Expression of Killer Immunoglobulin Receptor Genes among HIV-Infected Individuals with Non-AIDS Comorbidities

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Abstract

Combined antiretroviral therapy (cART) increased the life expectancy of people living with HIV (PLHIV) and remarkably reduced the morbidity and mortality associated with HIV infection. However, non-AIDS associated comorbidities including diabetes, hypertension, hyperlipidemia, and cardiovascular diseases (CVD) are increasingly reported among PLHIV receiving cART. Killer cell immunoglobulin receptors (KIRs) expressed on the surface of natural killer (NK) cells have been previously implicated in controlling HIV disease progression. The aim of this study is to investigate the role of KIRs in developing non-AIDS associated comorbidities among PLHIV. Demographic and behavioral data were collected from voluntary participants using a standardized questionnaire. Whole blood samples were collected for KIR genotyping. Hypertension (29.5%) and hyperlipidemia (29.5%) followed by diabetes (23.7%) and CVD (9.7%) were mainly reported among our study participants with higher rate of comorbid conditions observed among participants > 40 years old. The observed KIR frequency (OF) was ≥90% for inhibitory KIR2DL1 and KIR3DL1, activating KIR2DS4 and the pseudogene KIR2DP1 among study participants. We detected significant differences in the expression of KIR3DS4 and KIR3DL1 (p = 0.038) between diabetic and nondiabetic and in the expression of KIR2DL3 between hypertensive and normotensive HIV-infected individuals (p = 0.047). Moreover, KIR2DL1 and KIR2DP1 were associated with significantly reduced odds of having CVD (OR 0.08; 95% CI: 0.01-0.69; p = 0.022). Our study suggests the potential role of KIR in predisposition to non-AIDS comorbidities among PLHIV and underscores the need for more studies to further elucidate the role of KIRs in this population.

1. Introduction

The use of combined antiretroviral therapy (cART) significantly reduced the morbidity and mortality associated with human immunodeficiency virus (HIV) infection [1]. The former contributed to an increase in the life expectancy of people living with HIV (PLHIV) approaching that of HIV-negative individuals [2–4]. Consequently, the global proportion of people aging with HIV was estimated to reach 21% [5]. These data suggest a demographic shift affecting HIV management and care. With the increasing proportion of people living and aging with HIV, non-AIDS (acquired immunodeficiency syndrome) comorbidities have been increasingly reported among treated PLHIV leading to an increased number of deaths exceeding those of AIDS-related deaths [6–9]. These comorbidities include cardiovascular disease (CVD) [10–15], liver disease [16, 17], renal disease [11, 14, 18], diabetes [10–13, 15, 19], and neurocognitive abnormalities [20, 21], as well as non-AIDS defining malignancies including liver, brain, anal, and lung cancers [22, 23]. While biological aging was suggested to start earlier among HIV infected individuals (55 vs. 65 years) [24], the subsequent pathway leading to disease manifestation among treated and aging PLHIV is not fully understood.

Killer cell immunoglobulin receptors (KIRs) are highly polymorphic type 1 transmembrane glycoproteins expressed on the surface of natural killer (NK) cells [25]. The balance
between the inhibitory and activating signals received from KIRs regulates the function of NK cells. Genes encoding KIRs are located in the leukocyte receptor complex (LRC) on chromosome 19q13.4 [26]. There are 16 recognized genes that encode for KIR of which 7 are activating (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5A, KIR2DS5B, and KIR3DS1), 8 are inhibitory (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, and KIR3DL3), and one (KIR2DL4) predominately activating but can transmit inhibitory signals [27]. Two KIR haplotypes are defined in humans: haplotype A and haplotype B [27, 28]. While haplotype A encodes inhibitory receptors (KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL3, KIR2DP1, KIR3DP1, and KIR2DL4) and one activating receptor KIR2DS4, haplotype B carries a variety of gene combinations and encodes more activating receptors compared to haplotype A. These include KIR3DL3, KIR2DS2, KIR2DL2, KIR2DL5B (inhibitory) KIR2DS3, KIR2DP1, KIR2DL1, KIR3DP1, KIR2DL4, KIR3DS1, KIR2DL5A (inhibitory), KIR2DS5, KIR2DS1, and KIR3DL2 [29].

Despite the high allelic polymorphism of KIR genes, almost all individuals possess the following genes referred to as framework loci: KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2 [27, 28]. Each full-length haplotype is characterized by a centrally located 10–15000 bp region containing a recombination hotspot [30]. The latter subdivides the haplotype into two regions, namely, proximal or centromeric (Cent) (5') and distal or telomeric (Tel) (3') regions which are bound by the framework loci. This recombination hotspot is thus the intermediate region between the centromeric and telomeric regions of the haplotype and bound by the four genes of the framework loci with KIR3DL3 and KIR3DP1 defining the centromere and KIR2DL4 and KIR3DL2 defining the telomere [31]. A centromeric B haplotype is defined by the presence of at least one of the following: KIR2DS2, KIR2DL2, KIR2DL5, KIR2DS3, or KIR2DS5 whereas a centromeric A haplotype by the sole presence of KIR2DL3. A telomeric B haplotype is defined by the presence of at least one of the following: KIR3DS1, KIR2DL5, KIR2DS3, KIR2DS5, or KIR2DS1; on the other hand, a telomeric A haplotype is defined by the presence of KIR3DL1 and/or KIR2DS4. Finally, a KIR group A genotype is defined by having centromeric A/A and telomeric A/A; other combinations were denoted KIR group B genotypes [32].

There is scarcity of data on the effect of aging on KIR expression. However, ample data exist on the association between KIR expression and susceptibility, severity, clinical course, or clearance of viral infections (H1N1 2009 influenza, hepatitis C virus, and hepatitis B virus) [33–38] as well as HIV. The proportion of homozygote KIR3DS1 was higher in HIV-exposed uninfected individuals compared to those with primary HIV infection [39], and higher frequencies of KIR3DS1 were associated with low viral load [40]. In addition, HIV-exposed individuals who carry homozygote KIR3DS1 without KIR3DL1 were characterized by a delayed seroconversion compared to exposed individuals with KIR3DL1/S1 heterozygous genotype [41]. Few studies reported the association between KIR gene polymorphism and susceptibility to type 1 diabetes mellitus (T1DM) [42, 43], hypertension [44], and acute ischemic stroke [45]. KIR2DL2 was suggested as a susceptibility factor whereas KIR2DL1 and KIR2DL3 as protective factors for T1DM. KIR2DL1 and KIR2DS1 were linked to a decreased risk of T1DM among Asians but not Caucasians [43]. Moreover, the expression of KIR2DS5 was suggested as protective against hypertension in a cohort of Chinese Han patients [44]. Higher frequencies of KIR2DL3, KIR2DL5B, KIR2DS2, and KIR2DS4 were reported among patients with ischemic stroke compared to healthy controls [45]. Similarly, the frequency of KIR2DL3 and KIR2DL4 was higher in patients with large artery atherosclerosis compared to controls [45].

The aim of this study is to investigate the relationship between KIR genes and non-AIDS comorbid conditions among a cohort of PLHIV in Lebanon. To our knowledge, there are no data on the relationship between KIR genes expressed among treated HIV-infected individuals and the risk of developing non-AIDS associated comorbid conditions.

2. Methods

2.1. Study Design and Ethical Approval. This study was conducted at the American University of Beirut (AUB). Voluntary participants were recruited from three recruitment sites: AUB-Medical Center (AUBMC), Lebanese American University Medical Center-Rizk Hospital (LAUMC-RH), and Soins Infirmiers et Developpement Communautaire (SICD)—a nongovernmental organization providing health services to HIV-infected individuals. Human subject approval was obtained for this study from the Institutional Review Board (IRB) of AUB and the Lebanese American University (LAU). All participants provided informed consent. A standardized questionnaire was administered to a total of 105 treated adult HIV-infected individuals between November 2018 and December 2019. Demographic and behavioral data including lifestyle, smoking, physical activity, substance use and abuse, coinfections, chronic diseases (cardiovascular disease, hypertension, diabetes, lipid and metabolic disorders, cancer, mental health, and others), first-degree family history (i.e., parents or siblings) of chronic diseases, polypharmacy data, and mental health data were collected.

2.2. DNA Extraction and KIR Gene Genotyping. Out of the 105 voluntary participants, 103 HIV-infected individuals provided whole blood samples between November 2018 and December 2019. DNA was extracted using the QIAamp DNA Blood Minikit (Qiagen, Germany) as per manufacturer’s instructions. The integrity of the extracted DNA was checked by gel electrophoresis, and its concentration was measured by NanoDrop 2000c (Thermo Fisher Scientific). The purified DNA was stored at -20°C. The Polymerase Chain Reaction– (PCR–) based KIR genotyping Sequence-specific Oligonucleotide Hybridization (SSO) Kit (One Lambda, Thermo Fisher, USA) was used to detect the presence and absence of 16 KIR genes, as per manufacturer’s instructions. Briefly, 2 μl of DNA at an adjusted concentration of 20 ng/μl was used along with the primer sets in a total volume of 20 μl per PCR reaction to amplify the alleles.
All amplifications were performed using SureCycler 8800 (Agilent Technologies) as per the manufacturer’s recommendation: 3-minute denaturation step at 96°C followed by 5 cycles of 96°C, 60°C and 72°C for 20 seconds each; 30 cycles of 96°C for 10 seconds, 60°C for 15 seconds, 72°C for 20 seconds; 1 cycle of final elongation step at 72°C for 10 minutes; and finally hold at 4°C. The presence and absence of the following gene loci and variants were determined: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DP1, and KIR3DP1. Data were acquired using Lumiplex LABScan 3D™ and analyzed using HLA Fusion™ Research Software Version 6.0.

2.3. Haplotype Classification and KIR Gene Frequencies. KIR haplotypes were defined as previously described [46]. Homozygous A haplotype (AA) was defined by the sole presence of KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4, KIR2DP1, KIR3DP1, KIR2DL4, KIR3DL2, and KIR3DL3 genes. Other KIR combinations were referred to as Bx haplotype (i.e., BB or BA). The frequency of KIR was calculated by direct count of the observed phenotype and referred to as observed frequency (OF). In addition, the estimated KIR gene frequency (KF) for the putative loci was calculated using the following formula: KF = 1 − √(1 − OF) based on the assumption of Hardy-Weinberg equilibrium [47]. We further analyzed participants with Bx genotype based on the expression of different KIR genes combinations. We numbered KIR combinations within the Bx genotype by a number (i.e., Bx1, Bx2, Bx3, etc.) and categorized these into centromeric and telomeric KIR clusters as previously described [30, 46].

The centromeric cluster consists of KIR2DS2, KIR2DL2, KIR2DL5B, and KIR2DS3 combination, while the telomeric half consists of KIR3DS1, KIR2DL5A, KIR2DS5, and KIR2DS1 genes. Bx genotype can exhibit centromeric KIR cluster (KIR2DS2, KIR2DL2, KIR2DL5B, and KIR2DS3 combination), telomeric KIR cluster (KIR3DS1, KIR2DL5A, KIR2DS5, and KIR2DS1 combination) or both [46]. In the analysis, we included the full length KIR2DL5 since we were unable to get the data pertinent to the frequency of KIR2DL5A and KIR2DL5B.

2.4. Statistical Analysis. We compared the frequency of expression of KIR genes and KIR haplotypes between individuals with and without non-AIDS associated comorbid conditions using Fisher’s exact test (FET). We performed clustering analysis to identify KIR clusters. We compared the frequency of the latter between individuals with and without comorbidities using FET. We also examined the relationship between KIR expression and clusters of KIR genes versus the presence or absence of non-AIDS-related comorbidities (specifically diabetes, hypertension, hyperlipidemia, and CVD) using multivariate logistic regression model while adjusting for associated risk factors. We corrected for multiple comparisons for post hoc tests using Bonferroni correction; thus, the calculated alpha was 0.05/12 = 0.004, whereby 12 is the total number of KIR genes included in the analysis. All analyses were conducted using STATA SE 13.0.

3. Results

3.1. Characteristics of Study Participants. The majority of our participants were 45-59 years (41.9%), males (82.9%) and heterosexuals (52.9%) (Table 1). The majority of our participants contracted HIV-1 and started cART more than 10 years ago (38.8%). The majority of our participants were 45-59 years (41.9%), males (82.9%), and predominantly heterosexuals (52.9%). The majority of our participants were 45-59 years (41.9%), males (82.9%), and predominantly heterosexuals (52.9%).

Table 1: Demographic and clinical characteristics of study participants.

| Characteristic                        | n   | %    |
|--------------------------------------|-----|------|
| Gender (n = 105)                     |     |      |
| Male                                 | 87  | 82.9 |
| Female                               | 18  | 17.1 |
| Age in years (n = 105)               |     |      |
| < 25                                 | 42  | 40.6 |
| 25-44                                | 44  | 41.9 |
| ≥ 45                                 | 19  | 18.1 |
| Sexual history (n = 102)             |     |      |
| Heterosexual                         | 54  | 52.9 |
| MSM                                  | 33  | 32.4 |
| Bisexual                             | 14  | 13.7 |
| HIV route of transmission (n = 105)  |     |      |
| Unprotected sex                      | 72  | 68.6 |
| Shared needle/syringe                | 7   | 6.7  |
| Others                               | 7   | 6.7  |
| Duration of HIV infection (n = 103)  |     |      |
| < 5 years                            | 9   | 10.3 |
| 5-10 years                           | 40  | 38.8 |
| > 10 years                           | 54  | 52.4 |
| Duration of cART (n = 105)           |     |      |
| < 5 years                            | 16  | 15.2 |
| 5-10 years                           | 42  | 40.6 |
| > 10 years                           | 47  | 44.8 |
| cART regimen (n = 102)               |     |      |
| NRTI + NNRTI                         | 47  | 46.1 |
| NRTI + INSTI                         | 47  | 46.1 |
| Others (PI + NRTI and/or NNRTI)      | 8   | 7.8  |
| ≥ 1 non-AIDS associated comorbidities (n = 105) | 44 | 41.9 |
| Hypertension                         | 31  | 29.5 |
| Hyperlipidemia                       | 31  | 29.5 |
| CVD                                  | 10  | 9.5  |
| Diabetes                             | 9   | 8.6  |
| Non-cART medication (n = 105)        |     |      |
| Antihypertensives                    | 31  | 29.5 |
| Lipid-lowering agents                | 31  | 29.5 |
| Hypoglycaemic agents                 | 9   | 8.6  |

MSM: men exclusively having sex with men; cART: combined antiretroviral therapy; NRTI: nucleoside/nucleotide reverse-transcriptase inhibitor; NNRTI: nonnucleoside reverse-transcriptase inhibitor; PI: protease inhibitor.
| Inhibitory KIR | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 2DS5 | 3DS1 | 2DP1 | 3DP1 |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| OF            | 95   | 52.4 | 89   | 100  | 64   | 90   | 100  | 100  | 44.7 | 52.4 | 34   | 90   | 40   | 43.7 | 95   | 100  |
| KLF           | 0.78 | 0.31 | 0.67 | 1    | 0.4  | 0.68 | 1    | 1    | 0.26 | 0.31 | 0.19 | 0.68 | 0.23 | 0.25 | 0.78 | 1    |

KIR: killer cell immunoglobulin receptor; OF: observed frequency calculated by direct counting; KLF: estimated KIR gene frequency calculated using the formula $1 - \sqrt{1 - \text{OF}}$. 

Table 2: The observed and estimated KIR gene frequencies among study participants.
Table 3: Distribution of KIR-gene profiles among study participants.

| Genotypes | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | N  | % (N = 103) |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|--------------|
| AA        | 2DL4 | 3DL1 | 2DS4 |      |      |      |      |      |      |      |      |      |      |      |     | 27 | 26.21        |
| Bx1       | 2DL4 |      |      | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 2   | 1.94         |
| Bx2       | 2DL4 |      |      | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DS2 | 2DL2 | 3DL3 |      |      |      | 3DP1 | 1   | 0.97         |
| Bx3       | 2DL4 | 3DL1 | 2DS4 |      |      |      |      |      |      |      |      | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 8   | 7.77         |
| Bx4       | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DS2 | 2DL2 | 3DL3 | 2DP1 | 2DL1 | 3DP1 |      | 7   | 6.8          |
| Bx5       | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DS2 | 2DL2 |      |      |      |      | 3DP1 |     | 2  | 1.94         |
| Bx6       | 2DL4 |      |      | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DL3 |      |      |      |      | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 4   | 3.88         |
| Bx7       | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 2   | 1.94         |
| Bx8       | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 5   | 4.85         |
| Bx9       | 2DL4 | 3DL1 | 2DS4 |      |      |      |      |      |      |      |      |      |      |      |      | 15 | 14.56        |
| Bx10      | 2DL4 | 3DL1 | 2DS4 |      |      |      |      |      |      |      |      |      |      |      |      |     | 3  | 2.91         |
| Bx11      | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 2   | 1.94         |
| Bx12      | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 16  | 15.53        |
| Bx13      | 2DL4 | 3DL1 | 2DS4 | 3DS1 | 2DS5 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 | 2DS3 | 3DL3 | 2DP1 | 2DL1 | 3DP1 | 3   | 2.91         |
| Bx14      | 2DL4 | 3DL1 | 2DS4 |      |      |      |      |      |      |      |      |      |      |      |      |     | 2  | 1.94         |
| Bx15      | 2DL4 | 3DL1 | 2DS4 | 3DS1 |      |      |      |      |      |      |      |      |      |      |      |      | 2   | 1.94         |
| Bx16      | 2DL4 | 3DL1 | 2DS4 | 3DS1 |      |      |      |      |      |      |      |      |      |      |      |      | 1   | 0.97         |
| Bx17      | 2DL4 | 3DL1 | 2DS4 | 2DS5 | 2DS1 | 3DL2 | 2DL3 | 2DS2 | 2DL2 |      |      |      |      |      | 3DP1 | 1   | 0.97         |

Gene presence or absence is represented by italic and empty boxes, respectively. Each number next to the genotype represents different KIR combination.
years ago. NRTI+NNRTI and NRTI+INSTI were the most commonly used drug regimens (Table 1). Forty-two percent of the study participants reported having one-to-four non-AIDS associated comorbidities with 29.5%, 29.5%, 9.5%, and 8.6% suffering from hypertension, hyperlipidemia, CVD, and diabetes, respectively (Table 1). Following the same trend, antihypertensive and lipid-lowering agents were the most commonly reported non-cART medications. Our results showed that the frequency of comorbid conditions increases with age among PLHIV; 19% of our participants above 40 years old reported one or two comorbid conditions (Table 4). On the telomeric side, the Tel-AA (53.4%), Tel-Bx2 (27.2%), and Tel-Bx1 (9.7%) were the most common profiles. We also detected higher frequency of Cent-B profiles (58%) than Tel-B profiles (48%) (Table 4).

3.3. KIR Genotypes and Non-AIDS Comorbid Conditions. For this analysis and thereafter, we studied the genes with enough variability (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, and KIR2DP1). We compared the frequency of KIR genes between participants with and without diabetes, hyperlipidemia, CVD, or hypertension. We detected the following significant differences: KIR3DL1 (inhibitory) and KIR2DS4 (activating) between diabetic and nondiabetic individuals (FET, \( p = 0.038 \)) and KIR2DL3 (inhibitory) between hypertensives and normotensives (FET, \( p = 0.047 \)) (Table 5). We also detected a significant difference among hypertensive males expressing KIR2DL3 (FET, \( p = 0.034 \)) (data not shown). Moreover, the expression of KIR2DL1 (FET, \( p = 0.043 \)), KIR2DP1 (FET, \( p = 0.043 \)), and KIR2DS3 (FET, \( p = 0.049 \)) was significantly

Table 4: Centromeric and telomeric gene profiles corresponding to combination of conventional KIR haplotypes.

(a)

| Genotype | KIR2DL3 | KIR2DS2 | KIR2DL2 | KIR2DS3 | KIR3DL3 | KIR2DP1 | KIR2DL1 | KIR3DP1 | N | % (N = 103) |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---|----------|
| AA       | KIR2DL3 |         |         |         |         |         |         |         | 47 | 45.63    |
| Bx1      | KIR2DS2 | KIR2DL2 | KIR2DS3 | KIR3DL3 | KIR2DP1 | KIR2DL1 | KIR3DP1 |         | 6  | 5.82     |
| Bx2      | KIR2DS2 | KIR2DL2 |         |         |         |         |         |         | 1  | 0.97     |
| Bx3      | KIR2DL3 | KIR2DS2 | KIR2DL2 |         |         |         |         |         | 16 | 15.53    |
| Bx4      | KIR2DS2 | KIR2DL2 |         |         |         |         |         |         | 4  | 3.88     |
| Bx5      | KIR2DL3 | KIR2DS2 | KIR2DL2 | KIR2DS3 | KIR3DL3 | KIR2DP1 | KIR2DL1 | KIR3DP1 | 27 | 26.21    |
| Bx6      | KIR2DL3 |         |         |         |         |         |         |         | 2  | 1.94     |

(b)

| Genotype | KIR2DL4 | KIR3DL1 | KIR2DS4 | KIR3DS1 | KIR2DS5 | KIR2DS1 | KIR3DL2 | N | % (N = 103) |
|----------|---------|---------|---------|---------|---------|---------|---------|---|----------|
| AA       | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 55 | 53.4     |
| Bx1      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 10 | 9.70     |
| Bx2      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 28 | 27.18    |
| Bx3      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 3  | 2.91     |
| Bx4      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 3  | 2.91     |
| Bx5      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 2  | 1.94     |
| Bx6      | KIR2DL4 | KIR3DL1 | KIR2DS4 |         |         |         |         | 2  | 1.94     |

Gene presence or absence is represented by italic and empty boxes, respectively. Each number next to the genotype represents different KIR combination.
|                | With diabetes (N = 9) | Without diabetes (N = 28) | p value | With hyperlipidemia (N = 31) | Without hyperlipidemia (N = 45) | p value | With CVD (N = 10) | Without CVD (N = 92) | p value | With hypertension (N = 31) | Without hypertension (N = 34) | p value |
|----------------|-----------------------|---------------------------|---------|-------------------------------|---------------------------------|---------|-------------------|----------------------|---------|-------------------------|---------------------------------|---------|
| **KIR genes**  |                       |                           |         |                               |                                 |         |                   |                      |         |                         |                                 |         |
| KIR2DL1        | 9 (25%)               | 27 (75%)                  | 1       | 29 (39.7%)                    | 44 (60.3%)                      | 0.563   | 8 (8.3%)          | 89 (91.7%)          | 0.074   | 27 (45%)                | 33 (55%)                          | 0.184   |
| KIR2DL2        | 5 (25%)               | 15 (75%)                  | 1       | 14 (35%)                      | 26 (65%)                        | 0.352   | 5 (9.4%)          | 48 (90.6%)          | 1       | 17 (53.1%)              | 15 (46.9%)                        | 0.46    |
| KIR2DL3        | 9 (26.5%)             | 25 (73.5%)                | 0.562   | 28 (41.2%)                    | 40 (58.8%)                      | 1       | 8 (8.8%)          | 83 (91.2%)          | 0.294   | 25 (43.1%)              | 33 (56.9%)                        | 0.047   |
| KIR2DL5        | 7 (28%)               | 18 (72%)                  | 0.687   | 20 (39.2%)                    | 31 (60.8%)                      | 0.805   | 5 (7.7%)          | 60 (92.3%)          | 0.49    | 21 (48.8%)              | 22 (51.2%)                        | 1       |
| KIR2DP1        | 9 (25%)               | 27 (75%)                  | 1       | 29 (39.7%)                    | 44 (60.3%)                      | 0.563   | 8 (8.3%)          | 89 (91.7%)          | 0.074   | 27 (45%)                | 33 (55%)                          | 0.184   |
| KIR2DS1        | 6 (31.6%)             | 13 (68.4%)                | 0.447   | 16 (42.1%)                    | 22 (57.9%)                      | 1       | 4 (8.9%)          | 41 (91.1%)          | 1       | 17 (53.1%)              | 15 (46.9%)                        | 0.46    |
| KIR2DS2        | 5 (25%)               | 15 (75%)                  | 1       | 14 (35%)                      | 26 (65%)                        | 0.352   | 5 (9.4%)          | 48 (90.6%)          | 1       | 17 (53.1%)              | 15 (46.9%)                        | 0.46    |
| KIR2DS3        | 4 (33.3%)             | 8 (66.7%)                 | 0.432   | 11 (42.3%)                    | 15 (57.7%)                      | 1       | 1 (2.9%)          | 33 (97.1%)          | 0.159   | 11 (55%)                | 9 (45%)                           | 0.591   |
| KIR2DS4        | 6 (18.2%)             | 27 (81.8%)                | 0.038   | *                             | 26 (38.2%)                      | 0.259   | 10 (10.9%)        | 82 (89.1%)          | 0.592   | 26 (45.6%)              | 31 (54.4%)                        | 0.463   |
| KIR2DS5        | 6 (33.3%)             | 12 (66.7%)                | 0.269   | 13 (39.4%)                    | 20 (60.6%)                      | 1       | 4 (10%)           | 36 (90%)            | 1       | 15 (51.7%)              | 14 (48.3%)                        | 0.622   |
| KIR3DL1        | 6 (18.2%)             | 27 (81.8%)                | 0.038   | *                             | 26 (38.2%)                      | 0.259   | 10 (10.9%)        | 82 (89.1%)          | 0.592   | 26 (45.6%)              | 31 (54.4%)                        | 0.463   |
| KIR3DS1        | 6 (35.3%)             | 11 (64.7%)                | 0.251   | 16 (44.4%)                    | 20 (55.6%)                      | 0.642   | 3 (6.8%)          | 41 (93.2%)          | 0.508   | 16 (55.2%)              | 13 (44.8%)                        | 0.324   |
| **KIR haplotypes** |                       |                           |         |                               |                                 |         |                   |                      |         |                         |                                 |         |
| AA             | 1 (10%)               | 9 (90%)                   | 0.393   | 8 (47.1%)                     | 9 (52.9%)                       | 0.585   | 3 (11.1%)         | 24 (88.9%)          | 0.722   | 7 (39%)                 | 11 (61%)                          | 0.418   |
| Bx             | 8 (29.6%)             | 19 (70.4)                 | 23 (39%) | 36 (61%)                      | 7 (9.3%)                        | 68 (90.2%) | 24 (51.1%)        | 23 (48.9%)          |         |                         |                                 |         |

CVD: cardiovascular disease. Significant p values (p < 0.05) of Fisher’s exact test are indicated by (*) and italic font.
different among participants with CVD who were >40 years of age. We detected a borderline significant difference ($p = 0.053$) in the expression of $KIR2DL1$ and $KIR2DP1$ among males with CVD (data not shown). Moreover, there was no significant difference in the expression of Bx or AA haplotypes among participants with or without any of the conditions above (Table 5). Collectively, these results suggest that $KIR3DL1$ and $KIR2DS4$ as well as $KIR2DL3$ can be associated with diabetes and hypertension, respectively, among people living and aging with HIV. These data also suggest that $KIR2DL1$, $KIR2DP1$, and $KIR2DS3$ are significantly associated with CVD among participants >40 years in our cohort.

We then analyzed in a multivariate analysis the relationship between KIR genes expression and the risk of having non-AIDS comorbidities. We adjusted for age, sex, and family history for the respective comorbid condition; for hypertension and CVD, we also adjusted for smoking and alcohol use. We applied Bonferroni correction, and thus, $p$ values of <0.004 were considered significant.

### 3.4. KIR Clusters and Comorbidities

Based on the KIR genes clustering analysis, we identified five KIR clusters: Cluster 1, Cluster 2, Cluster 3, Cluster 4, and Cluster 5 (Figure 1). Cluster 1 corresponds to the telomeric cluster containing $KIR3DS1$, $KIR2DS1$, and $KIR2DS5$. We detected significant difference in the expression of Cluster 4 between diabetic and non-diabetic participants ($p = 0.038$) and in the expression of Cluster 5 between hypertensives and normotensives.

#### Table 6: The relationship between KIR genes and non-AIDS associated comorbid conditions.

| KIR genes | Diabetes OR (95% CI) | p value | Hyperlipidemia OR (95% CI) | p value | Hypertension OR (95% CI) | p value | CVD OR (95% CI) | p value |
|-----------|---------------------|---------|---------------------------|---------|-------------------------|---------|----------------|---------|
| Inhibitory KIRs | | | | | | | | |
| KIR2DL1 | 1 | — | 0.3 (0.01-4.8) | 0.362 | 0.1 (0.01-1.78) | 0.129 | 0.08 (0.01-0.69) | 0.022 |
| KIR2DL2 | 1.1 (0.19-6.65) | 0.886 | 0.5 (0.16-1.4) | 0.179 | 1.3 (0.4-4.29) | 0.664 | 1.04 (0.25-4.31) | 0.954 |
| KIR2DL3 | 1 | — | 0.7 (0.12-4.29) | 0.721 | 0.08 (0.006-0.99) | 0.05 | 0.2 (0.03-1.46) | 0.113 |
| KIR2DL5 | 2.3 (0.3-17.48) | 0.422 | 1.3 (0.42-3.97) | 0.651 | 1.9 (0.51-7.12) | 0.341 | 0.6 (0.13-2.6) | 0.476 |
| KIR3DL1 | 0.14 (0.01-2.05) | 0.151 | 0.4 (0.08-2.06) | 0.272 | 0.6 (0.1-3.01) | 0.493 | 1 | — |

- Activating KIRs
- Pseudogenes

CVD: cardiovascular disease; OR: odds ratio; 95% CI: 95% confidence interval. We adjusted for age, sex, and family history of comorbid condition. For hypertension and CVD, we also adjusted for smoking and alcohol use. We applied Bonferroni correction, and thus, $p$ values of <0.004 were considered significant.

![Figure 1: Clustering analysis of KIR genes among study participants.](image-url)
Table 7: KIR cluster analysis.

| Cluster | With diabetes (N = 9) | Without diabetes (N = 28) | p value | With hyperlipidemia (N = 31) | Without hyperlipidemia (N = 45) | p value | With CVD (N = 10) | Without CVD (N = 92) | p value | With hypertension (N = 31) | Without hypertension (N = 34) | p value |
|---------|----------------------|---------------------------|---------|-----------------------------|-------------------------------|---------|-----------------|------------------------|---------|--------------------------|-------------------------------|---------|
| Cluster 1* | Yes | 6 (37.5%) | 10 (62.5%) | 0.136 | 13 (41.9%) | 18 (58.1%) | 1 | 3 (8.1%) | 34 (91.9%) | 0.744 | 14 (53.8%) | 12 (46.2%) | 0.456 |
|         | No  | 3 (14.3%) | 18 (85.7%) |         | 18 (60%) | 27 (60%) | 7 (10.8%) | 58 (89.2%) | 17 (43.6%) | 22 (56.4%) |                     |                  |         |
| Cluster 2 | Yes | 5 (25%) | 15 (75%) | 1 | 14 (35%) | 26 (65%) | 0.352 | 5 (9.4%) | 48 (90.6%) | 1 | 17 (53.1%) | 15 (46.9%) | 0.46 |
|         | No  | 4 (23.5%) | 13 (76.5%) |         | 17 (47.2%) | 19 (52.8%) | 5 (10.2%) | 44 (89.2%) | 14 (42.4%) | 19 (57.6%) |                     |                  |         |
| Cluster 3 | Yes | 4 (33.3%) | 8 (66.7%) | 0.432 | 11 (42.3%) | 15 (57.7%) | 1 | 1 (2.9%) | 33 (97.1%) | 0.159 | 11 (55%) | 9 (45%) | 0.591 |
|         | No  | 5 (20%) | 20 (80%) |         | 20 (40%) | 30 (60%) | 9 (13.2%) | 59 (86.8%) | 20 (44.4%) | 25 (55.6%) |                     |                  |         |
| Cluster 4 | Yes | 6 (18.2%) | 27 (81.8%) | 0.038* | 26 (38.2%) | 42 (61.8%) | 0.259 | 10 (10.9%) | 82 (89.1%) | 0.592 | 26 (45.6%) | 31 (54.4%) | 0.463 |
|         | No  | 3 (75%) | 1 (25%) |         | 5 (62.5%) | 3 (37.5%) | 0 (0%) | 10 (100%) | 5 (62.5%) | 3 (37.5%) |                     |                  |         |
| Cluster 5 | Yes | 9 (26.5%) | 25 (73.5%) | 0.562 | 28 (41.2%) | 40 (58.8%) | 1 | 8 (8.8%) | 83 (91.2%) | 0.294 | 25 (43.1%) | 33 (56.9%) | 0.047* |
|         | No  | 0 (0%) | 3 (100%) |         | 3 (37.5%) | 5 (62.5%) | 2 (18.2%) | 9 (81.8%) | 6 (85.7%) | 1 (14.3%) |                     |                  |         |
| Centromeric cluster | Yes | 4 (33.3%) | 8 (66.7%) | 0.432 | 9 (37.5%) | 15 (62.5%) | 0.804 | 1 (10%) | 31 (33.7%) | 0.165 | 10 (52.6%) | 9 (47.4%) | 0.758 |
|         | No  | 5 (20%) | 20 (80%) |         | 22 (42.3%) | 30 (57.7%) | 9 (8.8%) | 61 (59.8%) | 21 (45.6%) | 25 (54.4%) |                     |                  |         |

CVD: cardiovascular disease. *Cluster 1 is the same as telomeric cluster. Significant p values (p < 0.05) of Fisher’s exact test are indicated by (*) and italic font.
with age being a major risk factor. Consequently, more hypertension was predominantly reported among a small duration of HIV infection as associated risk factors. This study identified older age, male gender, higher BMI, and prolonged duration of HIV infection as associated risk factors. Recently, diabetes mellitus followed by dyslipidemia and hypertension was predominantly reported among a small group of HIV-infected individuals in western Saudi Arabia with age being a major risk factor. Consequently, more studies are needed to determine the prevalence of non-AIDS associated comorbid conditions among PLHIV in the MENA region.

We observed high frequency of KIR2DS4, KIR2DL1, KIR2DP1, and KIR3DL1 genes among our study participants. Our results were similar to previously reported data in the region specifically among healthy Lebanese [48, 57], Iranian [58, 59], and Turkish adults [60]. Similar results were also reported in Southern Brazil [61, 62] and Eastern Han populations in China [63]. Moreover, the AA genotype profile was the most frequent among our study participants similar to previously reported data in healthy unrelated individuals in Lebanon [57], Turkey [60], Iran [58], and Tunisia [64].

Several studies reported on KIR genes expressions among individuals with chronic conditions such as diabetes [42], hypertension [44], malignancies (colorectal cancer [65], biliary cancer [32], breast cancer [66], and leukemia [67, 68]), and primary immunodeficiency disorders such as common variable immunodeficiency (CVID) [69]. While the frequency of KIR genes did not differ between diabetics and healthy controls among the Chinese Han [70], Basque [71], and Saudi populations [72], the expression of KIR2DL3 was significantly different among British HIV-naïve children (<5 years) with type 1 diabetes compared to healthy controls [73]. We report a significant difference in the frequency of KIR2DS4 and KIR3DL1 between diabetic and non-diabetic HIV-positive participants. A recent meta-analysis showed that the expressions of KIR2DL1, KIR2DL2, and KIR2DL5 were significantly associated with susceptibility to T1DM [42]. KIR2DL2 was associated with increased risk of type 1 diabetes whereas KIR2DL1 and KIR2DL5 decreased the risk of the latter. However, we did not detect any significant difference in the expression of these genes between diabetics and non-diabetics among our study participants. While our results showed a significant difference in the frequency of KIR2DL3 between hypertensive and normotensive HIV-positive individuals, Wang et al. reported a significant association between the expression of KIR2DS5 and decreased risk of hypertension [44]. While our data did not reveal any association between the expression of KIR genes and

### Table 8: The relationship between KIR clusters and non-AIDS associated comorbid conditions.

| KIR clusters | Diabetes OR (95% CI) | p value | Hyperlipidemia OR (95% CI) | p value | Hypertension OR (95% CI) | p value | CVD OR (95% CI) | p value |
|--------------|---------------------|---------|-----------------------------|---------|-------------------------|---------|----------------|---------|
| Cluster 1*   | 2.7 (0.44-16.45)    | 0.283   | 1.27 (0.45-3.58)            | 0.649   | 1.7 (0.52-5.82)         | 0.373   | 0.8 (0.18-3.6) | 0.786   |
| Cluster 2    | 1.14 (0.19-6.65)    | 0.886   | 0.5 (0.16-1.4)              | 0.179   | 1.3 (0.4-4.29)          | 0.664   | 1.04 (0.25-4.31) | 0.954   |
| Cluster 3    | 2.4 (0.39-15.53)    | 0.34    | 1.2 (0.41-3.54)             | 0.715   | 1.6 (0.43-5.68)         | 0.501   | 0.2 (0.02-1.83) | 0.154   |
| Cluster 4    | 0.14 (0.01-2.04)    | 0.151   | 0.4 (0.08-2.06)             | 0.272   | 0.6 (0.1-3)             | 0.493   | 1              |         |
| Cluster 5    | 1                   |         | 0.7 (0.12-4.29)             | 0.721   | 0.08 (0.006-0.99)       | 0.05    | 0.2 (0.03-1.46) | 0.113   |
| Centromeric  | 2.4 (0.39-15.43)    | 0.34    | 0.9 (0.29-2.63)             | 0.82    | 1.2 (0.32-4.37)         | 0.811   | 0.2 (0.02-2.13) | 0.195   |

CVD: cardiovascular disease; OR: odds ratio; 95% CI: 95% confidence interval. *Cluster 1 is the same as telomeric cluster. We adjusted for age, sex, and family history of comorbid condition. For hypertension and CVD, we also adjusted for smoking and alcohol use. We applied Bonferroni correction, and thus, p values of <0.004 were considered significant.

(p = 0.047) (Table 7). We did not detect any significant difference between centromeric (Cent-A and Cent-B) and telomeric (Tel-A and Tel-B) clusters and any comorbid condition (data not shown). The multivariate analysis revealed that individuals expressing Cluster 5 have significantly reduced odds of hypertension (OR 0.08; 95% CI: 0.006-0.99; p = 0.05) (Table 8).

### 4. Discussion

PLHIV are at higher risk of developing non-AIDS associated comorbidities than the general population; moreover, the prevalence of these comorbidities among PLHIV increases with age [6, 8, 11]. Our data showed that the frequency of comorbid conditions was higher among HIV-positive individuals > 40 years compared to those ≤40 years of age (Abou Hassan et al. submitted manuscript). In accordance with previous reports from our group (Hammad et al. accepted manuscript JIDC) and worldwide [14, 49–52], the most commonly reported comorbidities among our study participants were hypertension and hyperlipidemia followed by CVD and diabetes.

A limited number of studies exists on the prevalence of chronic disease conditions in the Lebanese population. Recently, the prevalence of hypertension among Lebanese adults (n = 2014) was reported at 31%. The former was higher among older participants and those with higher body mass index (BMI) or reported to have CVD [53]. The prevalence of diabetes mellitus in Lebanon (n = 17,832) was reported at 7.95%. The prevalence of type 1 diabetes was estimated at 0.1% [54]. Studies on the prevalence of comorbidities among PLHIV in the Middle East and North Africa (MENA) region are currently lacking except for reports from Iran [55] whereby hyperglycemia was recently reported to be highly prevalent among a cohort of PLHIV. This study identified older age, male gender, higher BMI, and prolonged duration of HIV infection as associated risk factors [55]. Recently, diabetes mellitus followed by dyslipidemia and hypertension was predominantly reported among a small group of HIV-infected individuals in western Saudi Arabia with age being a major risk factor [56]. Consequently, more
risk of non-AIDS comorbidities, this could be due to ethnicity, sample size, and the population under study. Previous studies compared the frequency of KIR genes between individuals with a chronic condition and healthy subjects, while we compared the expression of KIR genes among HIV-infected subjects with and without non-AIDS comorbid conditions.

Despite the increased expression of centromeric and telomeric KIR clusters among hypertensive participants, there was no significant association between these clusters and non-AIDS comorbid conditions. This is probably due to our small sample size. A recent study in China showed that centromeric KIR cluster (KIR2DS2-2DL2-2DS3-2DL5) was significantly increased in children with B-cell acute lymphoblastic leukemia (B-ALL) compared to healthy controls and provided the first evidence that this gene cluster might increase the susceptibility to B-ALL in Chinese Han children [74]. This result suggests that KIR gene clusters might be a predisposing factor for susceptibility to diseases and warrants further investigation. To our knowledge, the relationship between KIR clusters among PLHIV and non-AIDS comorbid conditions was not previously explored.

Our study has several limitations. Our study lacks a control group of HIV-negative individuals; thus, we were unable to compare the frequencies of KIR genes between our cohort and HIV-naïve individuals. Our study is a cross-sectional study without historic clinical and medical data to assess the evolution of comorbid conditions and pertinent risk factors across time. Moreover, we were unable to investigate the interaction between KIRs and their putative human leukocyte antigen class I (HLA-I) ligand. The latter has been implicated in controlling HIV-disease progression [75–79] and in the control or the progression of other viral diseases including human influenza virus, viral hepatitis (HCV and HBV), and human cytomegalovirus (HCMV) [26, 77].

5. Conclusion

Additional studies with larger populations are needed to elucidate the role of KIRs in susceptibility or resistance to non-AIDS comorbid conditions among PLHIV. Importantly, the burden of non-AIDS comorbidities among people living and aging with HIV is critically needed in the region. These studies are important for the proper management and care of comorbid conditions among PLHIV. Understanding the molecular mechanisms governing the genetic factors modulating living and aging with HIV should be prioritized for screening and intervention to prevent and mitigate multimorbidities among people living and aging with HIV.

Data Availability

The clinical, biological, and demographic data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

FFA performed laboratory experiments, data collection, and analysis and wrote part of the manuscript. MBH performed data analysis and contributed to writing. KA guided and contributed to the analysis. NMM designed the study, oversaw all aspects of the study, edited, and finalized the manuscript. All authors reviewed, edited, and approved the final manuscript.

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