

The Distribution of Human Leukocyte Antigens Genotypes Among Nigerian Sickle Cell Anaemia Patients

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ABSTRACT

Background: Human leukocyte antigens (HLA) are involved in the modulation of immune responses, thus specific haplotypes, alleles and residues at different loci have been linked with disease susceptibility. There is no substantial data regarding HLA allele's linkage to sickle cell anaemia (SCA) patients from Nigeria.

Objective: This study was carried out to determine the HLA -A*, -B*, -C*, -DRB1* and -DQB1* allele frequency distribution among SCA patients of Nigerian origin.

Methods: This was an observational descriptive study of 169 registered SCA patients of the Sickle Cell Foundation Nigeria who met the European Society for Blood and Marrow Transplantation (EBMT) criteria for haematopoietic bone marrow transplantation and they were counseled for HLA tissue typing along with their siblings to know if there were any potential match among them that could donate stem cell for HSCT. Ten milliliters of venous blood were collected in a sterile Na-EDTA anticoagulant tube from each consenting subject and samples were collected and sent in batches to Istituto Mediterraneo Di Ematologia (IME), Rome, Italy for HLA study. Genomic DNA was isolated from the lymphocytes through QIAGEN DNA mini-isolation kit. HLA genotyping for HLA -A*, -B*, -C*, -DRB1* and -DQB1* loci typing was done using sequence specific oligonucleotide polymerase chain reaction.

Results: Sixteen alleles were detected at HLA A* locus, 24 alleles at HLA B* locus, 12 alleles at HLA C* locus, 13 alleles at HLA DRB1* locus, and 5 alleles at HLA DQB1* locus. The allele frequencies of these most common alleles were $\geq 10^{-1}$. The less common alleles have allele frequencies (AF %) of $\geq 10^{-2}$.

Conclusion: HLA (A*23, A*30 and A*02), (B*53 and B*15), (C*04 and C*07), (DRB1*15, DRB1*13, DRB1*11 and DRB1*03) and (DQB1*06, DQB1*05, DQB1*02 and DQB1*03) are the most frequent alleles among SCA patients in Nigeria.

Keywords: Sickle cell anaemia, bone marrow transplantation, human leukocyte antigen, sequence specific oligonucleotide polymerase chain reaction, allele

INTRODUCTION

Sickle cell anaemia (SCA) is one of the sickle cell disorders. It is an inherited autosomal recessive haemoglobinopathy,¹ characterized by the presence of the homozygous haemoglobin S gene.² SCA is the most severe genetic variant of the sickle cell diseases. Currently, hydroxyurea, blood transfusion and bone marrow transplantation are the major therapeutic intervention modalities, however bone marrow transplantation remains the most successful and the only curative therapy.³

SCA phenotypes are multigenic due to the diversity of pleiotropic genes involved in SCA pathology which are not identical in patients, thus the severity of SCA complications varies among individuals.^{1,3} Human leukocyte antigen (HLA) alleles have been established as adapting risk factors for vascular disease and occurrence of SCA complications including stroke, infections, Red Blood Cell (RBC) alloimmunization, skin ulcers,⁴ vaso-occlusive episodes, acute chest syndrome, avascular necrosis, leg ulcers, priapism and retinopathy.¹ However, heterogeneity of this phenotypes can also be modulated by other factors including environmental, affluence and population genetic diversity.¹ Thus, data on the distribution of pleiotropic genes, especially HLA genes which are important in the selection of compatible donors for transplantation of patients with SCA is of great importance.

The human leukocyte antigen (HLA) system is one of the most polymorphic immunological genetic systems in human genome and usually used in the anthropological analysis, disease linkage analysis, population genetics, forensic sciences, and organ transplantation, especially bone marrow transplantation.^{5,6} HLA plays a central role in the immune response, which may explain why specific HLA alleles may be linked to diseases, and why the high level of HLA genetic diversity may represent a selective response to pathogen-rich environments, among other possible mechanisms.⁷

Najat *et al.*⁴ established positive association between HLA-DRB1*100101 and the development of vaso-occlusive complications in SCA patients from Bahrain. HLA A*0102, A*2612, A*3301 have been reportedly linked with stroke in SCA patients from Brazil,¹ as HLA DRB1*0301, DRB1*0302, DRB1*1501, DQB1*0201, DQB1*0602 DPB1*0401 and DPB1*1701 were

prevalent in individuals with SCA of African-American origin.^{1,4,8} As specific HLA alleles and haplotypes may influence immune-mediated response associated with complications in SCA, we therefore hypothesize that specific HLA alleles and haplotypes may be characteristic of SCA. This study addressed the distribution of HLA genetic mapping in SCA patients of Nigerian origin.

MATERIALS AND METHODS

Subjects

This was an observational descriptive study of 169 registered SCA patients of the Sickle Cell Foundation Nigeria who met the European Society for Blood and Marrow Transplantation (EBMT) criteria for haematopoietic bone marrow transplantation and they were counseled for HLA typing along with their siblings to know if there were any potential donors among their siblings. Informed consent was obtained from all the participants of this study through the clinicians who ordered the HLA typing. Ten milliliters of venous blood was collected in a sterile tube (sodium-ethylene diamine tetraacetate (Na-EDTA) anticoagulant bottles 50IU/ml) from each subject and samples were prepared to obtain lymphocytes according to the method described by Kankonkar *et al.*⁹

HLA Genotyping

Genomic DNA was isolated from the lymphocytes through QIAGEN DNA mini-isolation kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol.¹⁰ HLA genotyping for HLA -A*, -B*, -C*, -DRB1* and -DQB1* loci antigen profiles were determined using sequence specific oligonucleotide polymerase chain reaction, a proposed standard protocol given by the XII International Histocompatibility Workshop in 1996.¹¹ HLA -A*, -B*, -C*, -DRB1* and -DQB1* loci typing was done using PCR-SSO probe (LABType – One Lambda, Canoga Park, CA) following the manufacturer's protocol.

In 10 µl PCR reaction tubes, HLA class I (A*, B* and C*) loci were amplified under the following thermal cycling conditions: at 96 °C for 3 minutes; 5 cycles at 96 °C for 20 seconds, 60 °C for 20 seconds, 72 °C for 20 seconds; 30 cycles at 96 °C for 10 seconds, 60 °C for 15 seconds, 72 °C for 20 seconds; and a final extension at 72 °C for 10 minutes. However, HLA class II (DRB1* and DQB1*) loci were amplified under the following thermal cycling conditions: 95 °C for 5 minutes; 35 cycles at 95 °C for 20 seconds, 58 °C for 20 seconds, 72 °C for 20 seconds, and a

final extension at 72 °C for 10 minutes. Corresponding sequence specific oligonucleotide probes of the HLA -A*, -B*, -C*, -DRB1* and -DQB1* genes (LABType – One Lambda, Canoga Park, CA) were used to detect all the PCR products as LABScan100 (One Lambda, Canoga Park, CA) was used to identify the intensity of fluorescence in the SSO reaction. HLA genotyping of each locus was finally established using HLA Fusion 2.0 software (One Lambda, Canoga Park, CA).

Statistical Analysis

The allele frequency (AF %) was calculated using the formula described by Kankonkar *et al.*⁹ as $AF = n+/(2N)$; where $n+$ is total number of individuals having the allele, N is the total number of individuals studied.

RESULTS

Demography of these subjects revealed they were all of Nigerian origin, however of different ethnicity majorly Yoruba, Igbo and Hausa. The percentages of male subjects were 56.8% while female subjects constituted 43.2%. Their mean age was 10.88±5.38.

The HLA -A*, -B*, -C*, -DRB1* and -DQB1* allele frequencies among sickle cell anaemia patients are shown in Figure 1 to 5 respectively. Sixteen alleles were detected at HLA A* locus (Figure 1), 24 alleles at HLA B* locus (Figure 2), 12 alleles at HLA C* locus (Figure 3), 13 alleles at HLA DRB1* locus (Figure 4), and 5 alleles at HLA DQB1* locus (Figure 5). In SCA patients, HLAA*23, HLAA*30 and HLAA*02 were the three most frequent alleles respectively at HLAA* locus; HLA B*53 and HLA B*15 were the two most frequent alleles respectively at HLAB* locus; HLA C*04 and HLA C*07 were the two most frequent alleles respectively at HLA C* locus; HLA DRB1*15, HLA DRB1*13, HLA DRB1*11 and HLA DRB1*03 were the four most frequent alleles respectively at HLA DRB1* locus; HLA DQB1*06, HLA DQB1*05, HLA DQB1*02 and HLA DQB1*03 were the four most frequent alleles respectively at HLA DQB1* locus. The frequencies of these most common alleles were $\geq 10^{-1}$. However, some alleles including HLA (A*68, A*74, A*03, A*36, A*34, A*33, A*66, A*01, A*29, and A*32 respectively), (B*58, B*44, B*35, B*07, B*57, B*49, B*45, B*42, B*14, and B*52 respectively), (C*16, C*06, C*03, C*02, C*08, C*15, C*17, C*18, and C*14 respectively), (DRB1*07, DRB1*08, DRB1*01, DRB1*09, DRB1*12, DRB1*14, DRB1*04, and DRB1*10 respectively), and DQB1*04 frequencies were $\geq 10^{-2}$.

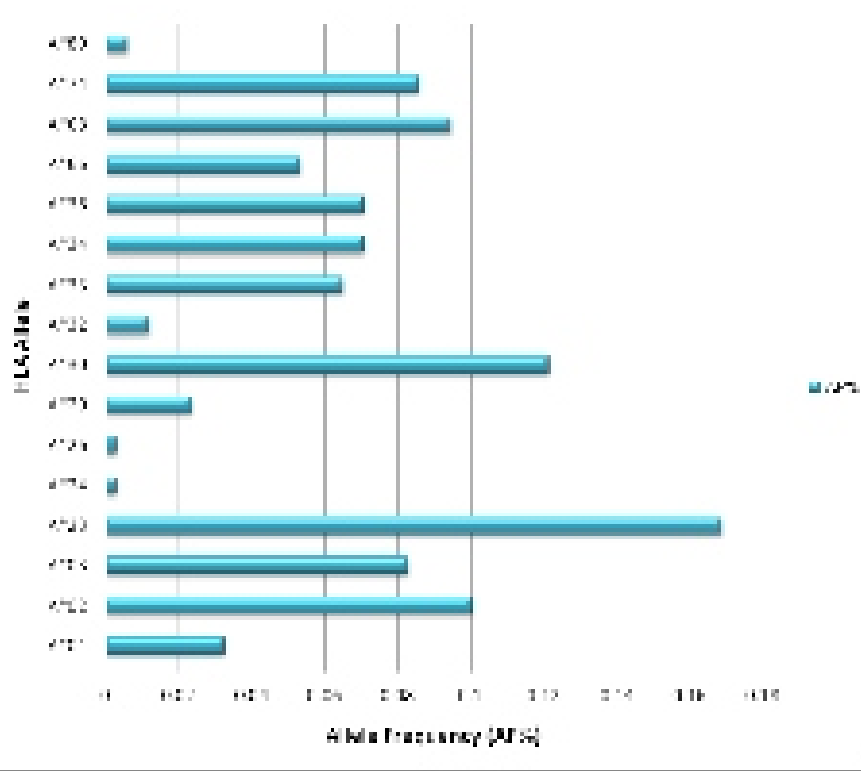


Figure 1: The HLA A* alleles frequency distribution in sickle cell anaemia patients

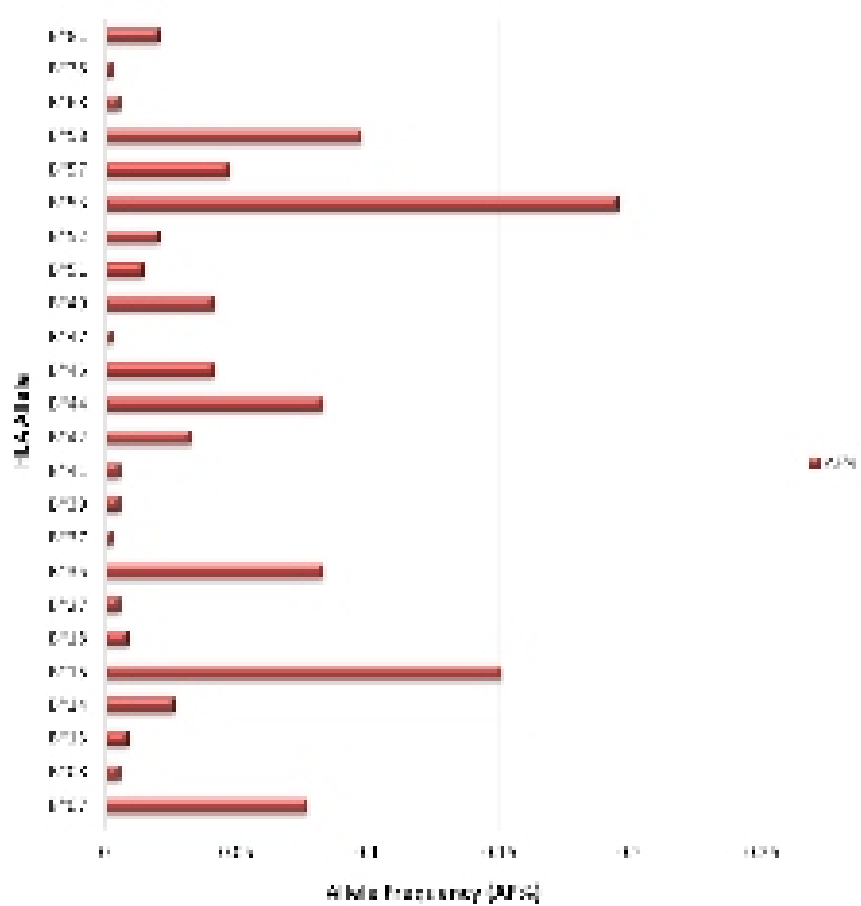


Figure 2: The HLAB* alleles frequency distribution in sickle cell anaemia patients

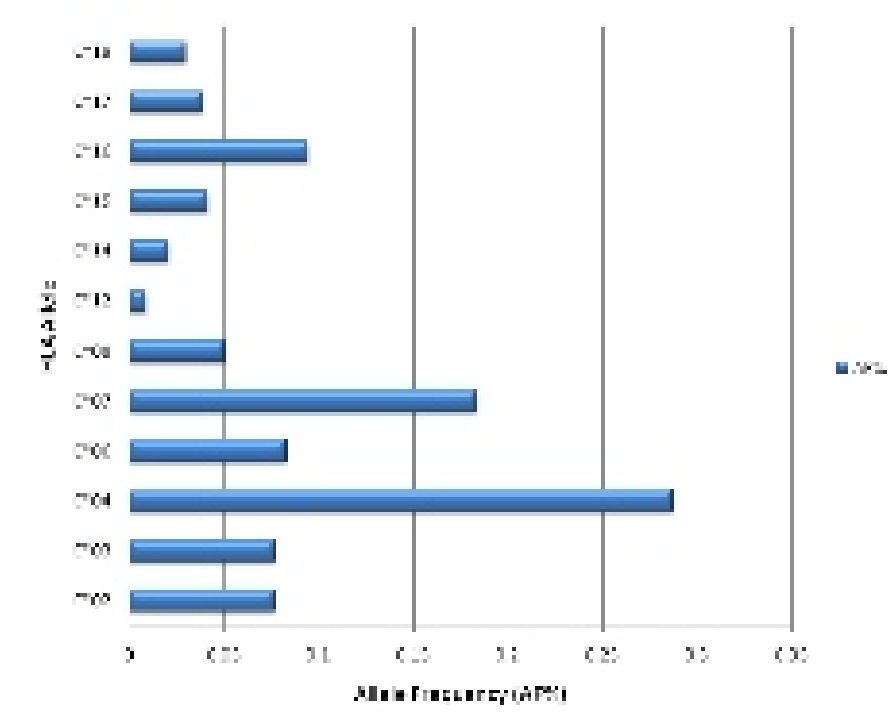


Figure 3: The HLAC* alleles frequency distribution in sickle cell anaemia patients

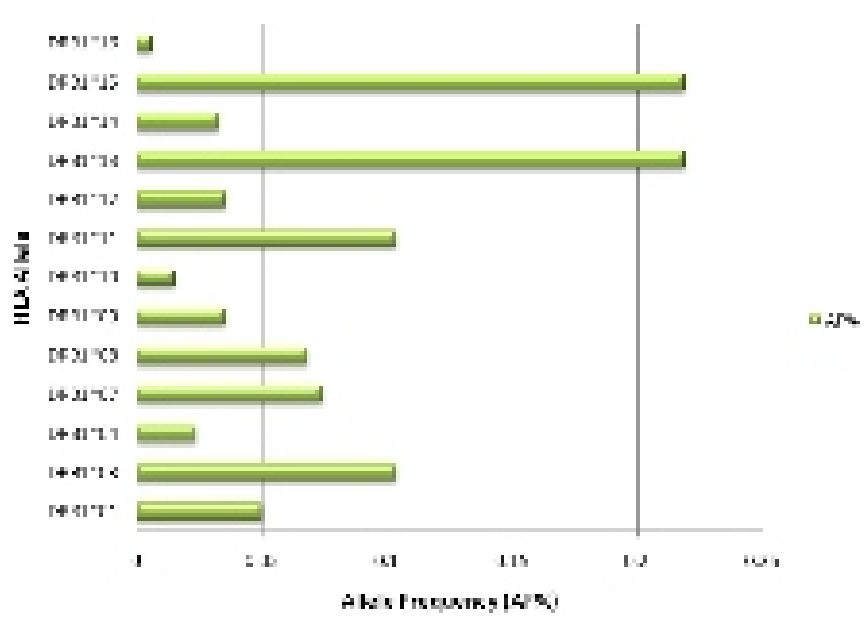


Figure 4: The HLADRB1* alleles frequency distribution in sickle cell anaemia patients

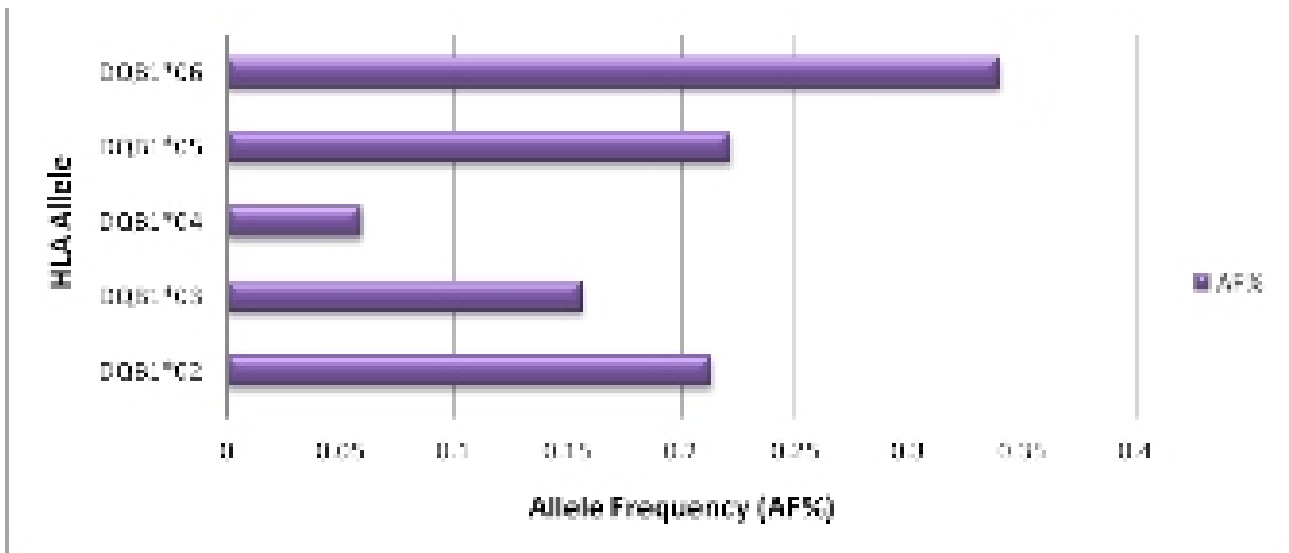


Figure 5: The HLA DQB1* alleles frequency distribution in sickle cell anaemia patients

DISCUSSION

Human leukocyte antigens are involved in the modulation of immune responses, thus specific haplotypes, alleles and residues at different loci have been linked with disease susceptibility.¹³ There is no substantial data regarding allele linkages to SCA patients from Nigeria. Hence, this study determined the HLA –A*, -B*, -C*, -DRB1* and -DQB1* allele frequency distribution among sickle cell anaemia patients of Nigerian origin.

The variability observed as a result of multiple alleles found at HLA A* (16 alleles), HLA B* (24 alleles), HLA C* (12 alleles), HLA DRB1* (13 alleles), and HLA DQB1* (5 alleles) loci could be due to the high level of genetic diversity present among the African population.¹⁴ A very confined alleles diversity at a specific HLA loci is usually common within a native population or social class group.⁹ Among the observed alleles HLA (A*23, A*30, A*02, A*68, A*74, A*03, A*36, A*34, A*33, A*66, A*01, A*29, and A*32) in Nigerian SCA patients, HLAA*23, A*30 and A*02 may be conserved in SCA patients. Allele A*01 and A*33 although with low frequency, but were found in sickle cell patients in this present study is in agreement with the study of Driss *et al.*¹ where HLA A*0102, A*2612, A*3301 were linked with SCA patients from Brazil. However, HLA (A*23, A*30 and A*33) which were found in this study have been tagged as “African-specific” alleles.¹²

In other HLA loci, (B*53 and B*15), (C*04 and C*07), (DRB1*15, DRB1*13, DRB1*11 and DRB1*03) and (DQB1*06, DQB1*05, DQB1*02 and DQB1*03) may be highly conserved among SCA patients as the probability of occurrence given by the allele frequency is one in every 10 (10⁻¹) SCA patients compared with other alleles (B*58, B*44, B*35, B*07, B*57, B*49, B*45, B*42, B*14, and B*52), (C*16, C*06, C*03, C*02, C*08, C*15, C*17, C*18, and C*14), (DRB1*07, DRB1*08, DRB1*01, DRB1*09, DRB1*12, DRB1*14, DRB1*04, and DRB1*10), and (DQB1*04) characterized by 1 in every 100 (10⁻²) SCA patients probability of occurrence. HLA-DRB1*10 was prevalent in SCA patients from studies in Bahrain⁴ and Kuwait.¹⁵ Some alleles reported in this present study corroborates the reports that suggested that HLA DRB1*0301, DRB1*0302, DRB1*1501, DQB1*0201, DQB1*0602 DPB1*0401 and DPB1*1701 were prevalent

in individuals with SCA of African-American origin.^{14,8} The study of Spinola *et al.*¹⁶ on Cameroonian population where A*02, A*23, A*30, B*53, B*58, C*04 and C*07 alleles were consistent further corroborates the specificity of some HLA class I alleles among Africans.

CONCLUSION

Considering the allele frequency distribution among sickle cell anaemia patients of Nigerian origin, HLA (A*23, A*30 and A*02), (B*53 and B*15), (C*04 and C*07), (DRB1*15, DRB1*13, DRB1*11 and DRB1*03) and (DQB1*06, DQB1*05, DQB1*02 and DQB1*03) are the most frequent alleles.

This study is the first to report HLA allele frequency distribution among sickle cell anaemia patients of Nigerian origin. It is therefore recommended that further studies should be done on a larger population to validate the findings of this current research. Haplotype frequency distribution among Nigerian population should be given prominent attention as it is the most useful information that guides bone marrow transplantation.

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