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Association between the killer cell immunoglobulin-like receptor a haplotype and childhood acute lymphoblastic leukemia

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ABSTRACT

Killer immunoglobulin-like receptors (KIRs) have the ability to regulate natural killer (NK) cell function through inhibition/activation mechanisms. Healthy human cells express HLA class I ligands on their surface, which are recognized by NK cells to avoid spontaneous cell destruction. The associations of KIRs and/or HLA class 1 ligands in leukemic patients have been studied in some populations, with some of these studies demonstrating an association of specific types with leukemia. KIRs and their corresponding HLA class 1 ligands were investigated in Saudi patients with ALL and AML and compared to healthy controls. The homozygous A haplotype was found significantly more often in ALL patients ≤18 years-old than in control individuals. No significant association was observed in KIRs and their corresponding HLA ligands in this study.

1. Introduction

Natural killer (NK) cells are lymphocytes that comprise approximately 10% of the total lymphocytes in peripheral blood circulation and are considered as a prototypical member of the group 1 innate lymphoid cells (ILCs) that are characterized by their capacity to produce interferon-γ and their functional dependence on the transcription factor T-bet. NK cells play a crucial role in recognizing and killing cancer/virally infected cells. Ninety percent of mature peripheral NK cells are a cytotoxic $CD56^{dim}$ and $CD16^{+}$ subset that express killer cell immunoglobulin-like receptors (KIRs) on their surface during late maturation [1–3]. KIRs have the ability to regulate NK cell function through inhibition/activation mechanisms. The action of killing is a dynamic process between activation and inhibition receptors governed by a fine balance of signals termed "licensing" due to interactions between KIRs and corresponding HLA class 1 ligands on the target cells [4–6].

Healthy human cells express HLA class I ligands on their surface, which are recognized by NK cells to avoid spontaneous cell destruction. Failure to recognize the suitable HLA ligand will activate NK cells to kill their target [7,8]. However, some evidence has proven that NK cells may act against tumor cells through stress molecules, the ligand for the C-type NKG2D. HLA class I-specific ligands include HLA-C group 1 (C1), HLA-C group 2 (C2) and HLA-Bw4 (Bw4) motifs that span residues 77–83 of the α1 domain, but HLA-C is the most important ligand for NK cell regulation [9]. KIR genes are located at chromosome 19q13.4, while HLA genes are located at chromosome 6p21, and the different locations for these two genes indicate independence of segregation [4,10]. NK cells expressing KIRs on their surface can develop in the absence of the corresponding HLA class I ligands without deletion, and this mechanism is called unlicensed, in which NK cells continue to generate in hypo-responsive function against normal autologous cells [11].

Sixteen KIRs genes have been identified in humans [4] and based on gene content, KIR haplotypes have been classified into two major groups, group A haplotype that encode a single activating receptor (KIR2DS4) and group B haplotypes that encode more activating receptor [12,13]. Saudis are a part of the Arab populations living in the Arabian Peninsula, including the countries of Yemen, Oman, the United Arab Emirates, Qatar, Bahrain and Kuwait. A previous study conducted a principle component to determine the genetic variations of the KIRs among Arabs, Africans and Asians showed that Arabs clustered in the middle between Africans and Asians [12].

Abbreviations: KIRs, killer immunoglobulin-like receptors; NK, natural killer; ALL, acute lymphoblastic leukemia; AML, acute myelogeneous leukemia.

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Leukemia is a group of cancer diseases that occur in the bone marrow and lead to the massive production of abnormal white blood cells that are not fully developed (blasts or leukemia cells). A recent study based on Saudi Cancer Registry data rated the incidence of Leukemia (6.9%) as the fifth most frequent cancer disease among Saudi patients. No exact cause for leukemia has been found, but several environmental factors such as infection at an early age, exposure to ionizing radiation or chemicals (e.g., benzene), and parental use of alcohol or tobacco were identified as risk factors for acute lymphoblastic leukemia (ALL) in children. Epidemiological studies suggest an individual genetic component may play an important role in determining the susceptibility for cancer development [14,15].

It has been shown that NK cells are responsible for remarkable effects in adult and pediatric leukemia patients. KIRs with the corresponding HLA class I ligands play an important and effective role as anti-leukemic agents through mediating inhibitory or activating signals [16]. Evidence has shown that NK cells have anti-leukemic activity in a marrow graft of AML patients who received T-cell–depleted transplantations from haplotype identical HLA donors, and a low incidence of relapse was observed in transplanted leukemic patients receiving partially HLA mismatched stem cells [17].

The associations of KIRs and/or HLA class 1 ligands in leukemic patients have been studied in some populations [15,18–21], but to our knowledge, no previous similar work has been performed in Saudi leukemic patients. The aim of the present study is to investigate the distribution of KIRs and HLA class I ligands in Saudi leukemic patients and to compare the results with those of healthy controls.

2. Material and methods

KIRs and HLA class 1 genotypes were identified in 116 Saudi patients with leukemia (74 male, 42 female), acute lymphoblastic leukemia (ALL) (67 patients, 49 ≤ 18 years), and acute myelogeneous leukemia (AML) (49 patients, 19 ≤ 18 years) aged between 1 and 64 years with a mean of 22.4 years. Diagnosis of leukemia was based on established criteria, including blood and bone marrow smears examinations in addition to immunophenotyping of myeloid and lymphoid cells to determine the type/subtype of leukemia. A total of 108 patients were in remission (ALL = 64, AML = 44), and 7 patients (ALL = 3 and AML = 5) were not determined at the time of blood collection. The dimorphic and clinical data were extracted from the hospital information system, and only Saudi patients were included in this study.

In addition, 152 (76 patients ≤ 18 years) healthy and unrelated individuals (age range 1–62 years) selected from donors at the Stem Cell Transplant Program of King Fahad Medical City (KFMC), Saudi Arabia, were used as controls. The study was approved by the Hospital Institutional Review Board, and informed consent was signed by the participants or their guardians. Genomic DNA was extracted from whole blood in EDTA using a MagNa pure compact instrument (Roche Diagnostics Ltd. Rotkreuz), and the DNA yield was measured using a NanoDrop® 2000c spectrophotometer for quantity and quality. KIR genes (KIR-2DL1, -3DL2, -2DL2, -2DL3, -3DL1, -2DS1, 2DS2, -3DS1, -2DL5, -2DP1, -2DS3, -2DS4, -2DS5, -2DL4, -3DL3 and -3DP1) were genotyped using three separate groups of specific primer sets targeting exons 3 + 4, 5 and 7 + 8 + 9 for PCR amplification, followed by reverse SSO oligonucleotide probes utilizing Luminex® technology to identify the KIR genes (One Lambda). HLA-A, HLA-B, and HLA-C were also genotyped targeting exons 2 and 3 using group specific primers for target sequence amplification and Luminex® technology for allele group identification (One Lambda). Multiple known DNA samples for KIR and HLA genotypes were run in parallel as controls, and exact results were obtained. The assignment of KIR haplotypes into AA or Bx genotypes (x can be either an A or B haplotype) and their profile IDs were extracted from www.allelefrequencies.net [22]. The frequency distribution of HLA class I ligands was assessed based on HLA-C typing into C1 and C2 groups. C1 was assigned if an individual had a C*01, C*03, C*07, C*08, C*12 or C*14 gene, whereas C2 was assigned if an individual had a C*02, C*04, C*05, C*06, C*15, C*17 or C*18 gene. HLA-C genes that were not able to be grouped (subtyped classification for HLA-C were needed) into C1 or C2 were ignored [12]. The Bw4 motif was assigned based on http//hla.alleles.org.

Using the SPSS program software version 19.0, the frequencies for the KIR genes and their ligands were derived using a direct counting method, and the Fisher's exact test and logistic regression methodology were considered. We did not examine deviation from

Table 1
Frequencies of KIR genes in AML and ALL patients compared with healthy controls.

Gene	Control n = 152 (%)	ALL patients n = 67 (%)	AML patients n = 49 (%)
KIR2DL1	149 (98)	67 (100)	49 (100)
KIR2DL2	105 (69.1)	36 (53.7)[a]	29 (59.2)
KIR2DL3	124 (81.6)	58 (86.6)	44 (89.8)
KIR3DL1	144 (94.7)	65 (97)	45 (91.8)
KIR3DL2	152 (100)	67 (100)	49 (100)
KIR2DS1	66 (43.4)	22 (32.8)	16 (32.7)
KIR2DS2	112 (73.7)	39 (58.2)[b]	28 (57.1)
KIR3DS1	54 (35.5)	21 (31.3)	15 (30.6)
KIR2DL5	100 (65.8)	33 (49.3)[c]	27 (55.1)
KIR2DP1	149 (98)	67 (100)	49 (100)
KIR2DS3	68 (44.7)	28 (41.8)	18 (36.7)
KIR2DS4	141 (92.8)	62 (92.5)	47 (95.9)
KIR2DS5	66 (43.4)	23 (34.3)	14 (28.6)
KIR2DL4	152 (100)	67 (100)	49 (100)
KIR3DL3	152 (100)	67 (100)	49 (100)
KIR3DP1	152 (100)	67 (100)	49 (100)

[a,b,c]Control vs ALL a:p = 0.0299, corrected p = 0.1495; b: p = 0.0237, corrected p = 0.1422; c: p = 0.0219, corrected p = 0.1095.

Table 2
Frequencies of KIR genes in presence or absence of HLA-C1, HLA-C2 and Bw4 in AML and ALL patients compared with healthy controls.

KIR-HLA ligand	ALL patients	AML patients	Controls
KIR2DL2 (C1-ligand)	n = 33 (%)	n = 26 (%)	n = 92 (%)
C1 homo	8 (24.2)	6 (30.1)	25 (27.2)
C1 het	16 (48.5)	8 (30.7)	39 (42.4)
No ligand	9 (27.3)	12 (46.2)	28 (30.4)
KIR2DS2 (C1-ligand)	n = 33 (%)	n = 26 (%)	n = 104 (%)
C1 homo	8 (24.2)	6 (30.1)	25 (24.0)
C1 het	16 (48.5)	8 (30.7)	46 (44.3)
No ligand	9 (27.3)	12 (46.2)	33 (31.7)
KIR2DL3 (C1-Ligand)	n = 51 (%)	n = 37 (%)	n = 117 (%)
C2 homo	13 (25.5)	6 (16.2)	25 (21.4)
C2 het	20 (39.2)	19 (51.4)	53 (45.3)
No ligand	18 (35.3)	12 (32.4)	39 (33.3)
KIR2DS1 (C2-Ligand)	n = 20 (%)	n = 13 (%)	n = 58 (%)
C2 homo	3 (15)	4 (30.8)	18 (31.)
C2 het	11 (55)	5 (38.4)	24 (42.4)
No ligand	6 (30)	4 (30.8)	16 (27.5)
KIR2DL1 (C2-Ligand)	n = 58 (%)	n = 43 (%)	n = 139 (%)
C2 homo	13 (22.4)	8 (18.6)	39 (28.1)
C2 het	25 (43.1)	18 (41.9)	64 (46.0)
No ligand	20 (34.5)	17 (39.5)	36 (25.9)
KIR3DL1 (Bw4-ligand)	n = 55 (%)	n = 39 (%)	n = 133 (%)
Bw4 homo	10 (18.2)	10 (25.7)	15 (11.3)
Bw4 het	23 (41.8)	21 (53.8)	69 (51.9)
No ligand	22 (40)	8 (20.5)	49 (36.8)
KIR3DS1 (Bw4-ligand)	n = 19 (%)	n = 11 (%)	n = 49 (%)
Bw4 homo	4 (21.1)	1 (9.1)	6 (12.2)
Bw4 het	5 (26.3)	7 (63.6)	22 (44.9)
No ligand	10 (52.6)	3 (27.3)	21 (42.9)