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Role of Killer cell immunoglobulin-like receptors (KIR) genes in stages of HIV-1 infection among patients from Burkina Faso

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Abstract: Objectives: A cluster of specialized KIR genes of specialized KIR genes has been shown to be associated with susceptibility or resistance to viral infections in humans. Therefore, this pilot study, this pilot investigation sought to determine the frequencies of KIR genes human immunodeficiency virus type 1(HIV-1) patients and establish their potential clinical involvement in disease progression and staging.

Methods: HIV-1 infected and healthy individuals were selected for this study. Hepatitis B surface antigen (HBsAg), anti-HCV antibodies and anti-HIV-1/2 antibody/antigen were screened using a 4th generation ELISA assay (Cobas e 411 Analyzer, Roche Diagnostics GmbH Mannheim, Germany). SSP-PCR was used to evaluate the frequencies of KIR genes. $CD4^+$ T counts and HIV-1 viral load were measured in patients using respectively BD FACSCount and Abbott m2000rt instruments.

Results: We found a significant association between the frequencies of *KIR2DL2* (OR=4.41; p < 0.001), *KIR2DS2* (OR=4.76; p < 0.001), *KIR2DS3* (OR=2.27; p=0.004), *KIR2DS4* (OR=1.76; p=0.026), *KIR3DS1* (OR=2.43; p=0.016) and HIV-1 infection; whilst the *KIR3DL1* gene (OR= 0.39; p < 0.001) was associated with protection against HIV-1 infection. HIV-1 replication was found to be associated with the presence of *KIR2DS2* (OR=6.08, p = 0.024). In contrary the pseudogene *KIR2DP1* (OR=0.39; p=0.026) were linked to a protective status with the highest number of lymphocyte T CD4 counts.

Conclusion: Our data showed that *KIR2DL2, KIR2DS2, KIR2DS3, KIR2DS4,* and *KIR3DS1* were significantly associated with HIV-1 infection whereas *KIR3DL1* was associated with protection against HIV-1 infection. Further investigations are needed to fully comprehend the clinical significance of KIR genes in HIV disease progression.

Keywords: KIR gene, HIV-1, T CD4, Viral Load, Burkina Faso

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Introduction

Despite progress in the availability of treatment modalities, human immunodeficiency virus (HIV) and AIDS remain persistent public health concerns in sub-Saharan Africa.

Recent reports from the WHO showed that HIV infections have claimed 35 million lives in 2018, making it one the most, making it one of the most serious life-threatening human diseases in both 20th and 21th century [1]. Sub-Saharan Africa (SSA) is one of the most affected regions by HIV infection globally, with an estimated 25.6 million people living with the disease [1]. In 2016, approximatively 95,000 individuals were living with HIV and 3,400 new infections were reported in Burkina Faso [2]. As HIV progresses, host immunity is depleted from its most effective immune cells ($CD4^+$ T cells), thus increasing the body's vulnerability to opportunistic infections. Antiretroviral treatment is therefore is therefore used to prevent viral spread and restore the CD4 T cell levels.

However, several studies have reported a high rates of therapeutic failures [3-5], and investigations have been conducted to develop more effective therapies to inhibit HIV replication in infected patients. Studies on host/pathogen interactions have contributed to improve our knowledge of HIV molecular pathogenicity in human. Currently, investigations have focused on understanding host-genetic factors that could potentially modulate cellular susceptibility to HIV replication. Some studies have shown that host genetic factors could confer susceptibility or protection against HIV infection [6, 7].

Natural Killer cells (NK) are known to play an important role by acting as the first line of protection against cells infected by virus and tumor cells. Upon, viral infection NK are activated through various and complexes mechanisms resulting in a potent response against the pathogen, followed by the activation of an adaptive immune response mediated by T and B lymphocytes (specific immunity) [8].

Killer-cell immunoglobulin-like receptors are members of highly polymorphic receptors found on the surface of NK cells. KIRs are important regulators of the cytotoxic effect of NK following activation cells by enabling differentiation between major histocompatibility (MHC) class I allelic variants, this allows NK cells to identify virally infected cells or neoplastic cells leading to their removals [9, 10]. Non-infected and healthy cells expressing HLA class I proteins are preserved through inhibitory "self-recognition" mechanisms that prevent their lysis, whilst infected cells and cancer cells lacking the HLA class I molecules on their surfaces are recognized and destroyed by lysis activating receptors [11]. However, cancer cells have also evolved to evade immune surveillance by using a similar pathway [12].

KIRs are also categorized based on the number of their extracellular immunoglobulin domains and the length of their cytoplasmic tails. A long cytoplasmic tail with two motifs Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) confers an inhibitory activity (2DL, 3DL); while a short cytoplasmic tail endows activating activity (2DS, 3DS) [11, 13]. Currently, KIR genes are divided into two haplotypes A and B depending on the presence of certain specific genes [14, 15]. Haplotype A is composed of *KIR3DL3*, *KIR2DL3*, *KIR2DP1*, *KIR2DL1*, *KIR3DP1*, *KIR2DL4*, *KIR3DL1*, *KIR2DS4*, and *KIR3DL2* genes, while all other haplotypes as described as haplotype B (14th International HLA and Immunogenetics Workshop, 2005). It has been demonstrated that KIR are associated with the pathogenesis of certain diseases including type 1 diabetes mellitus [16], hepatitis C virus (HCV) infection [17, 18], and hepatitis B virus (HBV) infection [19-21]. It has also been reported that KIR genes are associated with resistance to HIV-1 infection [22]. This pilot study was undertaken to evaluate the association between KIR genes and HIV disease progression in a cohort of HIV-1 infected patients from Burkina Faso. It is anticipated that this work will provide insights into the specific KIR genes involved in susceptibility or resistance to disease progression which will improve clinical management of HIV-1 patients in the country.

Material and Methods

Type and Population of study

This is a case-control study, which was conducted from January to June 2019. A total of 279 individuals were included in this investigation, which consisted of 145 HIV-1 infected patients and 134 seronegative individuals (HIV-1 negative) recruited at the Pietro Annigoni Biomolecular Research Center (CERBA/LABIOGENE) and the regional blood transfusion center of Ouagadougou (CRTS/O), respectively. All subjects were seronegative for hepatitis B (HBV) and C (HCV) infections.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by Burkina Faso' National Health Ethic Committee (reference number No2017-01-004).

Samples collection and Research for HBV, HCV and HIV viral markers

Blood samples were collected from HIV-1 infected patients and from healthy voluntary and non-remunerated blood donors at CRTS/O. Serological tests using four-generation ELISA Ag/Ab were performed for HIV, HCV and HBV screening and confirmation in the control group using the Cobas e 411 Analyzer (Roche Diagnostics GmH Mannheim, Germany) according to the manufacturer's protocol. Serological markers for HBV and HCV of HIV-1 infected patients were detected using HBV One Step Hepatitis B Virus Combo Test Kits (Abon Biopharm Guangzhou, Co., Ltd. China) and HCV Hepatitis C Virus Rapid Test Device (Abon Biopharm Guangzhou, Co., Ltd Chine), respectively.

Determination of Lymphocytes TCD4 and HIV-1 viral load

Lymphocytes T CD4 (CD4 cells) assay was performed using BD FACSCount (Becton Dickinson, San Jose, CA) following the manufacturer's protocol.

Viral RNA was extracted from 200 µL of plasma using the « Abbott HIV-1 *m-sample* system preparation Kit (Promega, USA) » according to the manufacturer's protocol. HIV-1 viral load was determined using the « Abbott HIV-1 Real Time kit » (Promega, USA) on the Abbott m2000rt system (Abbott Laboratories, Illinois, USA) according to the manufacturer's protocol.

Genomic DNA Extraction and Determination of KIR Genes by SSP-PCR (Sequences Specific Primer)

Whole blood was used for genomic DNA extraction using the salting-out method as previously described [23]. DNA purity and concentration were determined using a Biodrop (Isogen Life Science, NV/S.A, Temse, Belgium). Approximatively 100 ng/µl of DNA was used to amplify the subset of 16 targeted KIR genes using the SSP-PCR (Sequence Specific Primer PCR) method as previously described [24]. The PCR reactions were performed in 60 µL of the reaction mixture containing 100 ng/µL of DNA (variable volume), 7.5 µL of 10 × PCR buffer, 2.25 µL MgCl2; 0.6 µL of dNTPs and 0.375 µL of platinum™ DNA Taq polymerase in nuclease-free water. PCR reactions were performed on the GeneAmp® PCR system 9700 (Applied Biosystem, USA) according to the following amplification program: after initial denaturation for 3 min at 94°C; the amplifications were carried out respectively for 5 cycles, 21 cycles and 4 cycles of denaturation at 94°C, annealing at primer specific temperature for 15 secs (65°C and 60°C) or 1 min (55°C for 4 cycles step), and extension at 30 secs at 72°C or 2 min for 4 cycles step with a final extension at 72°C for 7 min. The PCR products were separated on 3% agarose gel and visualized under UV light at 312 nm using the Gene flash (Gene Flash syngenge Bio Imaging, USA). PCR products were validated against a positive internal control corresponding to the DRB1 gene fragment (**Figure1**).

Statistical analysis

The data was analyzed with the standard Statistical Package for Social Sciences (SPSS) software version 20.0. The $\chi 2$ test was used to compare variant frequencies between groups. Risk was estimated with Odds Ratio (OR) and 95% of confidence interval (95% CI). P-values < 0.05 were considered statistically significant. Association between KIR gene and HIV-1 infection was established by comparing frequencies between cases and controls using the $\chi 2$ test.

Results

The distributions of sociodemographic characteristics are shown in **Table 1**. The study population consisted of 279 people (145 HIV-1 patients and 134 control subjects) aged 18 to 68. In the study population females were more represented than males (62.70% versus 37.30%) and they had 1.86 fold increased risk to be infected by HIV-1 (OR = 1.86, 95% CI = 1.13-3.04, p < 0.013). Among HIV-1 infected patients group females were more infected by than males (69.70% versus 30.30%), whilst, 81.40% of HIV-1 infected patients aged ≥ 40 years. Individuals aged ≥ 40 years in the study population were 4.78 times more likely to be infected by HIV-1 compared to individuals aged ≤ 39 years (OR = 4.78, 95% CI = 2.78-8.18, p < 0.001). All subjects were seronegative for hepatitis B (HBV) and C (HCV) infections (**Table 1**).

The Immunological and virological parameters of HIV-1 infected patients were shown in **Table 2.** The $CD4^+$ counts were stratified according to the Centers for Diseases Control and Prevention criteria [25]. The baseline viral load level for failure or therapeutic success is 1000 copies/mL in accordance with WHO Consolidated Guidelines for the Use of Antiretroviral Drugs for the Treatment and Prevention of HIV Infections in 2016 [26].

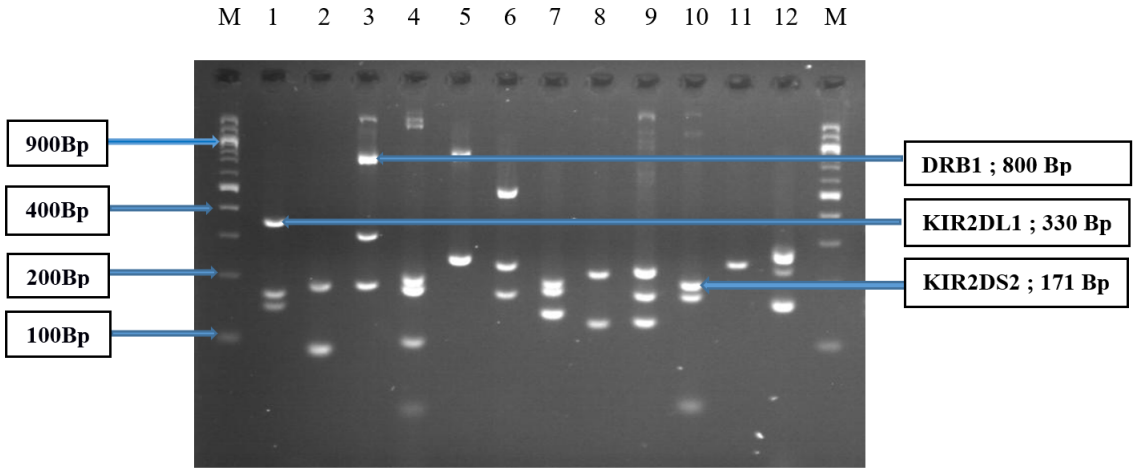

Figure 1: Gel electrophoresis of KIR genes PCR products.
M = DNA ladder 100 Bp. 1 - 12 = subset of 16
KIR genes, the interpretation of the gel was made based on the method described by Kulkarni (2010) [25].

Patients with viral load test results below the threshold should be considered as having suppressed viral loads but patients with a viral load greater than 1000 copies/mL after 12 months of treatment were defined as virological failures. Among HIV infected patients 68.8% (91/145) had T CD4+ counts ≥ 500 cells/mm^{3}and we found that 92.4% (134/145) of patients had HIV-1viral load < 1000 copies/mL. In this report, 62.7% (91/145) of HIV-1 infected patients should be considered as having suppressed viral loads and an effective immune system (CD4 ≥ 500cells/mm^3 and VL < 1000 copies/mL). All HIV-1 infected patients had started antiretroviral therapy (**Table 2**).

The frequency of KIR genes in the study population are shown in the **Table 3.** A total of 279 subjects (145 HIV-1 infected patients and 134 seronegative individuals) were genotyped for the KIR genes. Framework genes *KIR2DL4*, *KIR3DL2,* and *KIR3DL3* were present in all individuals. In the study population, we found that the frequency of inhibitory KIR genes, such as *KIR2DL2* (OR = 4.41; 95% CI = 2.6-7.48; p < 0.001); and activating KIR genes such as *KIR2DS2* (OR = 4.76; 95% CI = 2.68-8.48; p < 0.001), *KIR2DS3* (OR = 2.27; 95% CI = 1.29-3.97;p = 0.004), *KIR2DS4* (OR = 1.76; 95% CI = 1.07-2.89 ; p = 0.026), *KIR3DS1* (OR = 2.43; 95% CI = 1.18-5.01; p = 0.016) were more frequent in HIV-1 patients group than control subjects suggesting that these genes were associated with HIV-1 infection. In contrast, the inhibitory KIR gene *KIR3DL1* (OR = 0.39; 95% CI = 0.23-0.68; p < 0.001) was more frequent in the control subjects than HIV-1 infected patients group suggesting that *KIR3DL1* was associated with protection against HIV-1 infection (**Table 3**).

Table 4 shown the impact of KIR genes in HIV-1 infected patients according to the CD4$^+$ T cells counts. Lymphocytes T CD4$^+$ cells counts were stratified according to the Centers for Diseases Control and Prevention criteria. A total of 54 (37.2%) HIV-1 infected patients had CD4$^+$ T counts < 500 cells/mm^3 and 91 (68.8%) had CD4$^+$ T counts ≥ 500 cells/mm^3. A statistically significant difference was found when we compared KIR genes frequencies in patients with CD4$^+$counts < 500 cells/mm^3 and those with CD4$^+$counts ≥ 500 cells/mm^3, the pseudogene *KIR2DP1* (OR = 0.39; 95% CI = 0.18-0.85; p = 0.026) was more frequent in HIV-1 infected patients with CD4$^+$ T counts ≥ 500 cells/mm^3, suggesting that *KIR2DP1* was associated with protective status against T CD4 cell count decrease (**Table 4**).

The impact of KIR genes on the viral load of HIV-1 infected patient was show in the **Table 5.** The baseline viral load level for failure or therapeutic success is 1000 copies/mL in accordance with WHO Consolidated Guidelines for the Use of Antiretroviral Drugs for the Treatment and Prevention of HIV Infections in 2016 [26]. A total of 134 (92.4%) HIV-1 infected patients had HIV-1viral load < 1000 copies/mL and 11(7.6%) had HIV-1viral load ≥ 1000 copies/mL. A statistically significant difference was found when we compared KIR genes frequencies in patients with HIV-1 viral load < 1000 copies/mL and those HIV-1 viral load ≥ 1000 copies/mL, *KIR2DS2* gene (OR = 6.08 ; 95% CI = 1.26-29.22 ; p = 0.024) was less frequent in HIV-1 infected patients with HIV-1 viral load ≥ 1000 copies/mL, suggesting that *KIR2DS2* was associated with a higher risk to have HIV-1 viral load ≥ 1000 copies/mL.

Table 1: Sociodemographic characteristics of the study population.

Variables		HIV-1+ *n* (%)	Controls *n* (%)	Total *n* (%)	OR	95% CI	p-value
Gender							
#	**Male**	44 (30.30)	60 (44.80)	104 (37.30)			
	Female	101 (69.70)	74(55.20)	175(62.70)	**1.86**	**1.13-3.04**	**0.013***
Age (years)							
#	**≤39**	27(18.60)	70 (52.20)	97(34.77)			
	≥40	118 (81.40)	64 (47.80)	182(65.23)	**4.78**	**2.79-8.18**	< 0.001*
Serological Status							
	HIV+	145 (100)	0 (0.00)	145 (100)	-	-	
	HIV-	0 (0.00)	134 (20.0)	134 (100)	-	-	
	HBV-/HCV-	145(51.45)	134 (48.55)	279 (100)	-	-	

*Analysis by chi-square to obtain odds ratio values (OR) and confidence interval; HIV+: patients group with HIV-1 infection, HIV-/HBV-/HVC-: controls group without HIV-1; HBV and HCV infection CI: confidence interval; OR: odds ratio; #: reference; *: significant difference between groups (p < 0.05).*

Table 2: Immunological and virological parameters of HIV-1 patients.

Variables	CD4 < 500	≥ 500	VL < 1000	VL ≥ 1000	CD4 ≥ 500 and VL < 1000	CD4 < 500 and VL ≥ 1000
HIV-1+	54 (37.2)	91 (68.8)	134(92.4)	11(7.6)	91 (62.7)	11(7.6)

CD4 (Cells/mm3): lymphocyte T CD4, CD4 count was stratified according to the Centers for Diseases Control and Prevention criteria. VL (copies/mL): HIV-1 patients Viral Load.

Discussion

From our knowledge, this is the first study to assess the association between KIR genes and HIV-1 infection and disease progression in patients from Burkina Faso. Human immunodeficiency virus (HIV) remains a public health concern in sub-Saharan Africa. Decades of the fight against HIV and the availability of antiviral therapy in Africa have helped decrease the incidence and mortality of the disease. Despite all of these efforts, recent increases in new HIV cases have been reported in the continent [2]. Thereby, it is crucial to push towards , development of surrogates strategies to prevent a new HIV pandemic in this part of the world.

We conducted this pilot study to determine whether or not frequencies of KIR genes are associated with resistance or susceptibility to HIV infection in Burkina Faso. Ultime goal is to use is to use these findings to develop novel approaches to improve the clinical management of HIV patients.

The study population included 62.7% women and 37.30% men. In the HIV-1 positive group women were predominant with 69.70% **(Table 1).** Due to the high proportion of women in HIV-1 infected patients, certain studies suggested that women had an increased risk of being infected by HIV than men. According to World Health Organization, women are more likely to be infected with HIV in any type of sexual intercourse than men because of biological factors, the mucosal areas exposed during sexual intercourse are larger in women than in men [27]. Moreover, some, countries such as Burkina Faso, through the prevention of mother to child program of HIV infection, HIV test for all pregnant women is recommended. This could explain the high proportion of HIV-1 infected women. The proportion of HIV-1 infected patients aged ≥ 40 years were high than HIV-1 infected patients aged ≤ 39 years (81.40% versus 18.60%). Decreasing in HIV-related mortality since the introduction of combination antiretroviral therapy has resulted in increased life expectancy and an aging HIV-

Table 3: Frequency of KIR genes in HIV-1 patients and controls subjects.

KIR GENES n (%)		HIV-1+	Controls	Crude OR (95% CI)	p-value
Inhibitors					
KIR2DL1	-	52(35.9)	50(37.3)	1	
	+	93(64.1)	84(62.7)	1.06(0.65-1.73)	0.801
KIR2DL2	-	67(46.2)	106(79.1)	1	
	+	78(53.8)	28(20.9)	**4.41**(2.6-7.48)	**< 0.001**
KIR2DL3	-	73(50.3)	72(53.7)	1	
	+	72(49.7)	62(46.3)	1.15(0.72-1.83)	0.572
KIR2DL4	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
KIR2DL5A	-	77(53.1)	84(62.7)	1	
	+	68(46.9)	50(37.3)	1.48(0.92-2.39)	0.106
KIR2DL5B	-	70(48.3)	74(55.2)	1	
	+	75(51.7)	60(44.8)	1.32(0.82-2.12)	0.246
KIR3DL1	-	55(37.9)	26(19.4)	1	
	+	90(62.1)	108(80.6)	**0.39**(0.23-0.68)	**< 0.001**
KIR3DL2	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
KIR3DL3	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
Activators					
KIR2DS1	-	97(66.9)	88(65.7)	1	
	+	48(33.1)	46(34.3)	0.95 (0.58-1.56)	0.829
KIR2DS2	-	79(54.5)	114(85.1)	1	
	+	66(45.5)	20(14.9)	**4.76** (2.68-8.48)	**< 0.001**
KIR2DS3	-	97(66.9)	110(82.1)	1	
	+	48(33.1)	24(17.9)	**2.27** (1.29-3.97)	**0.004**
KIR2DS4	-	42(29.0)	56(41.8)	1	
	+	103(71.0)	78(58.2)	**1.76** (1.07-2.89)	**0.032**
KIR2DS5	-	94(64.8)	84(62.7)	1	
	+	51(35.2)	50(37.3)	0.91 (0.56-1.49)	0.71
KIR3DS1	-	117(80.7)	122(91.0)	1	
	+	28(19.3)	12(9.0)	**2.43** (1.18-5.01)	**0.016**
Pseudogene					
KIR2DP1	-	35(24.1)	40(29.9)	1	
	+	110(75.9)	94(70.1)	1.33(0.78-2.27)	0.344

+ = Presence of KIR gene; - = Absence of KIR gene; HIV-1= Human Immunodeficiency Virus type 1

Table 4: Impact of KIR gene on CD4 T cells count.

KIR GENE n (%)		< 500 cells/mm³	≥ 500 cells/mm³	Crude OR (95% CI)	p-value
Inhibitors					
KIR2DL1	-	20 (37.0)	32 (35.2)	1	
	+	34 (63.0)	59 (64.8)	0.92 (0.46-1.86)	0.82
KIR2DL2	-	27 (50.0)	40 (44.0)	1	
	+	27 (50.0)	51 (56.0)	0.78 (0.4- 1.54)	0.481
KIR2DL3	-	25 (46.3)	48(52.7)	1	
	+	29 (53.7)	43 (47.3)	1.29 (0.66-2.54)	0.453
KIR2DL4	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
KIR2DL5A	-	28 (51.9)	49 (53.8)	1	
	+	26 (48.1)	42 (46.2)	1.08 (0.55-2.13)	0.816
KIR2DL5B	-	28 (51.9)	42 (46.2)	1	
	+	26 (48.1)	49 (53.8)	0.8 (0.41-1.56)	0.507
KIR3DL1	-	19 (35.2)	36 (39.6)	1	
	+	35 (64.8)	55 (60.4)	1.21 (0.6-2.43)	0.6
KIR3DL2	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
KIR3DL3	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
Activators					
KIR2DS1	-	36 (66.7)	61 (67.0)	1	
	+	18(33.3)	30 (33,0)	1.02 (0.5-2.08)	0.964
KIR2DS2	-	30 (55.6)	49 (53.8)	1	
	+	24 (44.4)	42 (46.2)	0.93 (0.47-1.84)	0.842
KIR2DS3	-	39 (72.2)	58 (63.7)	1	
	+	15 (27.8)	33 (36.3)	0.68 (0.32-1.41)	0.295
KIR2DS4	-	14 (25.9)	28 (30.8)	1	
	+	40 (74.1)	63 (69.2)	1.27 (0.6-2.7)	0.535
KIR2DS5	-	33 (61.1)	61 (67.0)	1	
	+	21 (38.9)	30 (33.0)	1.29 (0.64-2.61)	0.471
KIR3DS1	-	45 (83.3)	72 (79.1)	1	
	+	9 (16.7)	19 (20.9)	0.76 (0.32-1.82)	0.535
Pseudogene					
KIR2DP1	-	19 (35.2)	16 (17.6)	1	
	+	35(64.8)	75 (82.4)	**0.39** (0.18-0.85)	**0.026**

+ = Presence of KIR gene; - = Absence of KIR gene; T CD4 = Lymphocyte T CD4

positive population [28]. Individuals aged ≥ 40 years in the study population were 4.78 times more likely to be infected by HIV-1 compared to individuals aged ≤ 39 years (**Table 1).** Serological testings for HIV, HBV and HCV were performed in controlled subjects; HBV and HCV tests were performed in HIV positive patients to rule out possible cases of infection with these viruses.

In accordance with WHO Consolidated Guidelines for the Use of Antiretroviral Drugs for the Treatment and Prevention of HIV Infections in 2016 [26], 62.70% of HIV-1 infected patients ($CD4^+$ ≥ 500 cells/mm^3 and viral load < 1000 copies/mL) should be considered as having suppressed viral load and 7.60% of patients ($CD4^+$ < 500 cells/mm^3 and viral load ≥ 1000 copies/mL) were defined as virological failure (**Table 2).** The therapeutic failure could be due to virus factors and/or host-genetic factors that could potentially modulate cellular susceptibility to HIV replication. HIV preferentially infects immune cells altering host ability to mount a potent immune response against opportunistic infections which lead to patient death. Thus, understanding how host-genetic factors and the innate immunity interact will be of crucial importance to better understand disease progression and improve patient health.

Our group has previously show that host genetic factors some studies in Burkina Faso have shown that host genetic factors could confer susceptibility or protection against HIV infection, Kagoné *et al.* (2014) in their study suggested a protective role of a variation of *DC-SIGN* promoter and genetic resistance to HIV-1 in serodiscordant couples, Compaore *et al. (*2016) demonstrate that some *APOBEC3G* variants were associated with HIV-1 infection [6, 7].

Natural Killer cells are known to play an important role by acting as the first line of protection against cells infected by virus and tumor cells. Killer cell immunoglobulin receptors regulate the Natural Killer cells cytotoxicity activity in the innate response against viral infections by interacting with their corresponding HLA class I molecules (ligands) express on target cells.

In the present study, we analyzed the relationship between KIR genes and HIV-1 infection and disease progression in Burkina Faso. The comparison between KIR genes frequencies in HIV-1 infected group and control group showed that inhibitory gene *KIR2DL2* and activators *KIR2DS2, KIR2DS3, KIR2DS4, KIR3DS1* were associated with HIV-1 infection. On the contrary, the comparison showed that the inhibitory gene *KIR3DL1* was associated with protection against HIV-1 infection (**Table 3**). Zwolinska *et al.* (2016) identified *KIR2DL3* gene as hallmark of protective immunity against HIV-1 infection and found that *KIR2DL5* and *KIR2DL2* might favor disease initiation and spread as well as increased risk of HIV-1 infection in women [22] however, our investigation suggests that *KIR2DL2* is associated with HIV-1 infection in both men and women. Conversely, in Zimbabwe, a study found a significant association between *KIR2DL2* and resistance to HIV infection [29] .Other investigations have determined that *KIR2DL2*, *KIR2DL5*, *KIR2DS5*, and *KIR2DS2* genes are associated with a reduced risk of mother-to-child transmission in HIV-1 positive Kenyan mothers [30]. In concordance with these findings, we found that *KIR2DS2* gene is associated with HIV-1 infection. The implication of certain inhibitory KIR genes in HIV-1 infection could be explained by the presence presence of their ligands HLA class I on targets cells, infected cells lacking the specific HLA class I molecules of inhibitory KIR gene on their surfaces, are recognized and destroyed by lysis activating receptors. Concerning the implication of certain activators KIR genes in HIV-1 infection, we could explain this by the presence of specific inhibitory KIR gene ligands on target cells or the lack of specific activators KIR gene ligands on targets cells, which could prevent them from destroying infected cells by lysis activating program. To evade the innate immune response, HIV-1 is able to increase the level of expression of HLA-C molecules, main inhibitory receptor ligands [31].

When we grouped the KIR gene frequencies according to CDC staging of CD4+ count (**Table 4**) and HIV-1 viral load according to WHO Consolidated Guidelines for the Use of Antiretroviral Drugs for the Treatment and Prevention of HIV Infections in 2016 **(Table 5),** we found that *KIR2DP1* was associated with protective status against T CD4 cells count decrease and *KIR2DS2* was associated with an higher risk to have HIV-1 viral load ≥ 1000 copies/mL.

Gaudieri *et al.* (2005) in Australia, found that the *KIR2DL2* and *KIR2DS2* genes were specifically associated with a rapid decrease in CD4+ count and rapid progression to AIDS [32]. However, Chavan *et al.* (2014) in India, found no association between KIR genes and CD4+ count [33]. Aditi *et al.* (2015) found that instead of being silent spectators, pseudogenes are differentially expressed in HIV-1 infection. While non-coding RNAs are now considered integral to the host response to viral infections, these findings indicate for the first time that RNAs derived from pseudogene transcripts are a part of the cellular non-coding RNA pool, and may regulate expression of genes implicated in viral infections [34]. Mechanisms use by *KIR2DP1* to protect lymphocyte TCD4 lyse must be studied. According to Kumud *et al.* (2016) in US, *KIR2DS2* gene was linked to high viral replication in children under age of 2 years [35] and Merino *et al.*(2014)

Table 5: Impact of KIR gene on HIV-1 viral load.

KIR GENE n (%)		VL < 1000 (copies/mL)	VL ≥ 1000 (copies/mL)	Crude OR (95% CI)	p-value
Inhibitors					
KIR2DL1	-	45 (33.6)	7 (63.6)	1	
	+	89 (66.4)	4 (36.4)	0.29 (0.08-1.04)	0.057
KIR2DL2	-	63 (47.0)	4 (36.4)	1	
	+	71 (53.0)	7 (63.6)	1.55 (0.43-5.55)	0.499
KIR2DL3	-	69 (51.5)	4 (36.4)	1	
	+	65 (48.5)	7 (63.6)	1.86 (0.52-6.64)	0.341
KIR2DL4	-	0(0.00)	0(0.00)	-	-
	+	145(100)	134(100)	-	-
KIR2DL5A	-	73 (54.5)	4 (36.4)	1	
	+	61 (45.5)	7 (63.6)	2.09 (0.59-7.49)	0.256
KIR2DL5B	-	66 (49.3)	4 (36.4)	1	
	+	68 (50.7)	7 (44.78)	1.7 (0.47-6.07)	0.415
KIR3DL1	-	51 (38,1)	4 (36.4)	1	
	+	83 (61.9)	7 (63.6)	1.08 (0.3-3.86)	0.911
KIR3DL2	-	0(0.00)	0(0.00)	-	-
	+	134(100)	11(100)	-	-
KIR3DL3	-	0(0.00)	0(0.00)	-	-
	+	134(100)	11(100)	-	-
Activators					
KIR2DS1	-	91 (67.9)	6 (54.5)	1	
	+	43 (32.1)	5 (45.5)	1.76 (0.51-6.1)	0.37
KIR2DS2	-	77 (57.5)	2 (18.2)	1	
	+	57 (42.5)	9 (81.8)	**6.08**(1.26-29.22)	**0.024**
KIR2DS3	-	89 (66.4)	8 (72.7)	1	
	+	45 (33.6)	3 (27.3)	0.74(0.19-2.93)	0.67
KIR2DS4	-	40 (29.9)	2 (18.2)	1	
	+	94 (70.1)	9 (81.8)	1.91 (0.4-9.26)	0.419
KIR2DS5	-	88 (65.7)	6 (54.5)	1	
	+	46 (34.3)	5 (45.5)	1.59 (0.46-5.5)	0.461
KIR3DS1	-	107 (79.9)	10 (90.9)	1	
	+	27 (20.1)	1 (9.1)	0.4 (0.05-3.23)	0.387
Pseudogene					
KIR2DP1	-	31 (23.1)	4 (36.4)	1	
	+	103 (76.9)	7 (63.6)	1.89 (0.52-6.91)	0.461

+ = Presence of KIR gene; - = Absence of KIR gene; VL = Viral Load

in US found that *KIR2DS4* gene was relatively associated with an increase in HIV-1 viral load [36]. Several studies have shown that KIR genes and HLA class I molecules can act in synergistically to affect HIV-1 progression in adults [33, 37]. This study provided useful clinical information that will improve the clinical management of HIV in Burkina. However, the main limitations of this study are that only KIR genes characterization, but not KIR/HLA combination, the non-detection of CCR5-Δ32 mutation and the seronegative individuals in our study were non exposed non-infected individuals. Thus, further studies are in dire need to fully comprehend the association between KIR genes and HIV infection, which will help improve outcomes of HIV patients in Burkina.

Conclusion

Our results suggest that *KIR2DL2*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR3DS1* genes were associated with HIV-1 infection. While *KIR3DL1* gene was associated with protection against HIV-1 infection. *KIR2DS2* gene was associated with HIV-1viral replication and the pseudogene *KIR2DP1* was associated with the protection against CD4+ count decrease. Our findings might be useful for predicting the precision medicine in HIV-1 patients on ART therapy. However, additional investigations on KIR/HLA interactions are needed to understand the molecular mechanisms governing KIR genes involvement to HIV infection in Burkina Faso.

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