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Human Leukocyte Antigen-C Class I Population -Based Ancestry Of Genetic Ethnic Diversity In Nigeria

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Abstract:

Background: *The genetic makeup of the Human Leukocyte Antigen (HLA) bears the marks of the history of each population. Data of HLA alleles that would portray the population-based ancestry of the genetic diversity of the Nigerians in her ethnic distribution is lacking. This study examined the distribution of HLA -C in diverse Nigerian ethnic groups amongst Serodiscordant and concordant HIV positive and negative couples in Nigeria.*

Methods: *A total 271 serodiscordant and concordant HIV positive and negative couples in the Nigerian major tribes who signed informed consent document were enrolled into this study. Extracted genomic DNA was sequenced on exons 2 and 3 DNA for high resolution HLA-C class 1 genotype using allele-specific primers and sequence-based typing (SBT) method. Data was analyzed with on-line Linkage disequilibrium and chi square.*

Results: *The highest frequency distribution of high resolution of HLA-C alleles observed in the HIV positive subjects were: HLA-C*040101 72(34.5%) followed by C*0701 57 (24.2%) and HIV negative subjects C*040101 106(39.0%) followed by C*0701 86(31.6%). Among the Ibo and Hausa ethnicities, extensive diversities of rare alleles not shared by other ethnicities in Nigeria were observed. HLA-C*010201 1(0.3%), C*07401 and C*0728 1(0.3%) were the rarest alleles identified only in the Ibos. Among the Hausas, the rarest alleles observed were: C*102308 1(2.0%), C*07195 1(2.0%) and C*0705 1(2.0%). Within the Yoruba tribes, only allele C*0606 (0.9%) was observed. In other ethnic groups, HLA-C*0611 1(1.2%) was observed. However, allele C*1607 1(0.3%) was shared by the Ibos and Yoruba tribes.*

Conclusion: *The frequencies of HLA-C identified from the Nigerian population were comparable to HLA-C alleles from the Kenyans, Gambian and black/Caucasian of South Africans population. This is the first-time the ethnic level ancestry information on high and low resolution of HLA-C genotyping is showing the Nigerian ethnic distribution in HLA-C subtype.*

Keywords: *HLA-C, Genotyping, Ethnic diversity, HIV positive subject, HIV negative subject, Nigeria*

I. INTRODUCTION

The Major Histocompatibility Complex molecules (MHC) are glycoproteins expressed at the surface of almost all vertebrate cells. The Human Leucocyte antigen (HLA) is the human version of the Major Histocompatibility Complex in man. HLA is complex, located at the short arm of chromosome 6 (6p21) and spans approximately 4.0 kilobases of DNA. It is known to be the most polymorphic genetic system in humans[2]. The biological role of the HLA class I and

class II molecules are to present processed peptide antigens to CD8 and CD4 cells respectively for stimulation and destruction. The HLA system is clinically important as transplantation antigens and controls a major part of the immune system in all vertebrates. HLA are also ligands of killer immunoglobulin-like receptors (KIR) on the surface of natural killer cells, forming a bridge between innate and adaptive immunity. Few studies in Western world and Africa have demonstrated HLA implications in some autoimmune diseases including: ankylosing spondylitis, reactive arthritis, psoriatic arthritis, inflammatory bowel disease, celiac sprue, Type 1 diabetes mellitus, and systemic lupus erythematosus. Others include: pemphigus vulgaris, rheumatoid arthritis, and HIV. However, some recent studies considered HLA complex as the most remarkable genetic marker that is closely related to AIDS progression and highlights the differences in genetic susceptibility of HIV-infected individuals.

Polymorphism of the human leukocyte antigens (HLA) represents a major barrier to organ and hematopoietic stem cell (HSC) transplantation. The described polymorphism within the HLA class 1 gene is located within the exons 2 and 3 of the HLA genes that encode the peptide binding groove at the points in which T-cells interact with molecules itself. This diversity has evolved as a mechanism to ensure on-going pathogen recognitions and eradication. It has also an important function on the routinely used high throughput HLA typing methods.

The cloning and sequencing of HLA class I and II genes has not only provided a clear picture of the molecular basis of allelic polymorphism, but also allowed the development of a variety of PCR-based DNA typing techniques. Such methods are now progressively replacing serological typing for assessing donor and recipient HLA compatibility in clinical transplantation. The polymorphic regions in each allele are located in the region for peptide contact, which is displayed to the lymphocyte. For this reason, the contact region for each allele of MHC molecule is highly variable, as the polymorphic residues of the MHC will create specific clefts in which only certain types of residues of the peptide can enter. This imposes a very specific link between the MHC molecule and the peptide, and it implies that each MHC variant will be able to bind specifically only to those peptides that are able to properly enter in the cleft of the MHC molecule, which is variable for each allele. In this way, the MHC molecules have a broad specificity, because they can bind many peptides. The evolution of the MHC polymorphism ensures that a population will not succumb to a new pathogen or a mutated one, because at least some individuals will be able to develop an adequate immune response to win over the pathogen. The variations in the MHC molecules are the result of the inheritance of different MHC molecules.

The genetic makeup of the HLA bears the marks of the history of each population globally including several waves of relocations. The distribution of human leucocyte antigen allele and haplotype is varied among different ethnic populations. Therefore, when it comes to matching human leukocyte antigen types, a patient's ethnic background is important in predicting the likelihood of finding a match. This is because HLA markers used in matching are inherited. And again, some ethnic groups have more complex tissue types than others. Hence, HLA based- ethnicity aids in shared genetic heritage and very critical in getting a good match in organ and tissue transplantation. Therefore, groups of HLA variants run in certain ethnic groups which is part of the reason it is often difficult for some people to find a good HLA match than others in transplantation[9]. Another study reported the most evident alleles in the populations that have maintained larger effective population sizes for longer periods of time.

Class I HLA-C genetic diversity of Nigerian populations remains incompletely described. Data of HLA alleles that would portray the population-based ancestry of the genetic diversity of the Nigerians in her ethnic distribution is lacking. This study examined the distribution of HLA -C in diverse Nigerian ethnic groups amongst serodiscordant and concordant HIV positive and negative couples in Nigeria.

II. MATERIALS AND METHODS

STUDY POPULATION RECRUITMENT

The population eligible to participate in the study comprises of serodiscordant couples. The positive partner may or may not be on antiretroviral drugs (ART). Some concordant couples where (both partners are HIV positive or HIV negative) were also recruited for control measures. Couples who gave informed consent and were recruited were: Patients registered with Nigerian Institute of Medical Research (NIMR) Yaba Lagos HIV clinic and Nnamdi Azikiwe University Teaching Hospital Nnewi Anambra State (NAUTH) HIV clinics and NIMR HCT (HIV Counseling and Testing Unit). Others are NIMR Workers Couples (NIMRWC) and Divine Grace Church Couples (DGCC) who were used as controls.

STUDY SITES

Samples were collected and processes in two government-owned health institutions namely: Nigerian Institute of Medical Research Yaba, Lagos State, Nigeria, and Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria. Molecular work was done at National Microbiology Laboratory, Winnipeg, Manitoba, Canada.

INCLUSION CRITERIA

The participants included in this study were: (1). those couples that have discordant HIV test result. (2) Some couples that have concordant HIV results (3). Have co-habited for at least 3 months and or may have more than one partner. (4). The women are aged between 16-50 and the men 16- 65 years. (5) The HIV positive partners can be or not be on ARV. (6). Willingness to participate and given informed consent.

EXCLUSION CRITERIA

Also excluded were (1). Those over/or under the required age group (2). Those, who have not co-habited for three months (3). Those who are not willing to participate and give informed consent.

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STUDY PROCEDURE AND INFORMED CONSENT

Participants were counselled and told to inform their partners about the research to facilitate their consent. Those whose partners turned out to be HIV positive were referred to HIV Counseling and Testing Unit (HCT) for confirmation of their seropositive status and finally referred to HIV clinic for management. However eligible partners (HIV positive and their seronegative partners) that signed the consent document were enrolled into the study. Informed consent was obtained during focus group discussions conducted in an appropriate meeting room.

ETHICAL APPROVAL

Approval was obtained from NIMR IRB and NAUTH Ethical Committee. The study did not disclose any confidential information pertaining to the participants to anyone except for the clinicians managing the participants in their various clinics.

ADMINISTRATION OF STRUCTURED QUESTIONNAIRE USING IN-DEPTH INTERVIEW (IDI) PROCEDURES AND FACE TO FACE INTERVIEWS

Structured questionnaires were administered to two hundred and seventy-one (271) consenting serodiscordant couples and concordant HIV positive and negative couples. Information on socio-demographic characteristics, Knowledge of partners HIV status, knowledge of STIs, laboratory tests and treatment status, sexual behaviours and practices were required for completion of the questionnaire.

METHODS OF SAMPLE COLLECTION

The study was a cross-sectional survey. A total of 271 (542) blood samples, were collected from 236 couples from Nigerian Institute of Medical Research. Seventy (70) blood samples, collected from 35 couples from Nnamdi Azikiwe University Teaching Hospital Nnewi Anambra State. Whole blood (7mls) by vein puncture was collected in EDTA containing bottles. Whole blood was used for HIV antibody detection. White cell pellet was separated from whole blood buffy coat by centrifugation at 2000 rpm for 15 minutes within 4 hours of blood collection and used for white cell pellet harvestion and DNA extraction. White cell harvesting from blood samples were done at both study centres while DNA extractions was done in NIMR.

SAMPLE SIZE CALCULATION

The sample size was determined using formula of Oyeka, (1992) based on the HIV prevalence rate of 3.4% according to National Agency for the Control of AIDS (NACA, 2009) as shown as : $N=Z^2\ Q\ (1-P)/\ D^2$ Where N= is the minimum sample size required, Z^2= Desired is the confidence interval of 95% $=1.96^2$ = 3.8416, P=maximum expected prevalence = 3.4% = 0.34, Q=100%-p= (1-0.34) = 0.658, D^2 = This is the highest tolerance error @ 5% = 0.052 = 0.0025, N= 3.8416 X 0.34X0.658/0.0025 = 343 Couples. Sample size required was 343. A total number of 360 couples registered for the programme, the number of couples that actually signed informed consent and got enrolled into the study were 271 couples which brought the total blood sample number to 542. The rest were lost to follow-up while a few male partners or their wives did not give consent.

III. LABORATORY INVESTIGATIONS

HIV 1/ 2 SCREENING TEST

HIV screening tests were performed for all clients who presented themselves as HIV sero negative subjects. HIV screening was determined with Determine HIV-1/2 (Inverness Medical, Japan Co, Ltd). It is an immunochromatographic test for qualitative detection of antibodies to HIV-1/2. If either partner is positive, then both are tested with a second rapid test, Unigold (Trinity Biotech PPLC Bray Ireland). If for any individual, two tests do not agree, a third rapid test is performed with HIV1/2 START-PARK, (CHEMBIO diagnostic systems INC, Medford NY, USA). If two of three rapid tests are negative, then the individual is uninfected. Any other combinations are considered indeterminate, the person returns in 1 month for repeat testing. Methodology for each test was based on manufactures manual.

DETERMINE® HIV-1/2 AG/AB COMBO SYSTEM (INVERNESS MEDICAL, JAPAN CO, LTD).

Test Procedure

Fifty microliter of sample (50µl) was added to the sample pad. The specimen mixes with a biotinylated anti-p24 antibody and selenium colloid-antigen conjugate. The presence of a red bar at the patient HIV antigen window site after about 20 minutes indicates a positive reaction ie presence of HIV antibody according to manufacturer's protocol [11].

UNI-GOLDUNI™ RECOMBIGEN® HIV SCREENING KIT (MICHIGAN REGIONAL LABORATORY SYSTEM (2007)

To assay qualitative Screening Test Kit for the Detection of Antibodies to HIV-1/2 in Human Finger stick and Venous Whole Blood, Serum and Plasma

Test Procedure

The kit was allowed to reach room temperature (15°C to 27°C) for at least 20 minutes if previously stored in the refrigerator. The devices were laid on a clean flat surface and was labeled with the appropriate patient information / ID. The sample was drawn up to the first gradation on the pipette using one of the disposable pipettes included in the kit. Holding the pipette vertically over the sample port, free falling drops of sample was added to into the sample port. The sample was allowed to absorb into the paper. After the blood sample, has been completely absorbed, holding the dropper bottle of Wash Solution in a vertical position, four (4) drops of Wash Solution was added to the Sample Port. The timer was set for 10 minutes to incubate time. The test result was read after 10

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minutes but not more than 12 minutes' incubation time. Red colour in the sample port at both the control and test shows the screening test is positive and valid. Red colour seen only at the control port shows that the result is negative. If no red color is seen in the sample port, the test is repeated with a fresh device.

HIV 1/2 STAT-PAK™ (CHEMBIO DIAGNOSTIC SYSTEMS INC, MEDFORD NY, USA)

To assay qualitative Screening Test Kit for the Detection of Antibodies to HIV-1/2 in Human Finger stick and Venous Whole Blood, Serum and Plasma.

Test Procedure

Chembio HIV 1/2 STAT-PAK™ test device was removed from its pouch and place on a flat surface. The test device was labelled appropriately with the patient's name or identification number. The 5 µL sample loop provided was used to draw the specimen, allowing the opening of the loop to fill with the liquid. Holding the sample loop vertically, ~5 µL of sample (serum, plasma or whole blood) was dispensed onto the sample pad at the SAMPLE (S) well of the device. The Running Buffer bottle was held vertically and 3 drops (~ 105 µL) was added to the sample well slowly. The test result was read after 10 minutes. In some cases, a test line in less than 10 minutes however, 10 minutes was needed to report a non-reactive result. The results were read in a well-lit area. Results produced after 20 minutes were invalid. When the test is complete, the test result shows pink/purple line in the CONTROL (C) area of the test device and on non-reactive as well as reactive samples. This control line serves as an internal control and gives confirmation of sample addition and proper test performance. Pink/purple lines in both the TEST (T) and CONTROL (C) areas indicated a reactive sample

WHITE CELL PELLET HARVESTION AND DNA EXTRACTION

White cell pellet was harvested from buffy coat on peripheral blood cells with the use of Ace-shocking solution. The white cells pellet was used for Qiagen DNA extraction. DNA was extracted from white cell pellet with genomic DNAQIAGEN kit as described by_Qiagen DNA-extraction manual, (2013). The DNA filtrate was placed in the cryovial and stored frozen at -800C or -200C for further analysis.

POLYMERASE CHAIN REACTION (PCR) FOR DNA AMPLIFICATION

PCR reactions were performed on 96-well plates. Each reaction was composed of 20µl of ddH2O, 5µl of gDNA, and 25µl of master mix. The master mix contained 22.75µl of 2X mix (120mM Tris-HCl, 3mM $MgCl_2$, 30mM $(NH_4)_2SO_4$, 200µM dNTPs, 0.2% gelatin, ddH_2O), 55 pmol of both the forward and reverse primer and 13.75 µl of Taq polymerase. For HLA-C typing, primers were set to detect polymorphisms in exons 2 and 3 of the class 1 genes according to *Shampo et al protocol.*

After DNA extraction by Qiagen method, millions of DNA copies were made by using Polymerase Chain Reaction, (PCR) which uses repeated cycles of heating and cooling to make many copies of a specific region of DNA. PCR reactions were performed on 96-well plates. Each reaction was composed of 20µl of ddH2O, 5µl of gDNA, and 25µl of master mix. The master mix contained 22.75µl of 2X mix (120mM Tris-HCl, 3mM $MgCl_2$, 30mM $(NH_4)_2SO_4$, 200µM dNTPs, 0.2% gelatin, ddH_2O), 55 pmol of both the forward and reverse primer (Primer structural sequence as shown below) and 13.75 µl of Taq polymerase. For HLA -C typing, primers are set to detect polymorphisms in exons 2 and 3 of the class 1 genes as these regions cover most of the know polymorphisms. The structural sequence of the primers used for HLA-C was CPCRR representing HLA-C Reverse Primers and CPCRF: representing HLA-C Forward Primers as shown below.

HLA gene Primer name	***Nucleotide Sequence 5' - 3' o***
HLA-C PCR Forward	*ARCGAGGKGCCCKCCCGGCGA*
HLA-C PCR Reverse	*CGTGGRAGGCCATSCCGGGAGAT*

The reaction plates were then put into a thermocycler and run on a PCR program which began with a five-minute initial denaturation at 96°C, followed by 44 cycles of one minute of denaturation at 96°C, one minute of primer annealing at 5°C below the Tm of the respective primers and two minutes at 72°C for elongation. Each PCR program finished with a final elongation step at 72°C for ten minutes. After three cycles, the target sequence defined by the primers begins to accumulate. After 30 -35 cycles, as many as a billion copies of the target sequence of HLA-C genes were produced from a single starting molecule respectively.

AGAROSE GEL ELECTROPHORESIS

This was used to confirm that PCR was successful in amplifying the desired HLA gene. Each sample as was run in a 1% (w/v) agarose gel in 1X TBE buffer and stained with 0.003% 10mg/ml Ethidium Bromide. A 1kb ladder was added to the first well of every row. Once the DNA had migrated to a sufficient distance down the gel, they were removed from the tank and illuminated using a BioRad™ UV Transilluminator 2000. The results were viewed with the BioRad™ Quantity One® software according to Roth's protocol.

PURIFICATION OF PCR PRODUCT

Following sequencing PCR, the PCR product was precipitated using 21µl of a mixture containing 5ml of 95% ethanol and 250µl of sodium acetate. To remove any buffers, salts and reagents. Finally, the contents of each well were transferred to MicroAMP™ plates and put in the Applied Biosystems™ 3130xl Genetic Analyzer for electrophoresis according to Agencourt® AMPure Protocol.

SEQUENCING HLA PCR BY USING SANGER SEQUENCING METHOD

Sequencing HLA PCR by using Sanger Sequencing was done according to Sanger Method[15.] This protocol is performed after the PCR products have been purified. HLA

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DNA sequencing is a powerful technique used to identify allele dissimilarity within the HLA genes[15]. Sequencing focused on the most polymorphic exons of the class 1 genes. These exons encode the antigen recognition site. This region of the HLA molecule binds antigenic peptides and interacts with the T cell receptor for the antigen and natural killer cell immunoglobulin-like receptors (KIR). To genotype HLA-C alleles, exons 2 and 3 were sequenced. Each sequencing PCR reaction contained 4µl of purified PCR product, 1.5µl primer at 3.2µM and 2µl of Applied Biosystems™. BigDye® Terminator V1.1[16] and the forward and reverse primers as shown on the table 1 below:

Gene	HLA Forward and Reverse Sequencing Primers	BigDye 1.1
HLA-C	*CSEQ5Forward: GGGGACBGGGCTGAC*	√
HLA-C	*CSEQ3Reverse: GCCGTCCGTGGGGGATG*	√

For sequencing-PCR, each primer (forward or reverse) was in separate reactions. Therefore, sequencing the two full 96-well plates was done one for each primer.

IV. METHOD

The BigDye v1.1 and primers were defrosted in a tray of ice.

Using a Pipette, 44.5ul of the BigDye and Primers was added to the first column of the plate

Using a multichannel pipette (10ul range), 3.5ul of master mix was pipetted to the remaining columns. Using a multichannel pipette 4ul DNA was added to each column and the plates were sealed with silicone foil and quick spoon and place on the thermocycler for 8 hrs.

Each PCR master plate of 96- wells was done twice. One 96 -well plate for reverse primers and another 96 -well plate for forward Primers. During the elongation steps, deoxynucleoside triphosphates (dNTPs) and smaller amounts of fluorescently labeled dideoxynucleoside triphosphates (ddNTPs) were added into the growing strand. When a ddNTP was added, the chain was terminated because there was no longer a 3' OH group B. The sequencing PCR program begins with an initial denaturation at 96°C for three minutes, followed by 80 cycles of denaturation at 96°C for thirty seconds, primer annealing at 50°C below its Tm for thirty seconds, and finally an elongation step at 60°C for four minutes. A total of 8 hrs. was spent to assay each HLA PCR Sequencing reaction. Since there are billions of copies of the DNA template, there will be a mixture of strands of many lengths.

ETHANOL PRECIPITATION

Following sequencing PCR, the PCR product was precipitated using 21µl of a mixture containing 5ml of 95% ethanol and 250µl of sodium acetate. This process removed any buffers, salts and reagents in the PCR product. Finally, the contents of each well were transferred to MicroAMP™ plates and put in the Applied Biosystems™ 3130xl Genetic Analyzer for electrophoresis.

BIOSYSTEMS™ 3130XL GENETIC ELECTROPHEROGRAM

Each respondent's sequenced PCR product were loaded in the Biosystems for electrophoresis and data analysis. Data analysis software processed the raw data in the *ab1 file, using algorithms which applies the Multicomponent analysis that separates the four different fluorescent dye signals into distinct spectral components by mathematically filtering fluorescence signal from dyes with emission spectra overlap Basecalling. The selected Basecaller processes the fluorescent signals then assigns a base to each peak (A, C, G, T, or N). Analyzed sample data was displayed as an electropherogram, a sequence of peaks in four colors. Each color represents the base called for the peak according to Applied Biosystem manual[16].

CONDONEXPRESS TM

CodonExpress is a genotyping software based on a Taxonomy-based Sequence Analysis (TBSA) to resolve HLA Heterozygous and homozygous combinations. CodonExpress was used to type the Sequence-Based HLA class 1 genes according to CodonExpress TM, as described by the University of Manitoba, Canada instruction manual [17].

SEQUENCHER V5 FOR DATA INTEGRITY

Data Integrity was done by using DNA Sequencer V5 Software. This was done to ensure that all data generated in this study are correct and to rule out all unresolved HLA typing ambiguities as previously reported Sequencher V5 analyzer.

STATISTICAL ANALYSIS

Data generated was entered into IBM SPSS statistics version 20. Cross-sectional analysis using Chi Square was used to identify association between genetic and non-genetic factors. Confidence interval was set at 95% with level of significance set at p value less than 0.05 ($P < 0.05$)[17]. Linkage disequilibrium (LD) was conducted with on-line software from HIV databases.

V. RESULTS

The sociodemographic characteristics of the 271 (542) study participants by site of recruitment are shown on table 1. Their ages ranged from 20 to 60 years with median age of 38 years. Over two-thirds were people of reproductive age group 31-40 years. Sixteen couples 16 (3.0%) out of 271 couples were engaged to be married but have been co-habiting and are serodiscordant. The ethnicity showed that Ibos 54.9% were more in the population among the serodiscordant couples because of the second site of recruitment (NAUTH) followed by the Yoruba (20.9%), only 9.3% and 14.9% were of the Northern and Other ethnic extractions respectively. Majority of the serodiscordant couples were Christians (75.1%) and employed (93.0%). HIV status showed 224 *(82.7%) serodiscordant, 26(9.6%) concordant HIV positive and*

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21(7.7%) concordant HIV negative couples as shown on table 1.

Sites: NIMR/NAUTH	Concordant HIV Positive		Concordant HIV Negative		Serodiscordant Couples		
Age group	**Total no - 52**	**Frequency (%)**	**Total no -42**	**Frequency (%)**	**Total no - 448**	**Frequency (%)**	**Total**
Age							
20-25	2	(0.3)	0	(0.0)	7	(1.3)	
26-30	2	(0.4)	2	(0.4)	56	(10.3)	
31-35	10	(1.9)	6	(1.1)	114	(21.0)	
36-40	14	(2.6)	10	(1.9)	118	(21.9)	
41-45	12	(2.2)	12	(2.2)	76	(14.0)	
46-50	4	(0.73)	7	(1.3)	46	(8.5)	
>50	8	(1.5)	5	(0.9)	31	(5.8)	
Total	**52(26)**	**(9.5)**	**42(21)**	**(7.8)**	**448 (224)**	**(82.7)**	
Sex							
Male	26	(4.7)	18	(3.32)	224	(41.35)	**49.37**
Female	26	(4.7)	24	(4.42)	224	(41.35)	**50.47**
Marital Status							
Engaged	0	(0.0)	0	(0.0)	16	(3.0)	**3.0**
Married	52	(9.54)	42	(7.8)	432	(79.7)	**97.0**
Ethnic Groups							
Ibo	36	(6.6)	23	(4.2)	239	(44.1)	**54.9**
Yoruba	15	(2.8)	10	(1.8)	87	(16.1)	**20.9**
Hausa	0	(0.0)	3	(0.6)	47	(8.7)	**9.3**
Others	8	(1.5)	2	(0.4)	73	(13.0)	**14.9**
Occupation							
Employed	58	(10.7)	35	(6.5)	411	(75.8)	**93.0**
Unemployed	4	(0.7)	0	(0.0)	34	(6.3)	**10.0**
Religion							
Christianity	54	(10.0)	30	(5.5)	323	(59.6)	**75.1**
Islam	8	(1.5)	5	(0.9)	65	(12.0)	**14.4**
Traditional	0	(0.0)	0	(0.0)	4	(0.7)	**0.7**
Others	0	(0.0)	0	(0.0)	53	(9.8)	**9.8**
Total	**542**	**(100)**					

Table 1: Sociodemographic Characteristics of Study Participant (n= 542)

PCR PRODUCT BANDS

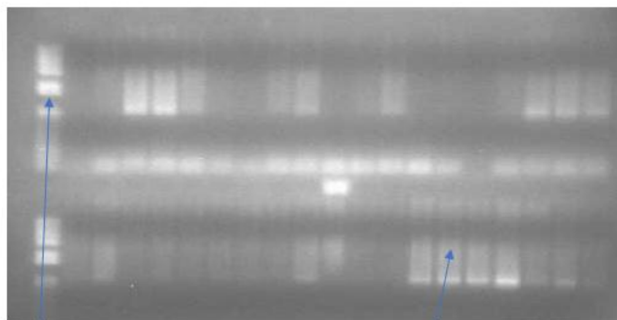

Figure 1: Post PCR product of HLA-C resolved on 2% agarose gel

The frequencies and cross tabulations of HLA-C alleles of high and low-resolutions in HIV positive and negative subjects are shown on table 2. The highest frequency alleles, observed in HIV negative subjects on low resolution were: C*04 124(49.3%) followed by C*07 86(31.6%). In high resolution, C*040101 106(39.0%) had the highest frequency followed by C*0701 86(31.6%). The least alleles are those alleles that have 0.4% frequency and they are: C* 010201 1(0.4%), C*120308 1 (0.4%), C*140101 1(0.4%).

The highest frequency alleles observed in HIV positive subjects were: C*04 92(39.0%) followed by C*07 81(34.3%) for low resolution. The highest frequency observed in the high-resolution alleles were C*040101 72(34.5%) followed by C*0701 57 (24.2%). The least alleles were those alleles that have 0.4% frequency and they are: C*0226 1(0.4%), C*030301 1(0.4%), C*030304 1(0.4%), C*041502 1(0.4%). The Chi-square analysis of HLA-C showed C*070201 as the allele most probable susceptible for HIV-1 predisposition (p=0.006). Other p values are >0.05 means that these alleles do not associate with either risk or projection of HIV transmission. Alleles such as: C*010201, C*120302, C*1606 were observed in HIV positive individuals and absent in HIV negative individuals. Also, HLA-C*140101, C*04120, C*0214, C*0802 were also observed in HIV negative individuals but absent in HIV positive individuals. The alleles absent on the HLA-C loci of the HIV negative or positive individuals were represented by zero (0).

n=272						
HLA-C Alleles in HIV Negative Individuals			**HLA-C Alleles in HIV Positive Individuals** (n=236)			
HLA Alleles	**No**	**Frequency %**	**HLA Alleles**	**No**	**Frequency %**	**P-value**
C*010201	1	(0.4)	C*010201	0	(0.0)	1.000
C*120301	13	(4.8)	C*120301	4	(1.7)	0.162
C*120302	0	(0.0)	C*120302	1	(0.4)	1.000
C*120308	1	(0.4)	C*120308	0	(0.0)	1.000
C*1203	14	(5.1)	C*1203	5	(2.1)	0.191
C*14	8	(2.9)	C*14	8	(3.4)	0.562
C*140101	1	(0.4)	C*140101	0	(0.0)	1.000
C*1402	6	(2.2)	C*1402	5	(2.1)	1.000
C*1403	1	(0.4)	C*1403	2	(0.8)	1.000
C*15	13	(4.5)	C*15	6	(2.5)	0.642
C*150501	10	(3.7)	C*150501	4	(1.7)	0.304
C*16	37	(13.6)	C*16	38	(16.1)	0.484
C*160101	31	(11.4)	C*160101	30	(12.7)	0.239
C*1607	0	(0)	C*1607	2	(0.8)	0.500
C*17	21	(7.7)	C*17	24	(10.1)	0.266
C*1701	21	(7.7)	C*1701	19	(8.0)	0.266
C*1704	0	(0.0)	C*1704	1	(0.4)	1.000
C*1801	14	(5.1)	C*1801	1	(0.4)	1.000
C*1802	0	(0.0)	C*1802	1	(0.4)	1.000
C*02	31	(11.4)	C*02	35	(14.8)	0.760
C*020201	1	(0.4)	C*020201	0	(0.0)	1.000
C*020202	5	(1.8)	C*020202	8	(3.4)	1.000
C*020203	1	(0.4)	C*020203	0	(0.0)	1.000
C*020205	0	(0.0)	C*020205	3	(1.8)	0.250
C*020213	0	(0.0)	C*020213	1	(0.4)	1.000
C*0202	7	(2.6)	C*0202	12	(5.1)	1.000
C*0205	0	(0.0)	C*0205	1	(0.4)	1.000
C*0210	22	(8.1)	C*0210	21	(8.9)	0.281
C*0214	1	(0.4)	C*0214	0	(0.0)	1.000
C*0226	1	(0.4)	C*0226	1	(0.4)	1.000
C*03	6	(2.2)	C*03	3	(2.1)	1.000
C*030201	11	(4.0)	C*030201	3	(2.1)	1.000
C*030301	0	(0.0)	C*030301	1	(0.4)	0.347
C*030304	0	(0.0)	C*030304	1	(0.4)	1.000
C*0303	0	(0.0)	C*0303	2	(0.8)	1.000
C*030401	1	(0.4)	C*030401	0	(0.0)	1.000
C*030402	14	(5.1)	C*030402	4	(1.7)	1.000
C*030418	1	(0.4)	C*030418	0	(0.0)	1.000
C*0304	16	(5.9)	C*0304	4	(1.7)	1.000
C*04	126	(49.3)	C*04	92	(39.0)	0.878
C*040101	108	(39.0)	C*040101	72	(34.5)	0.607
C*040128	1	(0.4)	C*040128	2	(0.8)	1.000
C*0407	2	(0.7)	C*0407	0	(0.0)	1.000
C*041502	0	(0)	C*041502	1	(0.4)	1.000
C*05	9	(3.3)	C*05	8	(3.4)	1.000
C*050101	4	(1.5)	C*050101	6	(2.5)	1.000
C*06	40	(14.7)	C*06	44	(18.6)	0.667
C*06/07	1	(0.3)	C*06/07	0	(0.0)	1.000
C*0602	33	(12.1)	C*0602	37	(15.7)	0.414
C*0606	0	(0.0)	C*0606	1	(0.4)	1.000
C*0611	1	(0.4)	C*0611	0	(0.0)	1.000
C*07	86	(31.6)	C*07	81	(34.3)	0.951
C*0701	64	(24.7)	C*0701	57	(24.2)	0.777
C*070123	0	(0.0)	C*070123	1	(0.4)	1.000
C*070201	7	(2.6)	C*070201	15	(6.4)	0.006
C*0704	1	(0.4)	C*0704	0	(0)	1.000
C*0705	0	(0.0)	C*0705	1	(0.4)	1.000
C*07195	1	(0.4)	C*07195	0	(0.0)	1.000
C*0728	1	(0.4)	C*0728	0	(0.0)	1.000
C*08	22	(8.1)	C*08	24	(9.5)	0.871
C*0802	14	(5.1)	C*0802	10	(4.2)	0.401
C*0804	7	(2.6)	C*0804	11	(4.6)	0.174

Table 2: Frequencies and Cross- tabulation of HLA-C genotype in HIV Negative and Positive Individuals

The distribution of HLA-C in diverse Nigerian ethnic groups among serodiscordant and concordant couples studied are shown on tables 3 and 4. Among the Ibo population, between HLA-group C1 and C2, the commonest allele, with the highest frequency observed was C*040101 107(36.6%)

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followed C* 070101 71 (24.3%) and the rarest alleles observed among the Ibos were: C*010201 1(0.3%), C*0728 1(0.3%) and C*07401 3(1.0%). HLA-C*1607 1(0.3%) was also identified only in Ibos and the Yoruba ethnic groups. Among the Yoruba population, between HLA-C1 and C2, the commonest allele, with the highest frequency was C*040101 46(41.1%) followed C* 070101 30 (26.8%) and the rarest allele observed among them was C*0606 1(0.9%). Among the Hausa population between HLA-C1 and C2, the commonest allele, with the highest frequency is C*040101 28(56.0%) followed C* 070101 12 (24.0%). The rarest allele observed among the Hausa population were: C*102308 1(2.0%), C*07195 1(2.0%) and C*0705 1(2.0%). The other states in Nigeria with diverse ethnic groups in the population such as: (Edo, Delta, Rivers, Cross River, Aqua Ibom, Benue) between HLA-C1 and C2, the commonest allele, with the highest frequency was C*040101 27(32.9%) followed C* 070101 20 (24.4%). The rarest allele observed among the population was C*0611 1(1.2%).

HLA-C and C2 Alleles and Frequencies %

Ibos n=298

HLA C1	Frequencies	HLA-C2	Frequencies
C*010201	1(0.3)	C*021010	1(0.3)
C*02	3(1.0)	C*03	1(0.3)
C*020202	5(1.7)	C*030201	2(0.7)
C*020203	1(.3)	C*030301	1(0.3)
C*020205	1(0.3)	C*030304	1(0.3)
C*0210	25(8.6)	C*030402	1(0.3)
C*022601	1(0.3)	C*04	2(0.6)
C*03	4(1.4)	C* 040101	24(8.2)
C*030201	6(2.1)	C* 040128	1(0.3)
C*030401	1(0.3)	C*0407	1(0.3)
C*030402	4(1.4)	C*05	4(1.4)
C*04	20(6.8)	C*050101	2(0.7)
C*040101	83(28.4)	C*06	5(1.7)
C*05	3(1.0)	C*060201	19(6.5)
C*050101	2(0.7)	C*07	13(4.5)
C*06	4(1.4)	C*0701	43(14.7)
C*060201	21(7.2)	C*070201	8(2.7)
C*07	5(1.7)	C*070401	3(1.0)
C*0701	30(10.3)	C*0728	1(0.3)
C*070201	2(0.7)	C*080201	9(3.0)
C*070401	1(0.3)	C*0804	10(3.4)
C*080201	2(0.7)	C*120301	5(2.7)
C*0804	3(1.0)	C*120302	1(0.3)
C*120301	5(1.7)	C*140101	1(0.3)
C*14	1(0.3)	C*140201	1(0.3)
C*140201	2(0.7)	C*15	3(1.0)
C*1403	1(0.3)	C*150501	7(2.4)
C*150501	1(0.3)	C*16	10(3.4)
C*16	1(0.3)	C*160101	27(9.2)
C*160101	4(1.4)	C* 17	1(0.3)
C*1607	1(0.3)	C*170101	17 (5.8)
BS	47(16.0)	C*1801	15(6.5)
		C*BS	29 (11.9)
Total 298			

Yoruba n=112

HLA C1	Frequencies	HLA-C2	Frequencies
C*02	2(1.8)	C*20202	1(0.9)
C*020202	(0.9)	C*03	1(0.9)
C*0205	1(0.9)	C*030201	1(0.9)
C*0210	8(7.1)	C*04	4(3.6)
C*0214	1(0.9)	C*040101	13(11.6)
C*03	5(4.5)	C*050101	2(1.8)
C*030201	3(2.7)	C*06	1(0.9)
C*030402	7(6.3)	C*060101	1(0.9)
C*04	3(2.7)	C* 060201	6(5.4)
C*040101	33(29.5)	C*07	1(0.9)
C*060201	7(6.3)	C* 0701	14(12.5
C*0606	1(0.9)	C* 070123	1(0.9)
C*0701	16(14.3)	C*070201	3(2.7)
C*070201	3(2.7)	C* 080201	6(5.4)
C*0804	1(0.9)	C* 0804	2(1.8)
C*120301	2(1.8)	C*120301	2(1.8)
C*140201	1(0.9)	C*140201	1(0.9)
C*150501	1(0.9)	C*15	2(1.8)
C*160101	1(0.9)	C* 150501	1(0.9)
BS	13(11.6)	C* 160101	18(16.1)
		C* 1607	1(0.9)
		C* 17	1(0.9)
		C*170101	13(11.6)
		C* 1704	1(0.9)
		C*1801	2(1.8)
		BS	12(10.7)
	Total	112	

The Ibo and Yoruba Ethnic Groups

Red highlights - Rare alleles

Turquois highlights- Two ethnicities shared alleles (Ibo and Yoruba)

Yellow highlights-Alleles with high frequencies

Purple highlights- Two ethnicities shared alleles (Ibo and other ethnic groups)

Green highlights- Most probable alleles associated with HIV acquisition

Table 3: Distribution of HLA -C Alleles in Diverse Nigerian Ethnic Groups Amongst Serodiscordant and Concordant HIV Positive and Negative Couples

HLA-C and C2 Alleles and Frequencies %

Hausa n=50

HLA C1	Frequencies	HLA-C2	Frequencies
C*02	1(2.0)	C*04	1(2.0)
C*020201	1(2.0)	C*040101	6(12.0)
C*020202	3(6.0)	C* 0407	1(2.0)
C*0210	2(4.0)	C*050101	1(1(2.0)
C*0226	1(2.0)	C* 060201	1(2.0)
C*030402	1(2.0)	C* 07	1(2.0)
C*04	2(4.0)	C*0701	7(14.0)
C*040101	22(44.0)	C*070201	1(2.0)
C*040128	1(2.0)	C*07195	1(2.0)
C*060201	3(6.0)	C*080201	5(10.0)
C*0701	5(10.0)	C*0804	2(4.0)
C*0705	1(2.0)	C*102308	1(2.0)
C*080201	1(2.0)	C*140201	1(2.0)
C*140201	1(2.0)	C*1403	1(2.0)
C*170101	1(2.1)	C*150501	3(6.0)
BS	4(8.0)	C*160101	2(4.0)
		C*170101	5(10.0)
		C*1801	6(6.0)
		BS	3(6.0)
Total 50			

Other ethnic groups n=82

HLA C1	Frequencies	HLA-C2	Frequencies
C*02	2(2.4)	C* 030402	4(4.9)
C*020202	3(3.7)	C*030418	1(1.2)
C*020205	2(1.8)	C*04	1(1.2)
C*020213	1(1.2)	C*040101	5(6.1)
C*0210	7(8.5)	C*040128	1(1.2)
C*03	2(2.4)	C*041502	1(1.2)
C*030201	2(2.4)	C*04NEW	1(1.2)
C*030402	1(1.2)	C*050101	1(1.2)
C*04	5(6.5)	C*06	2(2.4)
C*040101	22(26.8)	C*060201	9(11.0)
C*050101	2(2.4)	C*0611	1(1.2)
C*06	2(2.4)	C*07	3(3.7)
C*060201	10(12.2)	C*070101	12(14.6)
C*07	1(1.2)	C*070201	4(4.9)
C*070101	8(9.8)	C*08	2(2.4)
C*070201	1(2.4)	C*080201	1(1.2)
C*08	2(2.4)	C*0804	1(1.2)
C*120301	2(2.4)	C*120203	1(1.2)
C*160101	2(2.4)	C*14	2(2.4)
BS	5(6.1)	C*140201	3(3.7)
		C*1403	1(1.2)
		C*150501	1(1.2)
		C*16	1(1.2)
		C*160101	8(9.8)
		C*17	2(2.4)
		C*170101	4(4.9)
		C*1801	4(4.9)
		BS	4(4.9)
		Total 82	

The Hausa Population and other Ethnic groups

Red highlights - Rare alleles

Yellow highlights-Alleles with high frequencies

Purple highlights- Two ethnicities shared alleles (Ibo and other ethnic groups)

Green highlights- Most probable alleles associated with HIV acquisition

Table 4: Distribution of HLA -C Alleles in Diverse Nigerian Ethnic Groups Amongst Serodiscordant and Concordant HIV Positive and Negative Couples

VI. DISCUSSION

The frequencies of HLA-C alleles showed high resolution frequencies observed in HIV negative individuals were higher: C*040101 106(39.0%) followed by C*0701 86(31.6%) compared to the alleles frequencies observed in the HIV-1 positive individuals C*040101 72(34.5%) and C*0701 57 (24.2%). However, the result is consistent with a study done in Kenyan in Africa where C*0701 45(16.1%) and C*040101 35(12.5%) had the highest frequencies in the study population [18]. The alleles with the least frequencies in this study were represented by C*120302 1(0.2%), C* 010201 1(0.2%), C*070123 1(0.2%), C*0705 1(0.2). HLA- C*070501 1(0.4%) and C*010201 1(04%) were also the least identified allele in the same Kenyan population [20]. Interestingly, one of the rarest allele C*010201 1(0.3%) identified among the HIV negative individuals in this study, was the only allele with same frequency observed among Kenyan population[20]. In Harare Zimbabwe also, C*040101 (25.2%) was the most frequent allele observed in the population of 123 children and adolescent aged10-18 years[22] which is consistent with this study. In another study conducted in Kenya, there was evidence of interdependent protective effects of the HLA-C*040101-B*8101and HLA-C*120302-B*3910, haplotypes that cannot be explained solely by linkage to a protective HLA-B allele. In this present study, there was no statistical

significance linking HLA-C 040101/120302 to either as protective or resistant allele.

Ethnic distribution of HLA-C alleles portrays heterogeneity of the Nigerian populations in her ethnic distribution. Some specific HLA antigens are found in high frequencies amongst geographically related individuals while some alleles were identified in all the ethnic groups of Nigeria covered by this study. HLA-A: C* 040101 and C* 070101 had the highest diversity in distribution and the most frequent alleles because it was identified in some individuals across all the tribes in the Nigerian population studied. Within the Ibo (53.3%) and Yoruba (21%) ethnic groups, C*040101 107(36.6%)/C*0701 71 (24.3%) and C*040101 46(41.2%)/C*0701 30(26.8%) were identified as the most frequent alleles respectively. Also, within Hausa (9.5%) and other tribes (15.1%), HLA-C: C*040101 28(56.0%)/C*0701 30(24.0%) and C*040101 27(32.9%)/ C*0701 20(24.4%) were identified as the most frequent alleles respectively. This result is consistent with the findings of in Nairobi Kenyan where C*0701 (16.5%) and C*040101(12.5%) were among the most frequent alleles identified in the population of 140 women attending antenatal clinic[20]. Other high frequency alleles identified in this study that shows similarity in ethnic diversity were C*060201: Ibo: 40(13.7%), Yoruba 13(11.7%), Hausa 3 (8.0%) and other tribes 19(23.2%). Previous studies on allele representation were consistent with this study. They reported C*070201, C*120201, C*1202, C*0602 and C*0701, C*0205, C*150501 in a cross section of black/Caucasians in South African and Zambian population[23,24]. However, some specific alleles were found to be rare in some ethnic tribes while some alleles co-exist within some tribes in this study. The rarest allele among the Ibos were: C*010201 1(0.3%), C*0728 1(0.3%) and C*07401 3(1.0%). HLA-C*1607 1(0.3%) was also identified only in Ibos and the Yoruba ethnic groups. This allele, C*010201 1(0.4%) was also observed as the only allele seen in the 140 Kenyan antenatal patients[20]. Within the Yoruba ethnicity, the only rare allele identified was C*0606 1(0.9%). Within the Hausa ethnicity alleles, C*0705 1(2.0%), C*07915 1(2.0%) and C*102308 1(2.0%) were observed. In other states of Nigeria with diverse ethnicities: (Rivers, Calabar, Edo, Delta) allele C*0611 1(1.2%) was identified as the rarest allele. However, C*1403 was shared between the Ibo and other ethnicities. Interestingly, comparing the alleles identified in this study population with South Africa and East African populations, some similarities were seen in alleles such as: C*060201, C*040101, and C*1203/(C * 060201 (16.98%), C* 0701 (14.29%), C* 040101 (13.01%), C* 1701 (9.36%), C* 0210 (6.67%), C*160101 (5.95%), and C* 180101 (5.81%)[22]. In the Gambia, the HLA-C identified were: HLA-C*1601 and C*17 alleles which showed lower frequencies, whereas HLA- C*0304, C*0701, and C*0804 were higher in Cairo[25] which is also consistent with this study. There is also similarity comparing the alleles from this study with that identified from Tanzanians which has the highest frequency: C*0401, C*1601, C*0201 and C*1801. On the other hand, alleles, C*0302, C*0602, C*0701 and C*0704 were under represented[25]. Another report[26] showed that HLA alleles identified in French North African immigrants which include: C*0701, C*0702, C*1601 and C*0304 were also consistent with the alleles identified in this study.

VII. CONCLUSION

The study of HLA genetic diversity in Nigeria is of public health importance especially for donor searches, cord blood and haemopoietic stem cell transplantations which is done in most developed countries registries. Again, lack of adequate tools that can provide HLA typing information with the needed molecular work and cost-effectiveness, is one of the main obstacles to conducting large epidemiological studies. This deficiency is also reflected in not having a representation of Nigerian HLA ethnic genetic diversity population in the global HLA databases.

The result also confirmed the close relationship among populations in Nigeria with those from Kenya, Gambia and black/Caucasian of South Africans population, East Africa, migrant families from North Africa who reside in the Paris, European and Paleo-North African origin. This is the first-time a large ethnic based level ancestry information on high and low resolution of HLA-C genotyping is showing the Nigerian population in HLA-C ethnic distribution. However, the HLA antigens observed to have very high frequencies were HLA -C 040101 and 0701 among all the ethnic groups in Nigeria.

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