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Original Article

Insights into the Interplay between KIR Gene Frequencies and Chronic HBV Infection in Burkina Faso

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Abstract. ***Background/Objective:*** **Hepatitis B virus (HBV) infection is the leading risk factor for cirrhosis and hepatocellular carcinoma (HCC).The objective of this investigation was to assess the association between "Killer Cell Immunoglobulin-Like Receptor" (KIR) gene frequencies and chronic HBV infection.**
Methods: **Chronic HBV carriers and healthy patients were selected for this study. The viral load for HBV were performed, and SSP-PCR was used to characterize the frequencies of KIR genes.**
Results: **The study suggested that inhibitory genes KIR2DL2 (crude OR = 2.82; p < 0.001), KIR2DL3 (crude OR = 2.49; p < 0.001) and activator gene KIR2DS2 (crude OR = 3.95; p< 0.001) might be associated with chronic stages of HBV infection. Conversely the inhibitory genes KIR3DL1 (crude OR = 0.49; p = 0.0018) and KIR3DL2 (crude OR = 0.41; p = 0.005), the activator gene KIR2DS1 (crude OR = 0.48; p = 0.014) and the pseudo gene KIR2DP1 (crude OR = 0.49; p = 0.008) could be associated with immunity against HBV infection. Chronic HBV patients who are carriers for the KIR3DL3 gene (crude OR = 8; p = 0.048) were positive for HBeAg and patients who carried the KIR3DL2 gene (crude OR = 3.21; p = 0.012) had a high HBV viral load compared to the rest of the study population.**
Conclusion: **Our data showed evidence of a correlation between the risk of developing chronic HBV infection and certain KIR gene frequencies and also show that KIR3DL1, KIR3DL2, KIR2DS1 might confer a protective status against chronic HBV infection.**

Keywords: KIR, HBV, Chronic Hepatitis B, Burkina Faso.

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Introduction. Worldwide, chronic hepatitis B virus (HBV) infection is the leading cause of cirrhosis and hepatocellular carcinoma (HCC). Several factors might influence disease progression such as mixed infection or co-infection with other HBV genotypes or sub-genotypes, hepatitis C (HCV) and host immunity. To date, there is no accurate method to identify high risk groups for cirrhosis and HCC in Sub-Saharan Africa. Hepatitis B virus (HBV) infection is a major life-threatening disease in resource limited areas where access to vaccination, serological screening, and patient monitoring are daily challenges. According to the World Health Organization[1] in 2017, approximately 257 million people are suffering from chronic HBV infection (http://www.who.int/mediacentre/factsheets/fs204/en/). Also, every year roughly 1 million will succumb to chronic HBV (http://www.hepb.org/what-is-hepatitis-b/what-is-hepb/facts-and-figures/).[1] Chronic hepatitis C virus (HCV) infection is the most common risk factor for developing HCC in Western countries, but in contrast, both chronic HBV and hepatitis C (HCV) are highly prevalent in sub-Saharan Africa, resulting in about a quarter of all HCC cases worldwide. Sub-Saharan Africa and East Asia have the highest prevalence with about 6.2% of the adult population infected.[1-5] West Africa is a highly endemic region for HBV infection; the most common route of infection is a vertical transmission from mother to child followed by sexual intercourse in the adult population. The limited data on HBV epidemiology in Burkina Faso displayed a spatial distribution of HBV prevalence from 9% in Ouagadougou and Bobo-Dioulasso (Central and Western areas) to 14.4% in Fada N'gourma (Eastern area).[6-10] The persistence of chronic HBV infection is the main cause for developing liver cirrhosis and HCC, although, much remains to be learned on the molecular mechanisms of HBV pathogenesis. The progression of HBV infection to its chronic stages is associated with a complex interplay between the virus and its host. In host immunity, viral and epigenetic factors play a key role in the outcomes of chronic infection,[11,12] and in some cases, the infected host immune system can manage to suppress the virus. However, immune evasion strategies allow viral particles to escape immune clearance, as a consequence of the evolution of both the immune system and viral epitopes mutations.[13,14] Natural Killer (NK) cells are cytotoxic lymphocytes, major components of innate immunity that play an important role in the immune-mediated rejection process of virally infected cells and tumor cells.[15] Furthermore, NK cells function by secreting cytokines that will, in turn, modulate the immune response of the host against viral infection and aberrant cells by activating the adaptive immune effectors such as dendritic cells and T lymphocytes.[16] The human KIR gene locus is located on chromosome 19q13.4 in the Leukocyte Receptor Complex (LRC) and encodes approximately 15 KIR genes and two pseudo genes (2DP1, 3DP1).[17,18] These genes are divided in inhibitor genes (KIR3DL3, KIR2DL2, KIR2DL3, KIR2DL5B, KIR2DL1, KIR3DL1, KIR2DL5A, KIR3DL2) and activator genes (KIR2DS2, KIR2DS3, KIR3DS1, KIR2DS5A, KIR2DS5B, KIR2DS4, KIR2DS1); KIR2DL4 gene that can act as either an activator or inhibitor.[17,19] KIR receptors are glycoproteins found on the surface of NK cells involved in the activation or inhibition of the interactions between NK cells and the molecules of the Major Histocompatibility Complex (MHC) class I.[20,21] Healthy cells expressing MHC class I proteins are protected through inhibitory mechanisms that prevent their lysis by “self-recognition”, whereas cells infected by viruses and cancer cells lacking the MHC class I molecules on their surfaces are destroyed by lysis activating receptors.[22] KIR receptors are named according to the number of extracellular immunoglobulin domains they carry (2D or 3D) and the length of their cytoplasmic tail which can be long or short (L or S).The presence of a long cytoplasmic tail with two Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) confers inhibitory activity to inhibitory KIRs (2DL, 3DL) and the presence of a short cytoplasmic tail confers activating activity to KIR activators (2DS, 3DS).[23,24] KIR genes have been divided into two haplotypes A and B depending on the presence of specific genes. The latest haplotype definition has identified that 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). Previous studies have shown the involvement of KIR genes in the pathogenesis of some diseases such as type 1 diabetes mellitus,[25,26] hepatitis C virus infection[27,28] and perinatal transmission of HIV infection.[29,30] In China, a study has shown that the KIR2DS2, KIR2DS3 genes were associated with chronic hepatitis B virus infection, and the KIR2DS1, KIR3DS1, KIR2DL5 genes were considered protective genes facilitating HBV viral clearance.[31] Another report from Turkey showed that KIR2DL3 and KIR3DS1 genes could protect the host against infection with HBV.[32] Only one study has been conducted in West Africa and showed that Gambian carriers for the KIR3DS1 gene had a high risk of being positive for HBeAg as well as carrying a high HBV viral load, while KIR2DL3 gene carriers had low viral load.[33] In Burkina Faso, there are no reports on the association between the KIR gene frequencies and chronic carriage of HBV. Therefore, our pilot study sought to assess the interplay between the KIR gene frequencies and chronic HBV infection in the population of Burkina Faso and to identify which genes are strongly correlated to the risk of developing CHB. Better management of chronic HBV patient could prevent the rapid onset of liver cirrhosis and hepatocellular carcinoma in the Burkinabe population.

Material and Methods.

Ethical considerations. This investigation was approved by the National Health Ethic Committee of Burkina Faso (reference number No2017-01-004). Patients' written and informed consents were obtained according to the Helsinki Declarations. All results were used as parameters in the therapeutic management of patients.

Type and population of the study. This was a prospective study conducted from January to September 2017. A total of 244 individuals aged 18 and over and divided into two groups were included in this study. The first group consisted of 110 carriers for chronic HBV (HBsAg positive > six months) recruited at the Pietro Annigoni Biomolecular Research Center (CERBA / LABIOGENE). The second group included 134 negative controls subjects for HBV, HCV, and HIV recruited at the Regional Blood Transfusion Center of Ouagadougou (CRTS / O).

Samples collection and measurement of HBV, HCV and HIV viral markers. Chronic HBV patient blood samples were collected in dry and EDTA tubes and stored in the Infectious Diseases Research Unit of CERBA. Control blood samples were collected from healthy volunteer 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 cobas e 411 Analyzer (Roche Diagnostics GmbH Mannheim Germany) according to the manufacturer's protocol. After centrifugation at 3,500 rpm for 10 min, plasma was recovered for determination of HBV viral load and blood pellet for KIR gene research. The serum of HBV-positive patients was used to screen blood markers of HBV (HBsAg; HBeAg; anti HBe-Ab); using the HBV One Step Hepatitis B Virus Combo Test Kit (Abon Biopharm Guangzhou, Co., Ltd. China).

Extraction of viral DNA and Determination of HBV viral load. Viral DNA was extracted from 200 µL of plasma using the PureLink® Genomic DNA Extraction Kit (Life Technologies, CA USA) according to the manufacturer's protocol. The DNA samples were stored at -20°C until further analysis. Plasma viral load was determined using the 7500Fast Real Time PCR system (Applied Biosystems, USA) using the Genesig HBV Real Time Quantitative Kit Primer design kit (Southampton, United Kingdom).

Genomic DNA Extraction and Determination of KIR Genes by SSP-PCR (Sequences Specific Primer). Genomic DNA was extracted from the whole blood using the salting-out method and stored at -80°C until analysis as previously described.[34] 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 12 targeted KIR genes using the SSP-PCR method as previously described.[35] 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 × CPR buffer, 2.25 µL MgCl2; 0.6 µL of dNTPs and 0.375 µL of Platinum™ DNA Taq polymerase in nuclease-free water.[35] The PCR reactions were performed as follows: 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 sec (65°C and 60°C) or 1 min (55°C for 4 cycles step), and extension at 30 sec 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 apparatus (Gene Flash syngenge Bio Imaging, USA). PCR products were validated against a positive internal control corresponding to the DRB1 gene fragment.

Statistical analysis. Standard Statistical Package for Social Sciences (SPSS) version 20.0 was used for data analysis and interpretation. Changes were considered statistically significant at $p \leq 0.05$, using the Fisher Exact test. Odds ratio (OR) and confidence intervals (CI) at 95% were calculated to estimate the associations between the KIR gene frequencies and HBV chronic infection using Epi Info 7.

Results.

Sociodemographic characteristics of the study population. The study population comprises a total of 244 people (110 chronic HBV patients and 134 control subjects) aged from 18 to 73, with an average age of 34.75 ± 11.57. The mean age of the HBV patients was 38.55 ± 13.07 years and 31.62 ± 9.11 years in the control group. The percentage of men was 48.77% (119/244) and 51.23% (125/244) for women with a sex ratio of 0.95. The most heavily represented age groups in the study population were 25 to 39 years old with 51.23% (125/244) and the difference was statically significant (p = 0.017). There were slightly more men [53.64% (59/110)] infected with chronic HBV than women [46.36% (51/110)], as shown in **table 1**.

Biochemical and virologic features of chronic HBV patients. Liver function was assessed using the measurement of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme levels, and hepatitis B infection was determined by measurements of viral envelope antigen (HBeAg) and antibody (anti-HBeAb) levels. We tested HBsAg in all HBV chronic patients to confirm chronic HBV infection, and HIV, HBV, HCV tests were performed in controlled subjects to rule out possible cases of infection with these viruses. Viral load was below 2000 IU/ml for 57.3%, (63/110) of patients, and greater than 2000 IU/ml in 42.7% (47/110) of patients (**Table 2**). 17.27%, (19/110) of the patients were positive for HBeAg and negative for anti-HBeAb, suggestive of replicating virus, while 82.73% (91/110) of patients were negative for HBeAg and positive for anti-HBeAg, indicating chronic infection. In chronic hepatitis B patients who had HBeAg positive, 94.7% or (18/19) had a viral load greater than or equal to 2000 IU/mL compared to 5.3% (1/19) who had a viral load less than 2000 IU/mL. The univariate analysis showed that HBeAg is strongly associated with an

Table 1. Sociodemographic characteristics of the study population.

VARIABLES	cHBV	CONTROLS	TOTAL	p-value
	N=110(%)	N=134(%)	244(%)	
Sex				
Male	59 (53.64)	60 (44.78)	119 (48.7)	0.211
Female	51 (46.36)	74 (55.22)	125 (51.23)	
Age (years)				
<25	11(10)	38 (28.36)	49(20.08)	
25-39	55 (50)	70 (52.24)	125 (51.23)	0.017
>39	44(40)	26(19.40)	70(28.70)	
Serological Status				
HBV+	110 (100)	0 (0.0)	110 (100)	-
HBV-	0 (0.0)	134(100)	134 (100)	-
HIV+/HCV+	0 (0.0)	0(0.0)	0 (0.0)	-
HIV-/HCV-	110(45.08)	134(54.92)	244(100)	-

cHBV= chronic HBV.

Table 2. Biochemical and virologic characteristics of cHBV patients.

	Viral Load (IU/mL)		Univariate		Multivariable	
	<2000	≥2000	crude OR (95%CI)	crude P value	adj. OR (95%CI)	adj. P value
HBeAg						
negative	62 (68.1)	29 (31.9)	Ref.		Ref.	
positive	1 (5.3)	18 (94.7)	38.48 (4.90-302.33)	< 0.001	31.1 (3.66-264.65)	0.002
Trans (IU/mL)						
ALAT ≤40	57 (75.0)	19 (25.0)	Ref.		Ref.	
ALAT >40	6 (17.6)	28 (82.4)	14 (5.03-38.95)	< 0.001	12.02 (3.99-36.17)	< 0.001

increased viral load (Crude OR = 38.48, p < 0.001) and a multivariate age-sex-matched analysis supports that HBeAg is associated with increased HBV viral load (Adj -OR = 31.1, p = 0.002). A total of 34 patients had alanine aminotransferase (ALT) levels greater than 40 IU/mL, and 76 patients had ALT levels less than or equal to 40 IU/mL. Among patients who had ALT levels greater than 40 IU/mL, 82.4% (28/34) had a viral load greater than or equal to 2000 UI/mL versus 17.6% (6/34) who had a viral load less than 2000 IU/mL. Univariate analysis shows that elevated ALT levels are associated with increased HBV viral load (crude OR = 14, p < 0.001), a multivariate age-sex-matched analysis supports the assumption that elevation of ALT levels is associated with high values of HBV viral load (Adj -OR = 12.02, p < 0.001).

Characterization of KIR genes by SSP-PCR. A total of 16 KIR genes were determined by PCR-SSP. Our results shown the different frequencies of KIR genes between chronic HBV patients and controls subjects in this study (**Table 3**). Thus a univariate analysis shown that KIR genes such as KIR2DL2 (crude OR = 2.82 , 95%CI =1.61-4.96 , p < 0.001), KIR2DL3 (crude OR = 2.49 , 95%CI = 1.47-4.21 , p < 0.001) and KIR2DS2 (crude OR = 3.95 , 95%CI = 2.15-7.27, p < 0.001) were more frequent in chronic hepatitis B carriers than in control subjects however the KIR genes such as KIR3DL1 (crude OR = 0.49 , 95%CI = 0.28-0.89, p = 0.0018) and KIR3DL2 (crude OR = 0.41, 95%CI = 0.22-0.77, p = 0.005); KIR2DS1 (crude OR = 0.48, 95%CI = 0.27-0.86, p = 0.014) and the pseudo gene KIR2DP1 (crude OR = 0.49, 95%CI = 0.29-0.83, p = 0.008) were more frequent in controls than in chronic hepatitis B carriers (**Table 3**). An association has been found between the KIR genes and the viral replication marker of HBV, the univariate analysis shown that the carriers of the KIR3DL3 gene are likely to be HBeAg positive (crude OR = 8, 95%CI = 1.02-62.91, p = 0.048) (**Table 4**). We found a high frequency of the KIR3DL2 gene in chronic HBV patients who had a viral load greater than or equal to 2000 IU/mL, the univariate analysis shows that the KIR3DL2 gene is associated with the increase in the viral load HBV (crude OR = 3.21, 95% CI = 1.29-7.99, p = 0.012) (**Table 5**).

Discussion. Our study consisted in characterizing for the first time in a population of Burkina Faso, the KIR genes. The choice of the chronic hepatitis B population and negative blood donors for the HIV, HCV and HBV test allowed us to have a fairly representative sample of the general population of Burkina Faso, a country endemic to Hepatitis B[1] with a prevalence of almost 15%.[6-9,36,37] In addition, this choice is in the wake of studies that seek to understand how genetic factors and cells of the innate immune system are involved in cases of virus infection.[38-40] In Burkina Faso, a significant proportion of patients with chronic hepatitis B (cHBV) acquired the infection early in life through mother-to-child transmission.[41,42] The average age of our study population was 34.75±11.57 years. The most represented age group in our study population was 25 to 39 (51.23%) (p=0.017). In this group, they were a large number of women who were diagnosed following an antenatal check-up.

We have found that the presence of the viral replication marker is associated with viral loads greater than or equal to 2000 IU/mL (crude OR=38.48; p<0.001). As in others studies that have demonstrated that HBeAg detection is associated with hepatitis B virus replication;[43,44] a multivariate analysis adjusted for age and sex shows an association between HBeAg and the

Table 3. Frequency of KIR genes in chronic HBV patients and controls subjects.

KIR GENES **n (%)**	cHBV	CONTROL	crude OR (95%CI)	crude P value
Inhibitors KIR2DL1				
-	30 (27.3)	50 (37.3)	Ref.	
+	80 (72.7)	84 (62.7)	1.59 (0.92-2.74)	0.097
KIR2DL2				
-	63 (57.3)	106 (79.1)	Ref.	
+	47 (42.7)	28 (20.9)	2.82 (1.61-4.96)	< **0.001**
KIR2DL3				
-	35 (31.8)	72 (53.7)	Ref.	
+	75 (68.2)	62 (46.3)	2.49 (1.47-4.21)	< **0.001**
KIR2DL4				
-	33 (30.0)	36 (26.9)	Ref.	
+	77 (70.0)	98 (73.1)	0.86 (0.49-1.5)	0.589
KIR2DL5A				
-	70 (63.6)	84 (62.7)	Ref.	
+	40 (36.4)	50 (37.3)	0.96 (0.57-1.62)	0.878
KIR2DL5B				
-	61 (55.5)	74 (55.2)	Ref.	
+	49 (45.5)	60 (44.8)	0.99 (0.60-1.64)	0.971
KIR3DL1				
-	36 (32.7)	26 (19.4)	Ref.	
+	74 (67.3)	108(80.6)	0.49 (0.28-0.89)	**0.018**
KIR3DL2				
-	33 (30.0)	20 (14.9)	Ref.	
+	77 (70.0)	114(85.1)	0.41 (0.22-0.77)	**0.005**
KIR3DL3				
-	29 (26.4)	34 (25.4)	Ref.	
+	81 (73.6)	100(74.6)	0.95 (0.53-1.69)	0.86
Activators KIR2DS1				
-	88 (80.0)	88 (65.7)	Ref.	
+	22 (20.0)	46 (34.3)	0.48 (0.27- 0.86)	**0.014**
KIR2DS2				
-	65 (59.1)	114(85.1)	Ref.	
+	45 (40.9)	20 (14.9)	3.95 (2.15-7.25)	< **0.001**
KIR2DS3				
-	92 (83.6)	110(82.1)	Ref.	
+	18 (16.4)	24 (17.9)	0.9 (0.46-1.75)	0.75
KIR2DS4				
-	43 (39.1)	56 (41.8)	Ref.	
+	67 (60.9)	78 (58.2)	1.12 (0.67-1.87)	0.669
KIR2DS5				
-	66 (60.0)	84 (62.7)	Ref.	
+	44 (40.0)	50 (37.7)	1.12 (0.67-1.88)	0.668
KIR3DS1				
-	98 (89.1)	122(91.0)	Ref.	
+	12 (10.9)	12 (9.0)	1.24 (0.54-2.89)	0.611
Pseudogene KIR2P1				
-	51 (46.4)	40 (29.9)	Ref.	
+	59 (53.6)	94 (70.1)	0.49 (0.29-0.83)	**0.008**

+ = Presence of KIR gene; - = Absence of KIR gene; cHBV= Chronic Hepatitis B.

Table 4. KIR genes and Viral Markers.

KIR GENES n (%)	HBeAg-	HBeAg+	crude OR (95%CI)	crude P value
Inhibitors KIR2DL1				
-	25 (27.5)	5 (26.3)	Ref.	
+	66 (72.5)	14 (73.7)	1.06 (0.35-3.25)	0.918
KIR2DL2				
-	53 (58.2)	10 (52.6)	Ref.	
+	38 (41.8)	9(47.4)	1.26 (0.47-3.39)	0.653
KIR2DL3				
-	30 (33)	5 (26.3)	Ref.	
+	61 (67)	14 (73.7)	1.38 (0.45-4.18)	0.572
KIR2DL4				
-	30 (33)	3 (15.8)	Ref.	
+	61 (67)	16(84.2)	2.62 (0.71-9.7)	0.148
KIR2DL5A				
-	58(63.7)	12(63.2)	Ref.	
+	33(36.3)	7(36.8)	1.03 (0.37- 2.86)	0.962
KIR2DL5B				
-	49(53.8)	12(63.2)	Ref.	
+	49 (46.2)	7(36.8)	0.68 (0.25,1.89)	0.459
KIR3DL1				
-	32(35.2)	4(21.1)	Ref.	
+	59(64.8)	15 (78.9)	2.03 (0.62-6.64)	0.24
KIR3DL2				
-	31(34.1)	2(10.5)	Ref.	
+	60(65.9)	17 (89.5)	4.39 (0.95-20.24)	0.058
KIR3DL3				
-	28(30.8)	1(5.3)	Ref.	
+	63(60.2)	18(94.7)	8 (1.02,62.91)	**0.048**
Activators KIR2DS1				
-	72(79.1)	16 (82.4)	Ref.	
+	19(20.9)	3 (15.8)	0.71 (0.19-2.69)	0.615
KIR2DS2				
-	57 (62.6)	8(42.1)	Ref.	
+	34 (37.4)	11(57.9)	2.31 (0.84-6.3)	0.103
KIR2DS3				
-	75 (82.4)	17 (89.5)	Ref.	
+	16 (17.6)	2(10.5)	0.55 (0.12-2.63)	0.455
KIR2DS4				
-	39 (42.9)	4 (21.4)	Ref.	
+	52 (57.1)	15(78.9)	2.81 (0.87-9.14)	0.085
KIR2DS5				
-	53 (58.2)	13(68.4)	Ref.	
+	38(41.8)	6(31.6)	0.64 (0.22-1.85)	0.412
KIR3DS1				
-	80 (87.9)	18 (94.7)	Ref.	
+	11 (12.1)	1 (5.3)	0.4 (0.05-3.33)	0.4
Pseudogene KIR2P1				
-	44(48.4)	7(36.8)	Ref.	
+	47(51.6)	12(63.2)	1.6 (0.58-4.45)	0.363

+ = Presence of KIR gene ; - = Absence of KIR gene ; HbeAg+ = presence of HBeAg ; HBeAg- = absence of HBeAg

Table 5. KIR gene frequency and viral Load.

KIR GENES n (%)	VL< 2000 IU/mL	VL ≥ 2000 IU/mL	crude OR (95%CI)	crude P value
Inhibitors KIR2DL1				
-	17 (27.0)	13 (27.7)	Ref.	
+	46 (73.0)	34 (72.3)	0.97 (0.41-2.26)	0.937
KIR2DL2				
-	37 (58.7)	26 (55.3)	Ref.	
+	26 (41.3)	21(44.7)	1.15 (0.54-2.47)	0.721
KIR2DL3				
-	22 (34.9)	13 (27.7)	Ref.	
+	41 (65.1)	34 (72.3)	1.4 (0.62-3.19)	0.419
KIR2DL4				
-	23(36.5)	10(21.3)	Ref.	
+	40(63.4)	37(78.7)	2.13 (0.89-5.06)	0.088
KIR2DL5A				
-	43(68.3)	27 (57.4)	Ref.	
+	20(31.7)	20 (42.6)	1.59 (0.73-3.49)	0.245
KIR2DL5B				
-	35(55.6)	26(55.3)	Ref.	
+	28 (44.4)	21(44.7)	1.01 (0.47-2.16)	0.98
KIR3DL1				
-	22(34.9)	14 (29.8)	Ref.	
+	41(65.1)	33(70.2)	1.26 (0.56-2.85)	0.571
KIR3DL2				
-	25 (39.7)	8 (17.0)	Ref.	
+	38(60.3)	39(83.0)	3.21 (1.29-7.99)	**0.012**
KIR3DL3				
-	21(33.3)	8 (17.0)	Ref.	
+	42(66.7)	39(89.0)	2.44 (0.97-6.14)	0.059
Activators KIR2DS1				
-	49 (77.8)	39 (83.0)	Ref.	
+	14 (22.2)	8 (17.0)	0.72 (0.27-1.88)	0.501
KIR2DS2				
-	41 (65.1)	24 (51.4)	Ref.	
+	22 (34.9)	23 (48.9)	1.79 (0.83-3.86)	0.141
KIR2DS3				
-	55 (87.3)	37 (78.7)	Ref.	
+	8 (12.7)	10 (21.3)	1.86 (0.67-5.15)	0.233
KIR2DS4				
-	29 (46.0)	14 (29.8)	Ref.	
+	34 (54.0)	33 (70.2)	2.01 (0.91-4.46)	0.086
KIR2DS5				
-	37(58.7)	29 (61.7)	Ref.	
+	26(41.3)	18(38.3)	0.88 (0.41-1.91)	0.753
KIR3DS1				
-	56(88.9)	42 (89.4)	Ref.	
+	7(11.1)	5 (10.6)	0.95 (0.28-3.21)	0.937
Pseudogene KIR2P1				
-	32 (50.8)	19 (40.4)	Ref.	
+	31(49.2)	28(59.6)	1.52 (0.71-3.27)	0.282

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

viral load greater than or equal to 2000 IU/mL (Adj-OR=31.1 p=0.002). Similarly, alanine aminotransferase (ALT) levels greater than or equal to 40 IU/mL in chronic HBV patients were associated with viral load values greater than or equal to 2000 IU/mL (crude OR=14; p< 0,001), adjusted for age and sex we found a significant association (Adj-OR=12.02 p<0.001).

The main limitation of our study is that we have characterized only KIR genes, but not the

KIR/HLA combination. The frequency of inhibitory genes KIR2DL2, KIR2DL3, and activator gene KIR2DS2 was high in chronic HBV patients than in control group while the frequency of inhibitory genes KIR3DL1, KIR3DL2, the activator gene KIR2DS1, and the pseudo gene KIR2DP1 were high in the control group than in chronic HBV patients. In our study, the KIR genes, KIR2DL2, KIR2DL3, KIR2DS2, are associated with HBV chronic infection, and KIR3DL1, KIR3DL2, KIR2DS2, KIR2P1 are associated with protection against chronic HBV infection. The KIR3DL3 gene was linked to the HBeAg positive status of patients. There was a statistically significant correlation between the presence of KIR3DL2 gene and a high viral load of HBV. A Chinese study suggested that the activating genes KIR2DS2 and KIR2DS3 genes could be associated with chronic HBV infection, which induced a persistent yet weak inflammatory reaction that results in continuous injury of live tissues on chronic hepatitis and that inhibitory genes KIR2DL5 and activators genes KIR2DS1, KIR3DS1 could protect, thus facilitate HBV viral immune clearance.[31] On the other hand, a Turkish study showed that the inhibitory gene KIR2DL3 and activator gene KIR3DS1 could be protective against HBV infection.[32] Alongside this Turkish study; Gao et al. (2010) found that the combination of KIR2DL3 and HLA-C1 conferred protection against HBV infection and that the combination KIR2DL1 and HLA-C2 could be associated with HBV infection;[45] Di Bona et al. (2017) found that KIR ligand group HLA-A-Bw4 and HLA-C2 are associated with HBV chronic infection. Subjects possessing these alleles are more susceptible to be HBV chronic carriers while KIR2DL3 confer protection against HBV chronic infection.[46]

In our study, patients who carried the KIR3DL3 gene were associated with HBeAg positive status which is a viral replication marker of HBV. Whereas, those who carried the inhibitory KIR3DL2 gene had a high HBV viral load. In the Gambia, a study showed that carriers of the KIR3DS1 gene were HBV-positive and had a high viral load while KIR2DL3 gene carriers had a low HBV viral load.[33] Genotypes and haplotypes containing more activator genes would play an essential role in chronic infection or elimination of HBV.[47] Combinations of KIR genes and HLA molecules are associated with the development of hepatocellular carcinoma in patients infected with chronic hepatitis B virus.[48]

Conclusion. This investigation showed that KIR inhibitory genes KIR2DL2, KIR2DL3, and KIR2DS2 activator are associated with chronic HBV infection, while the inhibitory genes KIR3DL1, KIR3DL2, the activator gene KIR2DS1, and the pseudo gene KIR2DP1 are associated with protection against chronic infection by HBV. Also, the KIR3DL3 gene was linked to the HBeAg positive status; while the KIR3DL2 gene was associated with the evolution of HBV viral load in the context of Burkina Faso. However, KIR/HLA studies combined with additional genotyping of HBV are needed to investigate the molecular mechanisms by which KIR genes contribute to the infection or elimination of the hepatitis B virus.

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