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FREQUENCY OF HUMAN LEUCOCYTES ANTIGEN (HLA) B LOCUS IN UMBILICAL CORD BLOOD OF DIFFERENT ETHNIC SUBJECTS IN PORT HARCOURT

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ABSTRACT
The HLA system is known to be the most polymorphic genetic loci in humans. Distribution and frequencies of HLA alleles are highly variable among different human ethnic groups. The aim of the study was to determine the HLA B locus alleles and frequencies in umbilical cord blood in Port Harcourt, Rivers State, Nigeria. Frequencies of the HLA B locus alleles was determined in 90 unrelated umbilical cord blood samples using Sequence Specific Primer-Polymerase Chain Reaction(SSP-PCR) method. The results showed that a total of 25 HLA-B were found in the studied population. Out of which HLA B*53 (32.2%) was the most prevalent followed by B*15(28.8%), B*07(24.4%), B*35 (18.8 %) and B*76(13.3 %). The frequency of HLA B locus alleles among the participating ethnic groups in the study population like the Igbo, Hausa, Yoruba, Ikwerre, Ogoni, Ijaw, Efik and Fulani were also described. The most frequent alleles among the Igbos are HLA B*07(17.6%), HLA B*15(30.4%), HLA B*35(15.2%), HLA B*53(30.4%) and HLA B*76(13.0%). Among the Hausa are HLA B*07(28.6%), HLA B*15(14.3%), HLA B*27(28.6%), HLA B*35(28.6%), HLA B*46(14.3%), HLA B*52(14.3%), HLA B*53(28.8%) and HLA B*57(28.6%). Among the Yorubas are HLA B*07(23.5%), HLA B*15(29.4%), HLA B*35(23.5%), HLA B*53(29.4%) and HLA B*73(11.8%). Among the Ikwerre are HLA B*14, HLA B*15, HLA B*21, HLA B*35, HLA B*41, HLA B*42, HLA B*44, HLA B*53, HLA B*58 and HLA B*78 with allele frequencies of 12.5%, each. The common alleles for Ogoni are HLA B*15, HLA B*53(50.0%), HLA B*76(50.0%), HLA B* 18(25.0%) and HLA B* 58(25.0%). Among the Ijaw are HLA B*07(100%) and HLA A*35(100.0%). HLA B*07(80.0%), HLA B*15(20.0%) and HLA B*18(20.0%), HLA B*35(20.0%) and HLA B*76(40.0%) were found to be common among the Efik. Chi Square test was performed on data set to determine strength of association amongst the ethnic groups in HLA B locus allele frequency. The $\chi 2$ value was 1579.2, p value 0.0001. The HLA B locus alleles are statistically significantly different across ethnic groups in Nigeria. In conclusion, the result of the study suggests a high variability in HLA B locus in the studied Nigerian population.

KEYWORD: Human leucocyte antigen, stem cell, umbilical cord blood, PCR.

INTRODUCTION
The Umbilical cord blood, earlier believed to be a waste product and disposed after delivery, can now be collected from both vaginal deliveries and caesarean sections, either in-utero or ex-utero, without any risk to the donor, excess plasma and red cells removed, tested, HLA-typed, cryopreserved and stored to provide a readily available cord blood product (Sideri *et al.*, 2011). Allogenic transplantation with adult HSCs is deemed as a practice or technique of saving people's lives in the management of critical hematological diseases like hematopoietic malignancies, bone marrow failures and hereditary immunodeficiency disorders (Brunstein & Wagner, 2006). Notwithstanding, this technique is restricted by the handiness of acceptable human leucocyte antigen (HLA)-matched donors (Tse & Laughlin, 2005).

Cord Blood has also been observed to have low likelihood of passing on of some vital viral infections and minimized occurrence and seriousness of acute and chronic graft versus host disease (GvHD) than other origin of stem cell (Barker *et al.*, 2005). However with an international estimation of about 140 millions birth/year, umbilical cord blood could be rated as one of the most bountiful origin of stem cells when liken to other origins of stem cells (World Health Statistics, 2011). Umbilical cord blood stem cells collection is documented not associated with complicated moral, doctrinal or governmental interests, which makes them perfect for use in the clinical practice (Watt & Contreras, 2005; Ballen *et al.*, 2008).

Tissue compatibility is known by the major histocompatibility complex (MHC), better known as the Human Leucocyte Antigen (HLA) in humans. These are cell surface proteins that participates in a significant role in adaptive immune response (Han *et al*., 2003).These proteins creates a complex with the antigenic peptides that are present on the cell surface and the complex is acknowledged by T-cell receptors to sets off the adaptive immune response by instigating the death of the cell and/or making of antibodies or by presenting virus or pathogen- derived antigenic peptides to T cells and set off an immune response (Mehra *et al*., 2010). Then they also play a basic role in the reception and refusal of transplanted tissues (Klein & Sato, 2000).

The MHC is one of the largest multifarious or gene-dense section of the human genome and contains virtually 300 genes and pseudo genes, positioned in three sections called the class I, class II and class III sections. Approximately 20% of the proteins coded by the MHC have immune-related roles (Trowsdale, 2005). Immune retaliation caused by HLA discrepancies represent a major obstacle to hematopoietic stem cell transplantation (HSCT) (Lechler *et al*., 1990; Hurley *et al*., 2007). In hematopoietic cell transplantation from an unrelated donor, the likelihood of finding an HLA- matched donor is higher when the patient and donor emanates from the same ethnic group (Tiercy *et al.*, 2002). And because of the great polymorphism of HLA molecules, it has become clear that serologic typing techniques were completely deficient to cover all the heterogeneity present in the HLA system. The use of deoxyribose nucleic acid (DNA)-based method for HLA typing advanced the area forward. The DNA typing methods are based on the nucleotide sequence information of the polymorphic DNA segments, using Polymerase Chain Reaction (PCR) technology. The aim of the study is to determine the HLA B alleles and their frequencies in umbilical cord blood in Port Harcourt, Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area: The study was conducted in Port Harcourt metropolis, Rivers State Nigeria. Three Hospitals were randomly picked based on the number of child birth per month. One tertiary, one secondary and one primary health care facility. The hospitals are Braithwaite Memorial Specialist Hospital located at no 5-8 Harley Street, Old Government Reservation Area (GRA), Port Harcourt, Military Hospital located along Aba-Port Harcourt express road and Goodness and Mercy Maternity Centre located at No 30 Hilton Avenue off Eneka Road Rumunduru Port Harcourt(PH). The choice of the Hospitals was randomly based on hospital with high number of childbirth per month.

Subjects/Study population

Umbilical cord blood samples were collected from 90 women aged 17-40 years from three different Hospitals within Port Harcourt metropolis mentioned above. Umbilical cord blood samples were collected from normal delivery and caesarean sections Human Immuno Deficiency (HIV) free mothers while Umbilical cord blood were not collected from any complicated deliveries, HIV and Hepatitis B surface Antigen (HbsAg) positive patient of any age. The participants were informed on the objectives, benefits and procedure of the study and they were reassured of confidentiality. Each participant was also issued a questionnaire to fill out. A written and signed informed consent was obtained from each of the participants before enrolling into the study.

Experimental Design

A cross-sectional study design was employed in this study. Subjects were randomly selected without bias.

Sample Size Determination

Sample size was calculated using Power Analysis Statistical Software (PASS version 13, www. ncss.com). The effect size was set at 0.50 which is a moderate level for tests involving one proportion. The power of the study is 0.90. A sample size of 90 was obtained at the end of the calculation which has a 90% power to detect a difference (P1-P0) of 0.0487 using a two sided binomial test. Therefore 90 samples were used in the study.

Collection of Samples

The midwives in the delivery/labor room of the selected health facilities were trained and instructed to collect 5mls of umbilical cord blood into Ethylene Diamine Tetra Acetic Acid (EDTA) tubes from umbilical cord immediately after birth, from women who have consented to participate in the study. The blood sample was transported to the laboratory at room temperature and stored at 4^0C. The stored samples were pooled in batches and later transferred to Safety Molecular and Pathology Laboratory Enugu for processing and analysis.

Sample Analysis

Alkali Denaturation Test

The haemoglobin alkaline denaturation test was performed on the 90 Umbilical Cord Blood samples. This was done to confirm that the blood is of fetal origin.

Principle: The test is based on differences between maternal and fetal haemoglobin. Maternal blood contains adult haemoglobin composed of two alpha and two beta subunits (haemoglobin A or HbA; i.e., normal adult haemoglobin). Fetal blood contains fetal haemoglobin that composed of two alpha and two gamma subunits (haemoglobin F or HbF; i.e., normal fetal haemoglobin). This difference in composition gives the different types of haemoglobin chemical properties (in addition to the higher affinity HbF has for dissolved blood oxygen over HbA allowing baby to compete for oxygen better than mother).

Fetal haemoglobin is resistant to alkali (basic) denaturation, whereas adult haemoglobin is susceptible to such denaturation. Therefore, exposing the blood

specimen to sodium hydroxide (NaOH) will denature the adult but not the fetal haemoglobin. The fetal haemoglobin will appear as a pinkish colour under the microscope while the adult haemoglobin will appear as a yellow-brownish colour.

Procedure: 0.5 ml of the cord blood in a sterile test tube was mixed with a small amount of sterile water to cause haemolysis of the RBCs yielding free haemoglobin. The sample was next centrifuged for about 3minutes. The pink haemoglobin-containing supernatant was then mixed with 1ml of 1% NaOH for each 5 ml of supernatant. The colour of the fluid was assessed after 2 minutes. Fetal haemoglobin will stay pink and adult haemoglobin will turn yellow-brown since adult haemoglobin is less stable and will convert to haematin which has a hydroxide ligand.
All the samples tested were correctly confirmed to be of fetal origin.

Processing of Umbilical Cord Blood to Guanidium Isothiocynate (GITC)
Guanidine thiocynate ($NH_2C(NH)NH_2HSCN$) is a chemical compound used as a protein denaturant being a chaotropic agent. It is most commonly used in the extraction of DNA and RNA. GITC can also be used to lyse cells and virus particles in DNA extractions where its function, in addition to its lysing actions is to prevent activity of RNase and DNase enzymes by denaturing them, these enzymes would otherwise damage the extract.

Principle: Several molecular assays require nucleic acid from white cells. Red cell lysis using Ammonium chloride solution provides a faster means of harvesting white cells. The cell pellet is then lysed in Guanidium isothiocynate solution. The GITC lysate is stable for long term storage and amendable to various techniques of nucleic acid extraction. GITC requires activation by β Mercaptoethanol.

Procedure: The umbilical cord blood was transferred into a 15 ml tube and labeled accordingly. 10ml of already prepared cold 1x Red Cell Lysis Buffer (RCLB) was added to each sample and the tubes were closed properly and mixed by inversion. The 15ml tubes (sample tube) containing the 10mls 1xRCLB and umbilical cord blood was placed on already prepared ice bucket for 10min. After 10min the tube was centrifuge at 3000rpm for 7minutes and carefully the supernatant was decanted into the waste bucket, making sure the pellet (sub- natant) was not lost. 10ml of cold 1x RCLB was added into the cell pellet (sub-natant) and mixed by vortexing. The process was repeated three more times until there was no trace of RBC except the white cell pellet. 10ml of sterile PBS was then added to the cell pellet on each tube, mixed by vortexing and centrifuged at 3000rpm for 7min. The supernatant was decanted, then 5ml of sterile PBS was again added into each tube and mixed by vortexing and centrifuged at 3000rpm for 5min. The supernatant was decanted into the waste bucket (being careful not to decant the pellet) .The decanted tubes were drained on a clean towel by inversion. While draining, the GITC buffer was prepared by adding 10µl BME to the 1ml of GITC buffer. 1ml of the activated GITC buffer was added to a separate tube labelled accordingly to the sample no on the tubes for each sample. Using blunt end 18G needles and 2ml syringe the GITC lysate was homogenise 18-20 times. Sterile pasture pipettes was used to transfer the GITC lysate into 2ml cryovial tube and label accordingly for storage at minus 20^{o}C. The lysate can be used immediately for nucleic acid extraction or store at minus 20^{o}C freezer.

Extraction of Human Genomic DNA Using Reliaprep Blood gdNA Miniprep System
Principle of the Procedure: The ReliaPrep Blood gDNA Miniprep System provides a fast, simple technique for preparation of purified and intact DNA from mammalian blood. Samples are processed using a binding column in a microcentrifuge tube. Up to 200ul of blood can be processed per purification. The gDNA isolated is of high quality and can be used in common applications such as agarose gel analysis, restriction enzyme digestion and PCR analysis: HLA Typing, DNA sequencing, JAK2 mutation, e.t.c.

Procedure: Appropriate number of 1.5ml flipcap tube for samples and control was selected and labelled accordingly. 25µl of Proteinase K was dispense into each of the labeled tubes. 200ul of the GITC lysate was added and briefly mix by vortex. The mixture was allowed to stand for 1 minute. 200µl of the Cell Lysis Buffer (LBB) was added,the tube was closed and Mixed by vortexing for 10seconds. Ensuring that the mixture was mixed well. The mixture was incubated at 56^{o}C for 10minutes. 250µl of Binding Buffer was added to each tube. The tube was closed and mixed by vortexing for 10seconds. The required number of Spin Columns was Selected and labeled for each sample ensuring they are placed into Collection Tubes. The Lysates were then Transferred into the corresponding Spin Columns and centrifuged at 14000rpm for 1minute. The first flow through was discarded and new Collection Tubes added.

The Column was washed with 500µl of Column Wash Buffer and centrifuged for 3minutes at 14000 rpm. The last step (the washing) was repeated twice for a total of three washes. Discarding the flow through in each step.The Spin Column was placed in a clean Collection tube, centrifuged at 14000 rpm for 1minute to remove residual wash Buffer. Then the Spin Column was placed in a clean 1.5ml tube (Recovery tube). 200µl of sterile Nuclease-free water was added into each tube and incubated at room temperature for 1minute.Then centrifuge at 13000 rpm for 1minute. The purified genomic DNA was labeled and stored in the freezer at -20^{o}C or used for HLA typing immediately.

HLA Typing using Olerup SSP-PCR Method

Principle of the Procedure: HLA typing is an important test in solid organ, tissue and stem cell transplantation. Molecular based method remains the gold standard in HLA typing. Olerup HLA typing kit is an in vitro diagnostic kit for the typing of HLA class I & II alleles. It is based on polymerase chain reaction sequence specific primer (PCR-SSP). The technique couples the amplification process with allelic discrimination during the PCR process. This reduces the post amplification process to only gel electrophoresis. PCR-SSP provides a finer and higher resolution of the alleles than other PCR based methods. The result is qualitative which appears as positive or negative, which obviates the need for a more complicated result interpretation. It is a simple and elegant technique for HLA typing.

Prior to the PCR mix the quality of the gDNA sample was checked. The DNA sample should be in Tris EDTA (TE) buffer or sterile water. Good quality DNA is fundamental to achieving an optimum result with SSP Olerup system. Good quality DNA means that the ultra-violet spectrophotometric reading of the diluted DNA sample at OD260/280 should be between 1.7-1.9. However, the quality of the gDNA was confirmed using agar rose gel electrophoresis . 8ul of gDNA was mixed with 2ul of gel loading dye.

The 10ul mixture was loaded on a 2% gel and run on a 100v electrophoresis tank for 1hr. Visualize on a UV light to see the separation of the gDNA. The gDNA sample was therefore confirmed adequate.

PCR Mix for HLA B Locus

PCR mix was prepared according to the number of samples to be analyzed at a time for HLA B. PCR Buffer refers to the PCR Master mix that contains loading dye already. The PCR mix contains the PCR buffer (ul) (Master Mix),Water (ul),gDNA and Taq polymerase (ul).

HLA B locus has 32 alleles. The PCR mix for B locus contains water, gDNA, Taq polymerase and master mix according to the number of PCR reactions per test. Therefore the appropriate volume of each of the components of the PCR for each sample was mixed. For the 32 wells per sample 108ul of master mix was mixed with 72 ul of DNA sample, 177.1ul of dH2O and 2.9ul of Taq polymer. The reagents were properly mixed together by vortexing.

Setting up reaction: The Olerup tray contains allele specific primer sets. The tray was allowed to thaw and warm up. The PCR mix prepared as described above containing water, gDNA and Taq polymerase according to the number of PCR reactions per test. Place the Olerup PCR plate on PCR rack. The seal covering the plate was removed and kept neatly well for re-sealing after all pipetting. The Taq DNA Polymerase was removed from the freezer and keep chilled during setup (e.g., on ice). 10 ul of the prepared sample reaction mixture was dispensed into the appropriate wells of the Olerup SSP primer tray. The primer tray were covered with PCR caps making sure that all reaction wells were completely covered and also contains equal volumes of the reaction mix.

Thermal Profile

The PCR plate containing the Olerup primers and the PCR mixture was loaded on a thermal cycler. The thermal profile already programmed on the thermal cycler was set up and the loaded PCR plate left to run for 2hrs.

GEL ELECTROPHORESIS

At the end of the thermal cycling, the PCR plate was transferred to gel electrophoresis bench with the tray. The amplicons alongside a 100 bp DNA ladder was ran on a 2 % agarose gel placed in a 0.5x TBE (Tris Borate EDTA) buffer in an electrophoresis tank set at 100mAP for 45min. After the gel electrophoresis run then the gel was transferred to a Ultra violet (UV) illuminator light for visualization of the band separation then using the attached camera photographs of the gel pictures were taken .The pictures were saved with the sample number. After taking pictures of the gel then the gel was discarded properly. Same process was repeated till the 90 sample was completed for B locus.

Data Analysis

Graph pad prism 6.0 statistical software was used to analyze the data generated. Frequency of HLA B alleles and haplotypes were expressed in percentages. Non parametric chi-square were used to test the degree of association of categorical variables. An error probability ($p \leq 0.05$) was considered significant.

RESULT

Table 1 describes the demographics of participants in the study which includes the ethnicity, age range, number and frequency. 46 Igbo, 7 Hausa,17 Yoruba, 8 Ikwere, 4 Ogoni, 2 Ijaw, 5 Efik and 1 Fulani, with a frequency of 51.1%, 7,8 %, 18.9%, 8.9%, 4.4% 2.2%, 5.6% and 1.1% respectively. The mean age of the participant is 28.3 $\pm$ 5.5 years.

Table 1: Demographics characteristics of study participants.

Ethnicity	Number	Frequency (%)
Igbo	46	51.1
Hausa	7	7.8
Yoruba	17	18.9
Ikwere	8	8.9
Ogoni	4	4.4
Ijaw	2	2.2
Efik	5	5.6
Fulani	1	1.1
Age		
Mean (years)	28.3	
SD (years)	5.5	

A total of 25 HLA B locus alleles were found in the general study population. The most common alleles for HLA B locus alleles were HLA B*53, B*15, B*07, B*35 and B*76 with allele frequencies of 32.2%, 28.8%, 24.4%, 18.8 % and 13.3 % respectively.

Table 2: HLA-B Alleles Frequencies.

Alleles (n=90)	B*07	B*08	B*14	B*15	B*17	B*18	B*21	B*22	B*27	B*30	B*35	B*37	B*39	B*41	B*42	B*44	B*46	B*51	B*52	B*53	B*57	B*58	B*73	B*76	B*78
frequency	24.4	2.2	8.8	28.8	2.2	6.6	4.4	2.2	8.8	1.1	18.8	2.2	3.3	2.2	4.4	5.5	1.1	2.2	4.4	32.3	2.2	2.2	5.5	13.3	2.2

Table 3 describes the frequency of HLA B locus alleles among the participating ethnic groups in the study population like the Igbo, Hausa, Yoruba, Ikwere, Ogoni, Ijaw, Efik and Fulani in Nigeria. The most common HLA B locus among the Igbos are HLA B*07, HLA B*15, HLA B*35, HLA B*53 and HLA B*76 with allele frequencies of 17.6%, 30.4%, 15.2%, 30.4% and 13.0% respectively. Among the Hausa are HLA B*07, HLA B*15, HLA B*27, HLA B*35,HLA B*46, HLA B*52, HLA B*53 and HLA B*57 with allele frequencies of 28.6%, 14.3%, 28.6%, 28.6%, 14.3%, 14.3%, 28.8% and 28.6% respectively. The most common alleles among the Yorubas are HLA B*07, HLA B*15, HLA B*35, HLA B*53 and HLA B*73 with allele frequencies of 23.5%, 29.4%, 23.5%, 29.4% and 11.8% respectively. Among the Ikwerre are HLA B*14, HLA B*15, HLA B*21, HLA B*35, HLA B*41, HLA B*42, HLA B*44, HLA B*53, HLA B*58 and HLA B*78 with allele frequencies of 12.5%, 25.0%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 75.0%, 12.5% and 12.5% respectively. The common alleles for Ogoni are HLA B*15, HLA B*53 and HLA B*76 allele frequency of 50.0% each and HLA B* 18, HLA B* 58 each with an allele frequency of 25.0%. Among the Ijaw ethnic group the common HLA in the study population are HLA B*07 and HLA A*35 with allele frequency of 100.0% each. HLA B*07, HLA B*15 and HLA B*18, HLA B*35 and HLA B*76 were found to be common among the Efik with allele frequency of 80.0%, 20.0%, 20.0%, 20.0% and 40.0% respectively. Chi Square test was performed on data set to determine strength of association amongst the ethnic groups in HLA B locus allele frequency. The HLA B locus alleles are statistically significantly different across ethnic groups in Nigeria ($\chi 2$ value was 1579.2, p value 0.0001).

Table 3: Frequency of HLA B Locus Alleles in some Ethnic Groups in Port Harcourt.

	Frequency of distribution in percentage (%)																								
Tribe	B*07	B*08	B*14	B*15	B*17	B*18	B*21	B*22	B*27	B*30	B*35	B*37	B*39	B*41	B*42	B*44	B*46	B*51	B*52	B*53	B*57	B*58	B*73	B*76	B*78
Igbo n = 46	19.6	2.2	8.7	30.4	0.0	6.5	4.3	4.3	8.7	0.0	15.2	6.5	4.3	2.2	4.3	6.5	0.0	2.2	6.5	30.4	10.9	0.0	6.5	13.0	4.3
Hausa n = 7	28.6	0.0	0.0	14.3	0.0	0.0	0.0	0.0	28.6	0.0	28.6	0.0	0.0	0.0	0.0	0.0	14.3	0.0	14.3	28.6	28.6	0.0	0.0	0.0	0.0
Yoruba n = 17	23.5	5.9	17.6	29.4	11.8	5.9	5.9	0.0	5.9	0.0	23.5	5.9	0.0	0.0	5.9	5.9	0.0	5.9	0.0	29.4	0.0	0.0	11.8	5.9	0.0
Ikwere n = 8	0.0	0.0	12.5	25.0	0.0	0.0	12.5	0.0	0.0	0.0	12.5	0.0	0.0	12.5	12.5	12.5	0.0	0.0	0.0	75.0	0.0	12.5	0.0	12.5	0.0
Ogoni n = 4	0.0	0.0	0.0	50.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	25.0	0.0	50.0	0.0
Ijaw* n =2	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Efik n = 5	80.0	0.0	0.0	20.0	0.0	20.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40.0	0.0
Fulani N = 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nation-wide	24.4	2.2	8.8	28.8	2.2	6.6	4.4	2.2	8.8	1.1	18.8	2.2	3.3	2.2	4.4	5.5	1.1	2.2	4.4	32.3	2.2	2.2	5.5	13.3	2.2

*Chi Square test was performed on data set to determine strength of association amongst the ethnic groups in HLA B locus allele frequency. The $\chi 2$ value was 1579.2, p value 0.0001. The HLA B locus alleles are statistically significantly different across ethnic groups in Nigeria.

DISCUSSION

Our study which is aimed at determining the HLA type B alleles and frequencies in umbilical cord blood in Port Harcourt, Rivers State, Nigeria showed that out of the 90 umbilical cord blood samples analyzed, a total of 25 HLA-B alleles were identified. This is similar to a study by Madania *et al.,* (2014) among the Syrian population where he identified 28 for HLA-B . Also Hamed *et al.,* (2018) found similar result among the Mauritanian having 23 HLA B. However in contrast to these numbers a total of, 75 HLA-B alleles were identified by Ikeda *et al.,* (2015) among the Japanese .This may be as a result of increased number of sample size used for the study. Peterson *et al.,* (2014) also identified high numbers of HLA B among the East African population with a total of 113 HLA-B alleles that were identified . A total of 80 HLA-B alleles were identified by Mack *et al.,* (2011) among Americans.

With 25 different allele groups, HLA-B was showed a great level of polymorphism in the B locus from this study. For the B locus, the four most frequent alleles (above 10%) across the different ethnic groups represented in the study population are HLA B*53, HLA B*35, HLA B*07 and HLA B*15. The result showed that HLA B*53 and HLA B*15 are the most common alleles among the Igbo tribe followed by HLA B*07, B*35, HLA B*76 and HLA B*57.The Hausa also have similar high frequencies of HLA B*53, HLA B*07, HLA B*27, HLA B*35, HLA B*15, HLA B*46, HLA B*52, and HLA B*57. While the Yorubas have high frequencies of HLA B*53, HLA B*15, HLA B*07 and B*35 just like the Igbos. The other low number observed in other ethnic groups showed similar trends in HLA B allele frequencies distribution. These results are consistent with the result of Ellis *et al.,* (2001) in Côte d'Ivoire where he got a high frequency of HLA B*53 followed by HLA B*45, HLA B *15, HLA B*07 and HLA B*35. The HLA-B*07 allele which is the most frequent in Moroccans and Russians (Lebedeva, 2009; Gómez-Casado *et al.,* 2000) was the second most frequent allele among the studied Nigerian population. The B*35 allele which was in moderate frequency across the different ethnic groups captured in this study is also in high frequency in Iranians and Russians Tatars (Suslova *et al.,* 2007). Brick *et al.,* (2006) also reported same data for HLA B*15 and B*35 among Moroccan and Tunisian population. In contrast B*08 and B*51 which is among the low incident allele frequency among the studied Nigerian population has been reported to be high among the Syrian population by Madania *et al.,* (2014). Also from the result of the studied Nigerian population HLA B*17, B*22, B*30, B*39, B*37, B*46, B*78 showed a very low allele frequency across the different ethnic group.

CONCLUSION

This study on umbilical cord blood HLA B polymorphism has shown the different HLA B types and their alleles frequency among 8 different ethnic Nigerians living in Port Harcourt, Rivers State., the result of the study suggests a high variability in HLA B locus in the studied Nigerian population. Since Nigeria has been added to the world bone marrow registry and international search for allo- compatible marrow donor is now possible. The data from this study will therefore play a great role in addition to the data base on HLA allele types and frequencies. HLA B locus disease association could not be determined from the result of this study.

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