ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

HLA-C Class I Allele-Sharing and High Plasma Viral Load (HIV-1 RNA) in HIV-1 Transmission among Heterosexual Serodiscordant Couples in Nigeria

Otuonye NM[1]*, Luo Ma[2], Enabulele OI[3], Nwaokorie FO[4], Adedeji AM[5], Ifeanyi KA[6], Uwandu MO[7], Ojetunde MM[1], Akinbami JB[1], Bosede OT[8], Uzoma IC[9] and Odunukwe NN[10]

[1]*Central Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria*

[2]*National Microbiology Laboratory, Winnipeg, Manitoba, Canada*

[3]*Microbiology Department, University of Benin, Edo State, Nigeria*

[4]*Medical Laboratory Science Department, College of Medicine, University of Lagos, Idi Araba, Lagos, Nigeria*

[5]*Evaluation and Monitoring Unit, Nigerian Institute of Medical Research Yaba, Lagos Nigeria*

[6]*Immunology Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

[7]*Centre for Human Virology and Genomics, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria*

[8]*Molecular Laboratory, Haematology and Blood Transfusion Department, Obafemi Awolowo University Teaching Hospital, Ile Ife, Osun State, Nigeria*

[9]*Molecular Haematology Laboratory, Department of Medical Laboratory Sciences, University of Nigeria, Nsukka, Nigeria*

[10]*Clinical Science Department, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria*

***Corresponding Author:** Otuonye NM, Central Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

Received: January 15, 2023; **Published:** April 20, 2023

Abstract

Background: Few studies have documented serodiscordant couples sharing HLA-B at HLA-B loci and increased risk of HIV-1 transmission. This study aimed to determine the sharing of same HLA-C allele and high viral load associated with risk of HIV-1 transmission among heterosexual serodiscordant couples in Nigeria.

Methods: A total of 224 serodiscordants, 26 concordant HIV positive and 21 concordant HIV negative couples, who signed informed consent document were enrolled into this project. Extracted genomic DNA was used for HLA class 1 genotyping. Sequencing was done by Sanger method, using Biosystems™ 3130xl Genetic Analyzer. HLA-C Typing was done using Codon Express 2010. HIV-1 RNA and CD4 were analyzed. Data entry and statistical analysis was done with SPSS and Kaplan-Meier analysis.

Results: Couples age ranged from 20 - < 50 years. The most prevalent HLA-C alleles in the cohort were: C*040101 (34.5%) and C*07010 (24.2%). Serodiscordant couples sharing a single allele on HLA-C loci (HLA-C1 or C2) in the population were 55 (37.7%). Ten [10] (17.9%) serodiscordant and concordant HIV positive couples shared HLA-C alleles at C1 and C2 on HLA-C loci. Sharing HLA-C allele at group or at allele level on HLA-C loci was significant associated with HIV transmission among the couples ($p < 0.009$ and $p < 0.001$). HLA C*0701 allele was observed to be associated with high baseline HIV-1 RNA ($p < 0.003$) and no association was observed with HIV-1 RNA after 6 months on ARV (P = 0.086/HLA C*04 and C*0401 were associated with high baseline HIV-1RNA after 6 months on ARV ($p < 0.000$ and $p < 0.000$).

Conclusion: Couples who shared HLA-C allele were associated with various degrees of increased HIV1-RNA and low CD4+ counts at HLA-C loci which is independently associated with increased intra-couple HIV-1 transmission amongst serodiscordant couples in Nigeria.

Keywords: *HLA-C; Allele Sharing; HIV-1; Serodiscordant Couples; Heterosexual HIV-1 Transmission; Nigeria*

Abbreviations

MCH: Major Histocompatibility Complex; HLA: Human Leucocyte Antigen; Serodiscordant: One Partner is Infected with HIV and the Other is Not; Concordant: Both Couples can be Either HIV Positive or HIV Negative; PVL: Plasma Viral Load; CD4 Count: CD4 Lymphocyte Count; APC: Antigen Presenting Cells; KIR: Natural Killer Cell Immunoglobulin-Like Receptors

Introduction

Globally, 36.7 million people are currently living with HIV infection [1,2]. Nigeria has the 2nd largest HIV epidemic in the world with prevalence of 1.4% and has the highest new infection rates in sub-Saharan Africa [3]. WHO estimates that globally about half of HIV-positive people in long-term relationships have HIV-negative spouses resulting to having serodiscordancy. This has given room to most heterosexual HIV-1 transmission among Serodiscordant couples. HIV is a public health problem, over 65% of the world's HIV infection is found in sub Saharan Africa with heterosexual exposure as the primary mode of HIV transmission [4]. Again, an estimated 70% HIV-1 transmission occur between married partners therefore, cohabiting couples in Africa represent the world's largest HIV risk group [5].

Human Leucocyte Antigen (HLA) molecules play a key role in regulating the immune response towards infectious agents like HIV-1. HLA have been shown to influence transmission as well as the progression of HIV-1 towards AIDS [6]. The role of HLA-B in HIV-1 transmission have been documented extensively, however, the role HLA-C play, in HIV-1 transmission in Serodiscordant couples have been poorly studied in Nigeria. HLA is the gene complex encoding the Major Histocompatibility Complex (MHC) proteins in humans [7]. The cell-surface protein regulates the immune system and identifies the differences between healthy body tissue and foreign substances that may cause infections [7]. Humans synthesize two classes of HLA namely class I and class II. Both classes are closely linked to each other at the end of HLA region which lies on the short arm of Chromosome 6p21.31 [8]. Class I comprises of three major proteins namely: HLA-A, HLA-B, HLA-C, which bind and present endogenous peptides to $CD8^+$ T cells for destruction [6]. Class II are coded for a region called HLA-D namely: HLA-DP, HLA-DQ and HLA-DR. They are expressed on antigen presenting cells, they bind processed extracellular peptides and present them to $CD4^+$ T cells for destruction. Both class I and II are responsible for the compatibility or incompatibility of the tissues/organs transplant[8]. Previous authors reported the role of HLA-C and its association with HIV-1 transmission in discordant couples and mother-to-child cohort. Their study showed HLA-C*07 and C*04 to be higher, both in HIV-1-infected spouses and infants when compared to exposed uninfected spouses and infants [9]. Another study reported Serodiscordant couples sharing HLA-B at HLA-B loci and increased risk of HIV-1 transmission [10]. Genome wide studies identified some known HLA-C alleles that showed to have higher surface area and lower surface areas which associated with HIV-1 transmission [11].

The rational of the study is to determine the role of HLA-C in HIV-1 transmission among heterosexual Serodiscordant couples in Nigeria. We hypothesized that HLA-C has been implicated in HIV-1 heterosexual among Serodiscordant couples. This study will therefore: (1) Identify the dependent risk factor to heterosexual HIV-1 transmission in Serodiscordant couples. (2) Determine whether shared HLA-C alleles are associated with high HIV-1 RNA (plasma viral load). (3) Determine if the shared HLA alleles identified are linked to HLA-C high/low surface areas and HIV-1 transmission. The study is a cross sectional study.

Materials and Methods

Study population

A total number of 271 couples participated in the study. They comprise of *224 (82.7%) serodiscordant, while 26 (9.6%) concordant HIV positive and 21 (7.7%) concordant HIV negative couples were used as control.*

Inclusion criteria and exclusion criteria *were previously described [13].*

Study sites

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

Focus group discussion and consent documentation

Participants were counseled and told to inform their partners about the research - to facilitate their consent as previously described [12].

Ethical approval

Approval was obtained from NIMR IRB (IRB/12/176) and NAUTH Ethical Committee (CS/66/7/79). Informed consent was obtained from all the participants during couples' counselling and testing conducted in an appropriate meeting room.

Sample size determination and sample collection

The prevalence of Serodiscordant couple is in Nigerian is 7.7% [16]. Formula $N = z^2pq/d^2$ for a cross sectional study was used. It was multiplied by the design effect of 1.5 and attrition of 10%. Total sample size was approximately 271. Whole blood (7 ml) collection and processing was previously described [12].

Laboratory investigations

HIV-1 RNA Assay (plasma viral load)

Determination of the Amplicor HIV-1 MONITOR Test version 1.5 is an *in vitro* nucleic acid amplification test for the quantitation of RNA copies/ml from human was previously described [13].

CD4 count

Twenty (20 µl) microlitre of whole blood, 20 µl of CD4 mAb PE was added to Partec test tubes and incubated for 15 minutes in the dark as was previously described [13].

White cell harvestion and DNA extraction

White cell pellet was harvested from buffy coat on peripheral blood cells with the use of Ace-shocking solution and was previously described [13].

DNA amplification by thermocycler (PCR)

Copies of DNA 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 as was previously described [12].

Agarose gel electrophoresis

This was used to confirm that PCR was successful in amplifying the desired HLA gene. Each sample was run in a 1% (w/v) agarose gel in 1X TBE buffer and stained with 0.003% 10 mg/ml ethidium bromide and 1 kb ladder DNA as was previously described [12].

Purification of PCR product

Following sequencing PCR, the PCR product was precipitated using 21 µl of a mixture containing 5 ml of 95% ethanol and 250 µl of sodium acetate as was previously described [12].

Sequencing HLA using Sanger method [14]

Sanger technique was used to identify allele dissimilarity within the HLA class 1 genes [15] focusing on the most polymorphic exons 2 and 3. 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). Each sequencing PCR reaction contained 4 µl of purified PCR product, 1.5 µl primer at 3.2 µM and 2 µl of applied Biosystems™. BigDye® Terminator V1.1 and the forward and reverse primers as shown on the table 1 below, was previously described [12].

Gene	HLA forward and reverse sequencing primers	BigDye 1.1
HLA-C	CSEQ5Forward: GGGGACBGGGCTGAC	√
HLA-C	CSEQ3Reverse: GCCGTCCGTGGGGGATG	√

Biosystems™ 3130xl genetic electropherogram [15]

Each respondent's sequenced PCR product were loaded in the Biosystems for electrophoresis and data analysis as was previously described [12].

CondonExpress TM 24 [16]

CodonExpress is a genotyping software based on a taxonomy-based sequence analysis to resolve HLA heterozygous and homozygous combinations as was previously described [12].

Statistical analysis

Data generated were entered into IBM SPSS statistics version 20. Cross-sectional analysis using Chi Square was used to identify association between HLA-C genes, viral load and CD4 count as was previously described [12].

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, 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 employed (93.0%). HIV status showed 224 *(82.7%) Serodiscordant, while 26 (9.6%) concordant HIV positive and 21 (7.7%) concordant HIV negative couples* were added as control measures.

Variables	n = 542	Frequency	Frequency %
Age	20 - 25	9	1.6
	26 - 30	60	11.1
	31 - 35	130	24.0
	36 - 40	142	26.4
	40 - 41	100	18.2
	41 - 50	57	10.5
	> 50	44	8.2
Sex	Male	270	49.8
	Female	272	50.2
Marital Status	Engaged	16	3.0
	Married	526	97.0
Ethnicity	Ibo	298	54.9
	Yoruba	112	20.9
	Other Ethnicity	82	14.9
	Hausa	50	9.3
Occupation	Traders/Artisans	256	47.3
	Teaching/Civil servant	192	35.4
	Self employed	56	10.3
	Unemployed	38	7.0
Educational level	None	262	48.3
	Primary	73	13.5
	Secondary	110	20.3
	Tertiary	97	17.9

Table 1: *Sociodemographic characteristics of study participant.*

Table 2a showed serodiscordant couples sharing a single allele on HLA-C loci for HLA-C1 and C2 in the population studied. The trend is that 55 (37.7%) serodiscordant couples shared a single HLA-C allele at allele level which is represented by 1.

Couples Sharing One Allele at HLA-C1 or HLA-C2 A at HLA-C loci					
Sex F-M	C1	C2	HIV Status	C1	C2
F	C*0202	C*0401	POS	0	1
M	C*0401	C*0701	NEG	1	0
F	C*0602	C*0701	NEG	0	1
M	C*0401	C*0701	POS	0	1
F	C*0401	C*0701	NEG	0	1
M	C*0701	C*1601	POS	1	0
F	C*0201	C*0701	POS	0	1
M	C*0401	C*0701	NEG	0	1
F	C*0602	C*0701	POS	1	0
M	C*0602	C*1701	NEG	1	0
F	C*0602	C*0602	POS	1	1
M	C*0210	C*0602	NEG	0	1
F	C*0401	C*0804	NEG	1	0
M	C*0401	C*0401	POS	1	1
F	C*0804	C*1701	NEG	0	1
M	C*0701	C*1701	POS	0	1
F	C*0401	C*0401	POS	1	1
M	C*0401	C*0702	POS	1	0
F	C*0401	C*0701	NEG	0	1
M	C*0701	C*0804	POS	1	0
F	C*0401	C*1505	POS	1	0
M	C*0401	C*0702	NEG	1	0
F	C*0701	C*1701	POS	1	0
M	C*0201	C*0701	NEG	0	1
F	C*0602	C*1701	NEG	1	0

M	C*0602	C*0802	POS	1	0
F	C*0401	C*0401	NEG	1	1
M	C*0401	C*0701	POS	1	0
F	C*0401	C*0401	NEG	1	1
M	C*0401	C*0704	POS	1	0
Page 2 of table 2a					
F	C*0401	C*0401	POS	1	1
M	C*0401	C*0704	NEG	1	0
F	C*0401	C*1601	NEG	1	0
M	C*0210	C*0401	POS	0	1
F	C*0401	C*0501	POS	1	0
M	C*0401	C*0401	POS	1	1
F	C*0214	C*0701	NEG	0	1
M	C*0401	C*0701	POS	0	1
F	C*0401	C*1704	NEG	1	0
M	C*0401	C*0802	POS	1	0
F	C*0401	C*1801	POS	1	0
M	C*0401	C*1701	NEG	1	0
F	C*0210	C*0601	POS	0	1
M	C*0601	C*1801	NEG	1	0
F	C*1401	C*1601	POS	0	1
M	C*0401	C*1601	NEG	0	1
F	C*0401	C*0302	POS	0	1
M	C*0302	C*0802	POS	1	0
F	C*0401	C*0701	POS	1	0
M	C*1403	C*0401	NEG	0	1
F	C*0701	C*1701	NEG	1	0
M	C*0602	C*0701	POS	0	1
F	C*0401	C*1701	POS	1	0

M	C*0401	C*0702	POS	1	0
F	C*0401	C*1601	POS	0	1
M	C*0701	C*1601	NEG	0	1
F	C*0401	C*0210	POS	1	0
M	C*0401	C*0401	NEG	1	1
F	C*0702	C*1402	NEG	1	0
M	C*0401	C*0702	POS	0	1
F	C*0602	C*0602	NG	1	1
M	C*0210	C*0602	POS	0	1
F	C*0401	C*0804	POS	1	0
M	C*0401	C*0401	NEG	1	1
F	C*0701	C*0701	POS	1	1
M	C*0701	C*1801	NEG	1	0
F	C*0401	C*0804	NEG	0	1
M	C*0210	C*0804	POS	0	1
F	C*0602	C*1601	POS	1	0
M	C*0302	C*0602	NEG	0	1
Allele most commonly shared is C*0401. Sharing same HLA-C allele at allele level: p=0.031. OD value 3.200 Upper limit .819 and lower limit 406.					

Table 2a: *Prevalence of HLA-C1/C2 and Same Alleles Shared at Allele level among Serodiscordant Couples (Index-donor and Partner-recipient pairs) n=35 representing 55 couples who shared alleles*

On table 2b, 10 (17.9%) serodiscordant and concordant HIV positive couples shared HLA-C alleles at C1 and on C2 on HLA-C loci. Sharing HLA-C allele at group or at allele level on HLA-C loci was statistically significant: ($p < 0.009$ and $p < 0.001$ respectively. Odd ratio- at 95% interval = 3.200 upper limit .819 and lower .406/ OD = 4.287. Upper limit-10.037 and lower limit 1.831. There was no concordant HIV negative couples that shared allele.

Table 3 highlighted HLA-C alleles associated with high pVL and CD4 variation in HIV-1 positive subjects. HLA C*0701 allele was associated with high baseline pvl ($p < 0.003$) and no association was observed with pVL after 6 months on ARV ($P = 0.086$). HLA C*17 alleles was associated with high baseline pVL before and after 6 months on ARV ($p < 0.000$). HLA-C*04 were less significantly associated with high pVL before and after 6 months on ARV ($p = 0.086$ and $p = 0.060$). HLA C*04 and C*0401 were associated with high baseline pVL after 6 months on ARV ($p < 0.000$ and $p < 0.000$). HLA-C*06 and C*0602 alleles were moderately associated with high baseline pvl ($p = 0.006$ and $p= 0.006$) and significantly reduced at follow-up (0.279 and 0.219) respectively.

Table 2b. Prevalence of HLA-C1/C2 and Same Alleles Shared at Group level among Serodiscordant and Concordant HIV Positive Couples (Index-donor and Partner-recipient pairs) n=10 couples

Couples Sharing same	HLA-C1 and	HLA-C2 Alleles at HLA-C loci			
Sex F-M	C1	C2	HIV Status	C1	C2
F	C*0602	C*0701	POS	1	1
M	C*0602	C*0701	POS	1	1
F	C*0701	C*0701	POS	1	1
M	C*0701	C*0701	POS	1	1
F	C*0401	C*1701	POS	1	1
M	C*0401	C*1701	NEG	1	1
F	C*0701	C*0702	POS	1	1
M	C*0701	C*0702	POS	1	1
F	C*0210	C*0401	NEG	1	1
M	C*0210	C*0401	POS	1	1
F	C*0602	C*0602	NEG	1	1
M	C*0602	C*0602	POS	1	1
F	C*1601	C*0401	POS	1	1
M	C*1601	C*0401	NEG	1	1
F	C*1401	C*1601	POS	1	1
M	C*0401	C*1601	NEG	1	1
F	C*0701	C*0701	POS	1	1
M	C*0701	C*0701	POS	1	1
F	C*0401	C*0401	POS	1	1
M	C*0401	C*0401	NEG	1	1
Allele most commonly shared is C*0401 and 0701. Sharing same HLA-C allele at group level: p=0.001 by 10 couples (17.9%) Odd ratio: lower limit .0302 and upper limit .646					
Homozygosity of HLA-C alleles was identified with in these 10 couples.					

Mean Values HLA-C Alleles	No. of samples analysed HIV-1 RNA BL	No. HIV-1 RNA FU	No. CD4 BL	No. CD4 FU
C*0701 P. value	(40) 349,173.75 0.003	(32) 223,296.95 0.086	(44) 267.39 0.168	(31) 399.59 0.213
C*07 P. value	(67) 265,345.89.89 0.442	(56) 140,830.13 0.080	(75) 250.22 0.541	(50) 409.52 0.075
C*17 P. value	(18) 786,892.39 0.000	(17) 612,874.29 0.000	(21) 259.06 0.464	(16) 511.10 0.201
C*06 P. value	(5) 284087.50 0.006	(4) 1,054.00 0.219	(4) 263.75 0.426	(5) 501.40 0.231
C*0602 P. value	(4) 284,087.50 0.006	(4) 1,318.50 0.279	(3)250.33 0.221	(4) 514.00 0.321
C*0401 P. value	(9) 111,428.25 0.037	(8) 28,166.56 0.000	(8)266.50 0.986	(11) 464.09 0.890
C*04 P. value	(69) 153,605.40 0.091	(65) 30,370.62 0.060	(84) 221.72 0.318	(54) 477.87 0.262

Table 3: *HLA-C alleles associated with high HIV1-RNA (Plasma viral load) and CD4+ variation in HIV positive subjects n = 194.*

BL = Baseline CD4: CD4 count before 6 months before ARV.
BL = Baseline HIV-1 RNA: HIV-1 RNA 6 months before ARV.
FU = Follow- up CD4: CD4 count after 6 months on ARV.
FU = Follow- up HIV-1RNA: HIV-1 RNA count after 6 months on ARV.
Low viral loads = ≥ 40 - 500 copies/ml.
High viral load = ≤ 5000 copies/ml.
Low CD4 Count = 200 mm/3.
High/normal CD4 count = 500 - 120 mm/3.
No of samples analyzed.

Table 4 showed biological variables which revealed participants improvement on both CD4 count and viral load after 6 months on ARV: Baseline pVL ≤ 500 copies/ml (20.5%) after 6 months on ARV (FUP VL) ≤ 500 copies/ml (73.9%). Baseline CD4 count ≤ 200 (35.48%) after 6 months on ARV (FUP CD4) ≤ 200 (11%). However, 5.75% and 1.32% of the participants were still having > 1,000,000 copies/ml of pVL after six months on ARV.

Discussion

HIV-1 transmission associated with same HLA-C allele shared by serodiscordant couples

We observed that couples in heterosexual relationship who shared *HLA*-C allele at HLA-C loci at both allele level and group level were associated with increased risk of HIV-1 transmission. After adjustment for other genetic and non-genetic risk factors seen with heterosexual HIV-1 transmission, different degree of allele sharing at single (HLA-C1) or multiple HLA-C (HLA-C1 and -C2) loci between Index-donor and partner-recipient pairs were independently associated with intra-couple HIV-1 transmission ($p < 0.009$ and $p < 0.001$) respectively. It was also observed that the 21 concordant HIV negative couples that served as control did not share a single allele between

Variables	n = 542	Frequency	Frequency %
Baseline viral load for the Index			
HIV-1 RNA in copies/ml	≤ 500	43	20.5
	501 - 100,000	89	42.6
	100,001 - 1,000,000	68	32.5
	1,000,001 - 10,000,000	9	4.4
Follow-up viral load after 6 months on ARV for the Index			
HIV-1 RNA in copies/ml	≤ 500	167	73.9
	501 - 100,000	44	19.4
	100,001 - 1,000,000	13	5.75
	1,000,001 - 10,000,000	3	1.32
Baseline CD4 cells/mm^3 for the Index			
	≤ 200	88	35.48
	201 - 400	131	52.82
	401 - 600	19	7.66
	601 - 820	10	4.03
Follow-up CD4 cells mm/3 after 6 months on ARV for the Index (FUPCD4)			
	≤ 200	30	11.0
	201 - 400	68	26.0
	401 - 600	97	37.0
	601 - 800	40	15.0
	801 - 1000	24	9.0
	1001 - 1350	6	2.0

Table 4: *Biological variables: Baseline and follow-up viral load and CD4 count of the participants.*

Biological variables showed participants improvement on both the CD4 count and the viral load after 6 months on ARV: Baseline viral load ≤ 500 copies/ml (20.5%) after 6 months on ARV (FUP) ≤ 500 copies/ml (73.9%). Baseline CD4 count ≤ 200/mm^3 (35.48%) after 6 months on ARV (FUP CD4) ≤ 2003 (11%).

the couples. This result is consistent with the findings from studies in which sharing of HLA-B by Zambian couples in a heterosexual relationship was independently associated with HIV-1 transmission ($p < 0.0001$) [17]. Their findings were connected to the selective pressure by HLA-B alleles on transmitted HIV infections which has effect on B locus polymorphism in HIV-1. The findings in this study showed that Serodiscordant couples that shared HLA-C may be more predisposed to acquiring HIV-1 infection from their Index- compared to couples who do not share same HLA-C. The implication of sharing same allele by couples showed that these couples share HLA-C genes at family level, amino acid level and at polymorphism level within HLA-C loci located at axon 2 and 3 and this is where HLA genes encode the peptide binding grove. At this point, T-cell interact with molecules at the Major Histocompatibility Complex. It's the same MCH polymorphism that guarantees that a whole community will not accede to any new pathogen or a mutated one so that individuals will be able to develop adequate immune response in order not to be vulnerable to any new pathogen [18]. However, as these couples have shared alleles at

group level, there seem to be less genetic variation between the couples, so through sexual act may share diseases in common such as HIV infection. We observed 5 couples who shared HLA-C alleles in homozygous form. However, in another study, homozygosity at the HLA-A or -B locus was found among with rapid progression to late-stage of HIV-1-related conditions in Rwandan women ($p = 0.003$) [4]. Another study had a contrary report that homozygous genotypes is either HLA class I and HLA class II, do not exert any harmful effect on HIV-1 disease progression [11].

Additionally, HLA sharing by Serodiscordant couples can be very useful in tissue and solid organ transplant especially where a close relative will be required to donate an organ/tissue for kidney or Haploidentical Stem Cell Transplant for sickle cell diseases patients [19].

HLA-C alleles associated with increased HIV-1RNA and HIV transmission in serodiscordant couples

From previous report, 35' un-translated region allele was implicated to HLA-C surface expression. HIV positive subjects with high-expressing HLA-C alleles have high CD4 counts, low viral loads and progress slowly to AIDs. On the other hand, the HIV positive subjects with low HLA-C low surface expression is implicated with high viral load and low $CD4^+$ T cell counts which leads to rapid progression to HIV-1 disease [16,20]. This study is consistent with these previous studies because there were certain HLA-C alleles implicated with reduced surface expression, associated with high pvl and reduced CD4+ counts before and after 6 months on ARV. These include: HLA- C*07, C*17and C*04. These HLA-C allele were observed in both HIV positive males and females' subjects who shared HLA-C alleles with their partners. It was also observed that all the HLA-C alleles implicated with different degrees of allele sharing at HLA-C loci between Index-donor and partner-recipient pairs were associated with high pVL as shown on table 3. This also imply that HIV-1 pVL can independently or in association with different HLA-C allotypes contribute to HIV-1 transmission among Index-donor and partner-recipient pairs.

High viral load and low CD4 count implicated in HIV-1 transmission in serodiscordant couples

We observed a massive reduction of baseline viral (pVL) load (73.9%) and improved CD4 count (63%) after 6 months the participants received ARV. The improved CD4 count and reduced pVL is very critical in the improvement of the health of the Index spouse and will reduce the rate of heterosexual HIV transmission from the Index spouse to the partner. Having an undetectable pVL ($\leq$ 500 copies/ml, 73.9%), confirms that the risk of HIV becoming resistant to ARV is very minimal, the immune system is improved and there would be very low risk of having opportunistic infections. Our result is consistent with three previous studies that reported reduction in the pVL being an important target for HIV prevention. A study of heterosexual Uganda HIV Serodiscordant couples showed a direct relationship between the risk of HIV acquisition and donor pVL. No transmissions were observed when the pVL was below a critical threshold of 1,500 copies/ml [21,22]. Other previous reports showed that being consistent on ARV with pVL remaining at undetectable level (20 cells/mm^3), the risk of passing HIV to their sexual partners fell by 96% [22,23]. However, of concern are a few participants whose pVL remained unchanged after six months on ARV (5.75%/ 1.32%), because in this state, and without the use of condom, the Index can easily transmit HIV to the negative partner, if the Index donor is still stable having a very high plasma viral load.

Limitation of Study

There is need to study the role of HLA-A and its role in HIV-1 transmission in Serodiscordant couples.

Conclusion

Couples who shared HLA-C allele were associated with various degrees of increased pVL and CD4+ counts and were independently associated with accelerated intra-couple HIV-1 transmission amongst Serodiscordant couples in Nigeria.

Contribution of Authors

Otuonye NM-Conceived the idea, wrote the proposal.

Luo Ma-Provided all PCR and sequencing reagent.

Enabulele OI-Chief Supervisor the project.

Nwaokorie FO-Contributed in Proposal writing.

Chinweokwu K-Collected and processed samples at site 2: HIV clinic Nnamdi Azikiwe University Teaching Hospital Nnewi campus Anambra State Nigeria.

Uwandu MO-Contributed in sample collection.

Adedeji AM-Contributed in statistics analysis.

Ojetunde MM-Sample collection.

Ayoola JB-Data collation and entries.

Bosede OT-Data collation and entries.

Uzoma Chinwe Ijeoma-Contributed in article writing.

Odunukwe NN-Co project supervisor.

Competing Interests

All authors declare no competing interests.

Bibliography

1. Kaiser HJ. The Global HIV/AIDS Epidemic Global Health Policy (2017): 1-20.
2. UNAIDS (2019).
3. Avert Global Hiv and Aids Statistics (2017).
4. Allen S., *et al*. "Promotion of couples' voluntary counselling and testing for HIV through influential networks in two African capital cities". *BMC Public Health* 7 (2007): 349.
5. Bagala A. "Married Couples Top HIV Infection Rates". The Daily Monitor. Kampala (2006).
6. HLA-C major histocompatibility complex, class I, C Homo sapiens (human) Gene ID: 3107, updated (2019).
7. Black CJ and Tomás Cabeza de Baca. Major histocompatibility complex (2018).
8. Davis DM. "The Compatibility Gene (ebook)". How Our Bodies Fight Disease, Attract Others, and Define Our Selves (2014).
9. Meuleman T., *et al*. "Paternal HLA-C is a risk factor in unexplained recurrent miscarriage". *American Journal of Reproductive Immunology* 79.2 (2018).
10. Dorak TM., *et al*. "Transmission of HIV-1 and HLA-B allele-sharing within serodiscordant heterosexual Zambian couples". *Lancet* 363.9427 (2004): 2137-2139.
11. Zipeto D and Beretta A. "HLA-C and HIV-1: Friends or foes". *Retrovirology* 9 (2004): 39.
12. Otuonye NM., *et al*. "Human Leukocyte Antigen-C and Haplotypes: Associations with Resistance and Susceptibility to HIV-1 Infection among Serodiscordant Couples in Nigeria". *EC Microbiology* 18.3 (2022): 24-36.

13. Otuonye NM., *et al*. "Plasma Viral Load of HIV-1 in Microbial Infection and Antimicrobial Resistance among Heterosexual Serodiscordant Couples in Nigeria". *Fortune Journals of Health Sciences* 5.2 (2022): 254-270.

14. Sanger FN and Coulson A. "DNA sequencing with chain-terminating inhibitors". *Proceedings of the National Academy of Sciences of United States of America* 74 (1977): 5463-5467.

15. Applied Biosystems. DNA Sequencing by Capillary Electrophoresis Chemistry Guide: (Second Edition), Applied Biosystems, Foster City, California (2009): 450.

16. Codon Express TM. "CodonExpress Genotyping Software Manual: Genotyping Based on Taxonomy-based Sequence Analysis (TBSA) to resolve HLA heterozygous combinations". University of Manitoba, Canada (2010): 20.

17. Dorak TM., *et al*. "Transmission of HIV-1 and HLA-B allele-sharing within serodiscordant heterosexual Zambian couples". *Lancet* 363.9427 (2004): 2137-2139.

18. Davis DM. "The Compatibility Gene (ebook)". How Our Bodies Fight Disease, Attract Others, and Define Our Selves (2014).

19. Mary Carmelle Philogene MC and PhD and Brennan DC. Kidney transplantation in adults: HLA matching and outcomes (2022).

20. Apps R., *et al*. "HLA-C cell surface expression and control of HIV/AIDS correlate with a variant upstream of HLA-C". *Nature Genetics* 41 (2013): 1290-1294.

21. Brenner BG., *et al*. "Risk of HIV transmission in discordant partners". *Journal of HIV Therapy* 12 (2007): 19-21.

22. Aidsmap. HIV and AIDS Information: CD4, viral load and other tests (2016).

23. Quinn TC., *et al*. "Viral load and heterosexual transmission of Human Immunodeficiency Virus type 1". *New England Journal of Medicine* 342 (2000): 921-929.

Volume 19 Issue 5 May 2023
All rights reserved by Otuonye NM.*, et al.*