction

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ABSTRACT

Objective: Neutrophil alloantigens are responsible for a number of immune clinical disorders such as neonatal alloimmune neutropenia, febrile transfusion reaction and transfusion related acute lung injury. Recent progress enabled typing of neutrophil antigens by deoxyribose nucleic acid based techniques. In this study we report for the first time the frequency distribution of human neutrophil antigen system-one in Saudis.

Methods: Blood samples were collected from 100 successive healthy Saudi male blood donors presenting to Regional Laboratory and Blood Bank, Dammam, Kingdom of Saudi Arabia in 1997. Human neutrophil antigen typing was carried out using polymerase chain reaction – sequence specific primer method.

Results: Our results show that human neutrophil antigen system-one is highly polymorphic in this population and similar in its distribution to the Hispanic and Native Americans but different from the Caucasians, Indians and Africans.

Conclusion: We found the polymerase chain reaction-sequence specific primer method to be a practical technique for neutrophil alloantigen typing. We recommend introducing this technology in tertiary care hospitals to allow easier diagnosis of immune neutropenias.

Keywords: Neutrophil antigens, polymerase chain reaction, neonatal neutropenia, human neutrophil antigen.

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Polymorphism in neutrophil alloantigens is responsible for alloimmune clinical disorders such as neonatal alloimmune neutropenia (NAN), febrile transfusion reaction (FTR) and transfusion related acute lung injury (TRALI). The phenotype of neutrophil alloantigens may also underlie autoimmune clinical disorders such as autoimmune neutropenia of infancy (AIN).[1] These neutrophil specific antigens are not probably present on the surface of the neutrophil for the sole reason of causing immune disorder. They are expected to participate in neutrophil functions such as clearance of antigen - antibody (Ag-Ab) complexes by acting as constant fragment of immunoglobulin molecule (Fc) receptors. Whether polymorphism of these antigens contributes to the effective function of the neutrophil in terms of variable rate of immune complex clearance is not known.[2] Interestingly, complete absence of the clinically most important neutrophil antigen (NA) system is not associated with severe infections, which may be explained by compensation from other Fc receptor species. These patients are however at risk of developing isoimmune antibodies resulting in alloimmune neutropenias. In one study, NA null phenotype was associated with mild tendency to recurrent infections and autoimmune diseases in some patients.[3] Typing neutrophils for their private antigens has been revolutionized in the past decade after the introduction of genotyping technology. The new technology avoided many limitations associated with

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the conventional serotyping methods. Among the different polymerase chain reaction (PCR) techniques available, both sequence specific primer (SSP)[4] and sequence specific oligonucleotides (SSO)[5] were applied for this purpose. Despite this significant progress in typing neutrophil alloantigens, many uncertainties still remain. Molecular knowledge of other neutrophil alloantigens is lacking which is necessary to extend the PCR technology beyond the NA system. Major progress in this field was achieved by the introduction of new nomenclature system for neutrophil alloantigens by the Granulocyte Antigen Working Party of the International Society of Blood Transfusion (ISBT) who met in Spain in 1998.[6] This new nomenclature included the well-defined granulocyte alloantigens and was called human neutrophil antigen (HNA) system.[6] The molecular knowledge available to that date allowed the designation of 5 HNA loci: HNA-1 to HNA-5. Three alleles: a, b and c were recognized for the HNA-1 locus while only one possible allele was recognized for each of the loci, HNA-2 to HNA-5.[6] The extent of neutrophil alloantigen polymorphism varies in different nations. Hessner[7] demonstrated that NA2 is the predominant allele in white Americans (Caucasians), black Americans (African ancestry) and Asian Indians while NA one is more common in Native Americans and Hispanics. Japanese also have high frequency of NA-1 allele.[8] Variations in frequency distribution of neutrophil alloantigens may be attributed to the genetic make-up of a nation reflecting their racial origin modified by genomic interchange through inter-marriages and migration. There is no reason to implicate natural selective factors, as no particular advantage is obvious from any single phenotype over the others. The extent of polymorphism in the Middle East and Arab countries has not previously been studied. The Kingdom of Saudi Arabia (KSA) represents an interesting genetic environment. The genetic make-up of the residents of this country is that of Arabic ancestry modified through inter-marriages with residents from neighboring countries. The Indian ß-globin gene cluster haplotype is a common example in sicklers from Qatif and Al-Hassa, in the Eastern Province of KSA[9] while African ß-globin gene cluster haplotype is commonly seen in Benin, Central West Africa.[10] It would, therefore, be interesting to study the distribution of HNA in the KSA. In this study we evaluated the frequency distribution of HNA-1a and HNA-1b in the Kingdom using PCR-SSP technique. Human neutrophil antigen-one system is clinically the most important and the best understood at a molecular level among the other HNA systems. Knowledge of local HNA-one polymorphism may help to predict the clinically significant HNA antibodies in the regions as well as being of anthropological significance.

Methods.

Blood samples. Five to 10cc of citrate, phosphate and dextrose (CPD) blood samples were collected from 100 successive healthy Saudi male blood donors presenting to the Regional Laboratory and Blood Bank, which is located in Dammam, in the Eastern Region of KSA.

Deoxyribose nucleic acid extraction. Deoxyribose nucleic acid (DNA) was extracted using the salting-out technique with ethanol precipitation as described in previously reported literature.[11]

Deoxyribose nucleic acid amplifications. Extracted DNA was amplified in a Perkin Elmer Gene Amp 9600 using the PCR-SSP technique described by Hessner.[7] The reaction mix was 500ng template DNA, 0.6µM forward primer, 0.6µM reverse primer, 200µM deoxyribonucleoside 5-triphosphate (dNTP), 1µ Taq polymerase, 0.3µM positive control primer for human growth hormone gene, 10µM PCR buffer and water. Two sets of primers were included for HNA-1a and HNA-1b amplification. **Table 1** shows the sequence of these primers. Amplification was run for 30 cycles, each consisting of 94°C for one minute, 63°C for one minute and 72°C for one minute. A final extension time was extended to 10 minutes. The expected size of the amplified products are 118bp for HNA-1a and 171bp for HNA-1b.[7] Detection of the amplified product was achieved by electrophoresis of the amplified product on 2% agarose gel with ethidium bromide staining. Amplified products of HNA-1a and HNA-1b were recognized based on their molecular weight size, the former being 53bp shorter.

Results.

The results of this study are shown in **Table 2**. Gene frequencies were calculated by dividing the sum of homozygosity for an allele plus the half of the heterozygosity for the same allele over the total. Using this formula, the gene frequency for HNA-1a was found to be 0.53 and HNA-1b was 0.47.[12] **Figure 1** shows the agarose gel electophoretogram of 3 amplified DNA samples. Amplified products were the expected size when compared to a MW marker included in well number 8, among the 97 amplified samples, no single case of NA-null phenotype was detected.

Discussion.

To our knowledge this is the first study of its type in Arab countries. Results shown in **Table 1** and calculated gene frequencies demonstrate that both HNA-1a and HNA-1b alleles occur at high frequency in KSA, indicating that HNA-1 gene system is highly polymorphic in this population. We may anticipate from this fact that HNA-1 system may be important clinically. Recently we reported a case of neonatal alloimmune neutropenia due to HNA-1b antibody.[13] In the primer design of this PCR-SSP method, we utilized the advantage of allele

Table 1 - Primers used for amplifications of constant fragment receptor 3 b (FcRIIIb).

Allele	Sequence	Annealing position	Product size
HNA-1a-F*	5'CTCAATGGTACAGGGTGCTC-3'	126 - 147	118bp
HNA-1b-F*	5'CTCAATGGTACAGGGTGCTT-3'	126 - 147	171bp
HNA-1a-R**	5'GGCCTGGCTTGAGATGAGGT-3'	227 - 246	-
HNA-1b-R**	5'CACCTGTACTCTCCACTGTCGTT-3'	277 - 299	-

F* - forward primer, R** - reverse primer, HNA - human neutrophil antigen, bp - bone pair, C - cytosine, T - thymine, A- adenine, G - guanine

Table 2 - Frequency distribution of human neutrophil antigen system in Saudis.

Genotype	N	Genotype Frequency (%)
HNA-1 (a^+, a^+)	10	(10)
HNA-1 (a^+, b^+)	82	(85)
HNA-1 (b^+, b^+)	5	(5)
No amplification	3	-
Total	**100**	**(100)**

N - number,
HNA - human neutrophil antigen,
a^+, b^+ - refer to the presence or absence of a or b alleles at HNA locus.

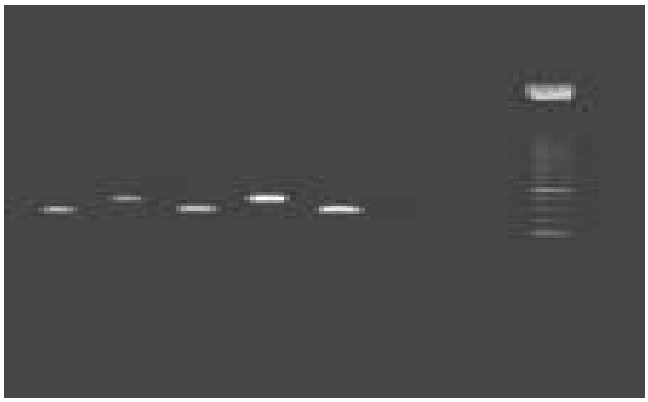

Figure 1 - Agarose gel electrophoretogram of 3 deoxyribose nucleic acid samples. Well number 1 and 2 - sample one of human neutrophil antigen-one (a^+, b^+), well number 3 and 4 - sample 2 of human neutrophil antigen-one (a^+, b^+), well number 5 and 6 - sample 3 of human neutrophil antigen-one (a^+, a^+), well number 7 - negative control (no amplification), well number 8 - 50 bone pair molecular weight ladder. a^+, b^+ - refer to the presence or absence of a or b alleles at human neutrophil antigen locus.

Table 3 - Genotype frequency of human neutrophil antigen one system in Saudis versus other ethnic groups.

Ethnic group	Sample size	Genotype frequency			Gene frequency	
		HNA-1 (a^+, a^+)	HNA-1 (a^+, b^+)	HNA-1 (b^+, b^+)	HNA-1a	HNA-1b
Saudis*	97	0.10	0.85	0.05	0.53	0.47
White Americans[7]	90	0.11	0.51	0.38	0.37	0.63
Asian Indian Americans[7]	92	0.16	0.28	0.55	0.30	0.70
Native Americans[7]	98	0.20	0.71	0.09	0.55	0.45
African Americans[7]	99	0.16	0.30	0.54	0.31	0.69
Hispanic Americans[7]	99	0.17	0.72	0.11	0.53	0.47

* - results obtained from current study, HNA - human neutrophil antigen, a^+, b^+ - refer to the presence or absence of a or b alleles at HNA locus.

specific primers, which render the amplification process allele specific thus simplifying downstream detection of the amplified product. Most PCR-SSP methods utilize a single common primer, which results in amplified products of the same size. In this method, the reverse primers were intentionally designed to be different to hybridize at different positions and to yield amplified products of different sizes, which enhanced their detection. This helped to eliminate false interpretation due to contamination or non-specific amplification. **Table 3** compares the gene frequency of HNA-1a and HNA-1b in Saudis from the current study with other populations. As can be seen, HNA-1a is more common in Saudis, which make it closer to the gene frequencies in Native Americans and Hispanics but clearly different from the distribution in Caucasians, Indians and Africans. History suggests the Hispanic origin of Native Americans,[14] which may explain similarities in the genetic make-up. Besides, inter-marriages were probably common between Arabs and Hispanics during the Moslem Empire dominance in Spain. We have recently noticed a peculiar genetic make-up for Saudis regarding human platelet alloantigens (HPA),[14] which was different from other populations in the East or West. Although, this study does not report any case of NA-null phenotype, the detection of this rare phenotype requires larger sample. In our hands, we found that PCR-SSP method is a practical method for neutrophil alloantigen genotyping. It may be useful to diagnose immune neutropenias and transfusion reaction such as TRALI. Genotyping may also be relevant in donor recipient matching for granulocyte transfusions and in prenatal diagnosis of NAN.

In summary, we found high frequency of both HAN-1a and HNA-1b alleles in Saudis. This may anticipate the clinical significance of HNA-1 system in immune neutropenias in this population. Introducing such technology in our hospitals will allow easier diagnosis of such conditions. Further clinical studies to evaluate the clinical significance of neutrophil antibodies and the gene frequencies of other HNA systems in Saudis are needed.

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