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Original article

# Evaluation of the relationships between HLA-G 14 bp polymorphism and two acute leukemia in a Saudi population

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## ABSTRACT

The non-classical Human Leukocyte Antigen G (HLA-G) is an immunomodulatory molecule, with low polymorphism frequency and restricted tissue distribution. Its higher expression was associated with immunoinhibitory properties causing tolerance for cancer progression. The rs371194629 is a 14 bp insertion/deletion polymorphism (*Ins/Del*) in exon 8 of the 3' untranslated region (3'-UTR) of the HLA-G gene, has been associated with the stability and the expression level of HLA-G mRNA. In this study, we evaluated the relationship between the HLA-G 14-bp *Ins/Del* polymorphism and two common blood cancers including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) in a population from Riyadh in Saudi Arabia. The study groups include 145 patients diagnosed for ALL, 98 patients diagnosed for AML and 115 healthy individuals. For all these individuals, the 14 bp *Ins/Del* polymorphism was genotyped using PCR methodology. mRNA gene expression of the HLA-G was assessed using quantitative RT-PCR. Logistic regression model analysis for HLA-G 14 bp *Ins/Del* polymorphism shows no statistically significant correlation with AML and ALL. For the HLA-G mRNA expression, while slightly increased level among healthy individuals compared to patients was observed, no statistically significant differences were obtained. Although our results did not show association between HLA-G with the two leukemia cancer diseases, the role of HLA-G gene awaits further investigations including large number of subjects with more clinical data combinations.

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## 1. Introduction

Cancer is becoming more frequent and lethal in both developed and developing countries, with global economic impacts (Siegel et al., 2021). Blood cancers account for nearly 10% of all newly diagnosed cancer cases in the world (Eid et al., 2021). The most

common type of pediatric cancer and the most leading cause of cancer mortality in children is acute lymphoblastic leukemia (ALL) (Inaba et al., 2013). ALL affects both children and adults, with a peak of occurrence between 1 and 4 years (Malard and Mohty, 2020). While the most common type of leukemia malignancy among adults is acute myeloid leukemia (AML). It is characterized by the proliferation of myeloid blasts or progranulocytes that do not differentiate normally (Newell and Cook, 2021). Exogenous or endogenous exposures, genetic predisposition, and randomness all play a role in causation (Alkhouly et al., 2013; Inaba et al., 2013). Also, chromosome abnormalities and genetic alterations that impact lymphoid precursor cell formation and proliferation are directly involved in the abnormal proliferation. However, the genetic factors for these leukemia diseases remain unknown.

According to circumstantial evidence, the host immune system can actively inhibit or tolerate tumor development. The production of immunosuppressive cytokines or the modification of antigen-presenting molecules on neoplastic cells is commonly blamed for

**Abbreviations:** HLA, Human Leukocyte Antigen; ALL, Acute Lymphoblastic Leukemia; AML, Acute Myeloid Leukemia; *Ins/Del*, Insertion/Deletion; UTR, Untranslated Region; CML, Chronic Myelocytic Leukemia; CLL, Chronic Lymphocytic Leukemia; NK, Natural Killer Cells.

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tumor escape from host immune surveillance (Alkhouly et al., 2013). HLA-G is a non-classical HLA molecule of the class Ib and is located between HLA-A and HLA-F located on chromosome 6p21.3. It has eight exons and seven introns (Castelli et al., 2008).

The HLA-G protein has special features including the reduced allelic polymorphism, restricted tissue expression, and the existence of seven expression isoforms that could be membranous (G1, G2, G3 and G4) or soluble proteins (G5, G6 and G7) (Paul et al., 2000).

HLA-G has been reported for its suppressive function as it decreases the human immune response through a variety of mechanisms, including the inhibition of natural killer (NK) cells and T lymphocytes' cytotoxic activities (Hofmeister and Weiss, 2003). HLA-G plays a key role in fetal maternal immune tolerance by inhibiting maternal immunity through its expression in trophoblast cells at fetus-maternal interface during pregnancy (Moreau et al., 1998). Thus, reduced placental expression of HLA-G has been associated with pathological conditions such as recurrent spontaneous abortion (Peng et al., 2008). Outside the placenta and in physiological conditions, HLA-G expression has been documented in few immune-privileged tissue, such as the thymus and the cornea (Le Discorde et al., 2003). However, an ectopic expression of HLA-G molecules has been associated with pathological conditions such as cancer, autoimmune and inflammatory diseases (Zaborek-Lyczba et al., 2021).

Many studies have been conducted on the HLA-G polymorphism and its functional association with human diseases. Thus far, 94 HLA-G alleles have been identified (IPD-IMGT/HLA, R, 3.47, Marsh, 2022; <https://www.ebi.ac.uk/ipd/imgt/hla>).

Polymorphism sites in the HLA-G gene's non-coding regions, particularly in the 3' UTR exon 8, have been reported to influence its expression (Rizzo et al., 2014). The insertion or deletion of a 14-bp (5'-ATTGTTTCATGCCT-3') polymorphism in the 3' UTR of the HLA-G gene, also known as rs371194629, is one of the most important polymorphisms (Rousseau et al., 2003). It has been reported in the untranslated exon 8 and was involved in the stability and expression of mRNA. The *Del* allele stabilizes the mRNA with a consequent higher HLA-G expression, while the *Ins* allele destabilizes the mRNA and is linked with a lower levels of HLA-G expression (Svendsen et al., 2013). According to Chen et al. (2008), the *Ins* alleles was associated with lower HLA-G mRNA levels and *Del* allele with higher level. In recent years, a rising number of studies have investigated the relationship between HLA-G 14-bp *Ins/Del* polymorphism and malignancies in different populations (Adolf et al., 2021). In Saudi Arabia, few studies have reported the association of HLA-G 14 *Ins/Del* variant and cancer diseases including breast cancer and colorectal cancer have been reported (Al Omar and Mansour, 2019; Al Omar et al., 2015; Hassan et al., 2019). However, only a handful studies have been done on leukemia. In Saudi Arabia, so far, no studies had investigated the association between HLA-G and any leukemia diseases. In this study, we investigated the genotypic and allelic association of HLA-G 14 bp *Ins/Del* polymorphism with the two most frequent leukemia malignancies (ALL and AML).

## 2. Materials and methods

### 2.1. Ethics statement

The study was approved by the medical ethics committee at King Khaled Hospital University (KKHU) in Riyadh, Saudi Arabia, and was carried out in accordance with King Saud University's ethical guidelines (IRB-No. E-20-5346).

### 2.2. Patients and healthy groups

A total of 358 human whole blood samples were acquired for this study. The patients in this study were 243 patients, including 145 patients diagnosed with ALL (87 males and 58 females), and 98 patients diagnosed with AML (54 males and 44 females). The control group included 115 healthy individuals of both genders (77 males and 38 females) without history for cancer and another inflammatory or autoimmune diseases. The mean age of patients with ALL was  $19.20 \pm 14.8$  years, and with AML was  $27.56 \pm 18.5$  years, while the mean age of controls was  $22.45 \pm 20.18$  years. All patients were sourced from the Department of Pediatric Hematology-oncology section at King Khaled Hospital University (KKHU) in Riyadh, Saudi Arabia.

### 2.3. Genomic DNA extraction

3 ml of peripheral blood was taken from patients and healthy controls in ethylene diamine tetra acetic acid (EDTA) coated collection vials from each participant. The QIAamp DNA Blood Mini Kit (QIAGEN, GmbH, Hilden, Germany) was used to extract genomic DNA from peripheral blood, following the manufacturer's instructions.

### 2.4. Genotyping of the 14-bp *Ins/Del*

The 14-bp *Ins/Del* polymorphism (rs371194629) in exon 8 (3' UTR) of the HLA-G gene (Fig. 1) was genotyped using the primers GE14HLA-G: 5'-GTGATGGGCTGTTAAAGTGCACC-3' and RHG4: 5'-GGAAGGAATGCAGTTCAGCATGA-3' (Hviid et al., 1999). Polymerase Chain Reaction (PCR) was done in a volume of 20  $\mu$ l containing 1  $\mu$ l of DNA, 10  $\mu$ l of PCR GoTaq<sup>®</sup> Master Mixes 2X (Promega, India), 0.5  $\mu$ l of each primer (10 pmol) and 8  $\mu$ l of RNase-free water. The PCR reactions were performed in a T100<sup>™</sup> Thermocycler (Bio-Rad, Singapore). For amplification, the following thermocycling conditions were used: an initial denaturation phase at 95 °C (5 min), followed by 34 cycles of 94 °C (30 s), 60 °C (30 s), 72 °C (1 min), and 72 °C (5 min) as a final extension. The amplicons, which are either 210 bp in length for the 14-bp *Del* allele, or 224 bp in length for the 14-bp *Ins* allele, were migrated in a 3% agarose gel stained with ethidium bromide. The gel was visualized and photographed in a BioDocAnalyze (Biometra GmbH, Germany).

For DNA sequencing, six randomly selected samples including the three genotypes were sequenced using the same reverse primer used in PCR reaction. The PCR products were purified using EXO-SAP (New England Biolabs) and sequenced using the BigDye Terminator v.3.1 Sequencing Kit (Applied Biosystems, CA, USA) in an ABI Prism 3730 XL Genetic Analyzer (Applied Biosystems).

### 2.5. RNA isolation and cDNA synthesis

The PureLink<sup>™</sup> RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, USA) was used to isolate total RNA from human whole blood.

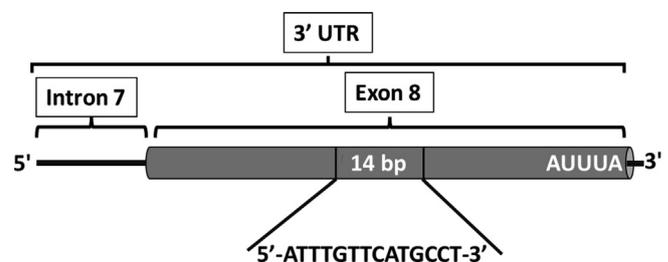


Fig. 1. Polymorphism in the HLA-G gene in 3' UTR. The 14-bp *Ins/Del* in exon 8.

The quantity and quality of extracted RNA were determined using a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific, USA). The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of RNA. A ratio of about 2.0 of pure RNA. For high-capacity cDNA synthesis, we used a reverse transcription kit (Applied Biosystems™, USA) according to the protocol described by the manufacturer. The kit is designed for real-time, two-step RT-PCR.

2.6. Quantitative RT-PCR analysis of HLA-G mRNA

For qRT-PCR analysis, we performed specific amplification of HLA-G transcripts from cDNA. For specific HLA-G amplification, the following sequences of primers were F: 5'-GTGTTCCGTGTCTCCTCT-3' and R: 5'-GACAGCGACTCGGCGT-3'. The housekeeping gene used in this analysis was for GAPDH gene using the couple of primers: F: 5'-TCTCCTCTGACTTCAACAGCGAC-3' and R: 5'-CCCTGTTGCTGTAGCCAAATTC-3'. For each PCR reaction, a total volume of 20 µl was used, which included 10 µl of 2X Fast SYBR® Green Master Mix, 1.5 µl of cDNA, 0.5 of each primer (5 pmol) and 7.5 µl of RNase-free water. The PCR reactions were performed in and ABI PRISM 7500 System Thermal Cycler (Applied Biosystems). The program of amplification was an initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 sb and 72 °C for 30 s, and a final extension for 10 mn at 72. For gene expression analysis, relative quantitation of HLA-G mRNA was done by the comparative CT method after normalization with the housekeeping gene (GAPDH). Relative expression level, determined as fold change, was expressed using the 2<sup>-(ΔΔCt)</sup> method (Schmittgen and Livak, 2008).

2.7. Statistical analysis

We tested the Hardy–Weinberg equilibrium (HWE) for 14 bp *Ins/Del* allelic and genotypic variations in control subjects. Odds ratios with confidence intervals of 95% were used to measure the strength of the association between the HLA-G 14 bp *Ins/Del* poly-

morphism and ALL and AML risk for all genotypes considering all the inheritance models (dominant, co-dominant, recessive, or log-additive). Analyses were performed using SNPStats software (Solé et al., 2006).

3. Results

The 14 bp *Ins/Del* HLA-G 3'UTR polymorphism was successfully genotyped for all the 243 patients with acute leukemia, including 145 ALL and 98 AML and 115 healthy controls. Fig. 2A shows a gel image of the genotyping result of the 14 bp polymorphism. Six PCR-genotyped samples from each group were selected for sequencing. Fig. 2B shows an example of electropherograms of the three HLA-G 14 bp *Ins/Del* genotypes, that confirm the classical genotyping methods. Allele frequencies of the *Del* and *Ins* are reported in Table 1. The genotype frequency of the HLA-G polymorphism was in Hardy–Weinberg equilibrium (HWE) for controls, AML and ALL patients. For the genetic and allelic association, we have tested five genetic models (allelic, codominant, dominant, recessive, overdominant and additive). Results are reported in Table 1. The allele distribution between control group and ALL and AML, did not show any significant difference. The *Del* allele appears slightly higher for all tested groups than the *Ins* allele, with a frequencies similar to those reported for many populations. For all the tested models of association, no significant differences were observed between controls and the two leukemia cancers.

Furthermore, we assessed the possible association between HLA-G 14 bp *Ins/Del* and the risk to ALL and AML by stratified analysis based on gender (Table 2) and age (Table 3). Once again, for both leukemia diseases, no significant association was observed with the studied polymorphism.

In order to assess the relationship between HLA-G gene expression and ALL diseases, we analyzed the HLA-G mRNA expression level in 12 ALL and 12 control individuals using relative quantitative RT-PCR. Fig. 3 shows the diagram of the mean fold of expression for healthy control and ALL patients.. In the control group, the level of HLA-G is two times higher than in patients. However, the difference did not reach significance (P = 0.22).

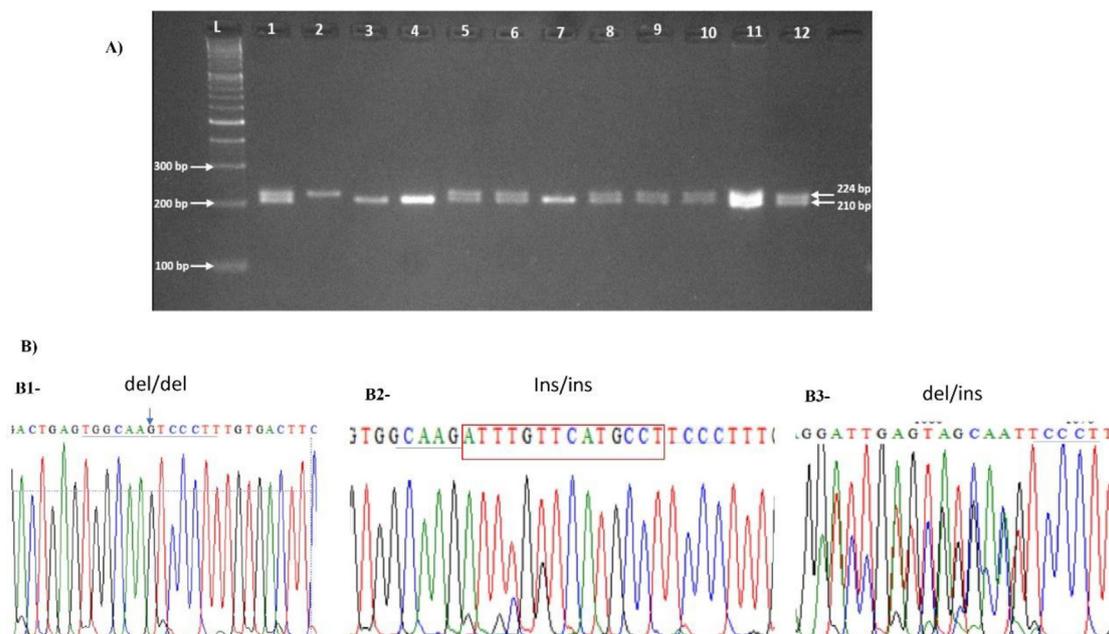


Fig. 2. Analysis of the 14bp *Ins/Del* polymorphism using gel electrophoresis (A). For each patient and control. In the gel, Lanes 1, 5, 6, 8–12 are heterozygous; Lane 2 homozygous *Ins/Ins* and lanes 3, 4 and 7 are homozygous *Del/Del*. (B) sequencing of the 3' UTR fragment, showing the electropherogram at the level of the 14 bp *Ins/Del*. B1, profile sequence of a homozygous *Del/Del*; B2, Profile of homozygous *Ins/Ins* and B3, profile of heterozygous *Del/Ins*.

**Table 1**

Allelic and genotypic association of HLA-G 14 bp INS/DEL polymorphism in health controls and ALL and AML patients, showing codominant, dominant, recessive, overdominant, and additive genetic models.

Genetic model	Allele/Genotype	Controls (N = 115) N (%)	ALL (N = 145) N (%)	OR (95% CI)	P value	AML (N = 98) N (%)	OR (95% CI)	P-value
Allele	Del	0.53	0.59	1.00	0.20	0.54	1.00	0.83
	Ins	0.47	0.41	0.79 (0.563–1.130)		0.46	0.959 (0.65–1.40)	
Codominant	Del/Del	37 (32.2%)	52 (35.9%)/	1.02 (0.58–1.79)	0.35	32 (32.6%)	1.00	0.96
	Ins/Del	48 (41.7%)	66 (45.5%)			42 (42.9%)	0.99 (0.53–1.85)	
	Ins/Ins	30 (26.1%)	27 (18.6%)			24 (24.5%)	1.08 (0.53–2.21)	
Dominant	Del/Del	37 (32.2%)	52 (35.9%)	1.00	0.53	32 (32.6%)	1.00	0.94
	Ins/Ins + Ins/Del	78 (67.8%)	93 (64.1%)	1.18 (0.70–1.98)		66 (67.3%)	1.02 (0.57–1.82)	
Recessive	Ins/Ins	30 (26.1%)	27 (18.6%)	1.00	0.15	24 (24.5%)	1.00	0.79
	Del/Del + Ins/Del	85 (73.9%)	118 (81.4%)	1.54 (0.86–2.78)		74 (75.5%)	1.09 (0.59–2.02)	
Overdominant	Del/Del + Ins/Ins	67 (58.3%)	79 (54.5%)	1.00	0.54	56 (57.1%)	1.00	0.54
	Ins/Del	48 (41.7%)	66 (45.5%)	0.86 (0.52–1.41)		42 (42.9%)	0.96 (0.55–1.65)	
Log-additive	–	–	–	1.23 (0.88–1.71)	0.23	–	1.04 (0.73–1.48)	0.84

ALL; Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CN: Control; OR: odds ratio; CI: confidence interval. Ins: 14 base pairs insertion; Del: 14 base pairs deletion.

**Table 2**

Correlations between HLA-G 14 bp *Ins/Del* polymorphisms and ALL and AML susceptibility stratified by gender.

Genetic model	Allele/Genotype	ALL (Male)	ALL (Female)	OR (95% CI)	P-value	AML (Male)	AML (Female)	OR (95% CI)	P-value
Allele	Del	0.57	0.61	0.837 (0.52–1.35)	0.46	0.53	0.55	0.95 (0.54–1.67)	0.86
	Ins	0.43	0.39			0.47	0.45		
Codominant	Del/Del	29 (33.3%)	23 (39.7%)	1.00	0.74	18 (33.3%)	14 (31.8%)	1.00	0.99
	Ins/Del	41 (47.1%)	25 (43.1%)	1.30 (0.62–2.72)		23 (42.6%)	19 (43.2%)	0.94 (0.37–2.38)	
	Ins/Ins	17 (19.5%)	10 (17.2%)	1.35 (0.52–3.50)		13 (24.1%)	11 (25%)	0.92 (0.32–2.66)	
Dominant	Del/Del	29 (33.3%)	23 (39.7%)	1.00	0.44	18 (33.3%)	14 (31.8%)	1.00	0.87
	Ins/Ins + Ins/Del	58 (66.7%)	35 (60.3%)	1.31 (0.66–2.62)		36 (66.7%)	30 (68.2%)	0.93 (0.40–2.18)	
Recessive	Ins/Ins	70 (80.5%)	48 (82.8%)	1.00	0.73	41 (75.9%)	33 (75%)	1.00	0.92
	Del/Del + Ins/Del	17 (19.5%)	10 (17.2%)	1.17 (0.49–2.76)		13 (24.1%)	11 (25%)	0.95 (0.38–2.40)	
Overdominant	Del/Del + Ins/Ins	46 (52.9%)	33 (56.9%)	1.00	0.63	31 (57.4%)	25 (56.8%)	1.00	0.95
	Ins/Del	41 (47.1%)	25 (43.1%)	1.18 (0.60–2.30)		23 (42.6%)	19 (43.2%)	0.98 (0.44–2.18)	
Log-additive	–	–	–	1.18 (0.74–1.89)	0.48	–	–	0.96 (0.56–1.63)	0.87

ALL; Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CN: Control; OR: odds ratio; CI: confidence interval. Ins: 14 base pairs insertion; Del: 14 base pairs deletion.

**Table 3**

Correlations between HLA-G 14 bp *Ins/Del* polymorphisms and ALL and AML susceptibility stratified by age.

Genetic model	Allele/Genotype	ALL (Age ≤ 18)	ALL (Age > 18)	OR (95% CI)	P value	AML (Age ≤ 18)	AML (Age > 18)	OR (95% CI)	P value
Allele	Del	0.59	0.58	1.03 (0.63–1.66)	0.90	0.58	0.52	0.97 (0.60–1.57)	0.90
	Ins	0.41	0.42			0.42	0.48		
Codominant	Del/Del	33 (36.7%)	19 (34.5%)	1.00	0.95	15 (40.5%)	17 (27.9%)	1.00	0.38
	Ins/Del	40 (44.4%)	26 (47.3%)	1.13 (0.53–2.39)		13 (35.1%)	29 (47.5%)	1.97 (0.76–5.11)	
	Ins/Ins	17 (18.9%)	10 (18.2%)	1.02 (0.39–2.68)		9 (24.3%)	15 (24.6%)	1.47 (0.50–4.33)	
Dominant	Del/Del	33 (36.7%)	19 (34.5%)	1.00	0.8	15 (40.5%)	17 (27.9%)	1.00	0.2
	Ins/Ins + Ins/Del	57 (63.3%)	36 (65.5%)	1.10 (0.54–2.21)		22 (59.5%)	44 (72.1%)	1.76 (0.74–4.18)	
Recessive	Ins/Ins	73 (81.1%)	45 (81.8%)	1.00	0.92	28 (75.7%)	46 (75.4%)	1.00	0.98
	Del/Del + Ins/Del	17 (18.9%)	10 (18.2%)	0.95 (0.40–2.27)		9 (24.3%)	15 (24.6%)	1.01 (0.39–2.62)	
Overdominant	Del/Del + Ins/Ins	50 (55.6%)	29 (52.7%)	1.00	0.74	24 (64.9%)	32 (52.5%)	1.00	0.23
	Ins/Del	40 (44.4%)	26 (47.3%)	1.12 (0.57–2.20)		13 (35.1%)	29 (47.5%)	1.67 (0.72–3.88)	
Log-additive	–	–	–	1.03 (0.64–1.64)	0.91	–	–	1.26 (0.73–2.18)	0.41

ALL; Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CN: Control; OR: odds ratio; CI: confidence interval. Ins: 14 base pairs insertion; Del: 14 base pairs deletion.

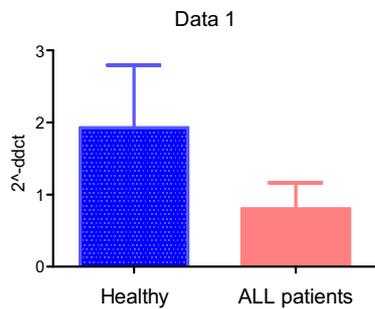


Fig. 3. Relative expression of HLA-G from blood samples of ALL patients compared to healthy individuals.

#### 4. Discussion

The prevalence of leukemia in Saudi Arabia is increasing every year. The number of cases passed from 297,000 to 437,033 between 1990 and 2018. The number of new cases in 2020 was 27885, while it was 24,485 in 2018 (Bawazir et al., 2019; Ferlay et al., 2021). Even though it is a result of screening methods progress, development of the immune therapy methods is necessary in order to improve leukemia control and treatment. HLA-G expression in both tumor and non-tumor cells is one of the mechanisms by which malignant cells escape detection by the host immune system (Amiot et al., 2011). This non-classical HLA-class I molecule was considered as an immunosuppressive checkpoint molecule, associated with immune evasion of malignant cells and was therefore inspected as a potential target for immunotherapy (Jasinski-Bergner et al., 2022). The level of expression of the HLA-G in some pathologic sites was associated in most of cases with unfavorable prognosis (Imani et al., 2018). While the relatively low polymorphism reported in coding or non-coding HLA-G molecule compared to classical HLA-genes, the most relevant polymorphisms were located within the regulatory untranslated regions of the gene, including the 3' and 5'UTRs. Studies on the association between HLA-G polymorphism in coding and non-coding regions with cancer, infectious or autoimmune diseases have confirmed, in most of them, the biological effect features of this polymorphism (Attia et al., 2020). Controversial results in associations were reported according to the disease and the ethnicity which could be attributed to the etiological processes of cancer, including gene-to-gene or gene-environment interactions, sample sizes, and the age of disease onset. Several studies have found that certain HLA-G gene variants are linked to cancer development (Yie et al., 2007; Zheng et al., 2021). One of the most interesting dimorphic site, was the 14 bp *Del/Ins* in the 3'UTR region, as it was associated with HLA-G mRNA stability and alternative splicing (Hviid et al., 2003; Rousseau et al., 2003). In this case control study, we have investigated relationships between the allelic and genotypic 14 bp *Ins/Del* polymorphism of the HLA-G and two main types of acute leukemia; ALL and AML. We have explored different genetic models in this study, however our results showed lack of any association between alleles or genotypes of the HLA-G 14 bp *Ins/Del* polymorphism and ALL and AML. To the best of our knowledge and instead of the large number of case/ control studies that have explored the association between the HLA-G 14 bp polymorphism and solid cancer diseases. This is the first case/control study exploring the association between HLA-G 14 bp *Ins/Del* polymorphism and leukemia diseases. In a cohort study, including only patients with chronic lymphoid leukemia. Roberta et al. (2014) showed that the *Del/Del* genotype was associated with higher expression of HLA-G and reduced overall survival, compared to those with other genotypes and the presence of HLA-G molecules in this leukemia environment, creates a favourable setting for

CLL expansion. The authors confirmed that in the presence of *Del/Del* patients have a poorer overall survival than those sharing either *Ins/Del* or *Ins/Ins* genotypes (Roberta et al., 2014). However, in another study, Własiuk et al. (2013) noted that higher levels of HLA-G on the surface and soluble are observed in AML patients especially on the surface of dendritic cells and CD4+ T cells and this high level of expression is not associated with any HLA-G 14 bp genotypes. In addition, we have performed an extensive analysis for the association between this HLA-G polymorphism and AML or ALL in subgroups according to age and gender. For all these analyses, we did not find any association considering these parameters. Considering that the functional feature of the HLA-G molecules are mostly associated with the level of expression, we have studied the mRNA expression and compared between ALL and healthy control. Our results did not show significant difference between these two groups, instead of the higher level among the control group. Unfortunately, we failed to obtain mRNA for AML patients. To compare with our results, we did not find in bibliography any case control study for HLA-G 14 bp genetic association and gene expression for ALL or AML diseases using healthy controls as reference group.

However, some studies have explored the relationships between these two aspects of HLA-G and some clinical and cellular parameters of variant leukemia. In some studies, the circulating blasts from CML and AML have been reported to express or to secrete HLA-G and the level of expression was associated with higher blast cells in the bone marrow and relapse (Caocci et al., 2017). Moreover, HLA-G expression has been suggested as predictive tumor biomarker to track disease progression or as a therapeutic target to improve immune responses to leukemia (Locafaro et al., 2014). Maki et al. (2008) pointed out that blocking HLA-G1 with a particular antibody enhanced the sensitivity of CLL samples to NK-mediated death, suggesting that HLA-G protects CLL cells from NK-mediated killing and so may contribute to their immunological escape in vivo. On the other hand, some studies reported opposite results. In one of these studies, patients with AML, CML, ALL, and CLL were selected for their HLA-G expression and they found that the HLA-G molecule was not expressed in newly separated human leukemia cells and hence plays no role in their immunological escape (Poláková et al., 2003). In the secreted forms of HLA-G, Giannopoulos et al. (2008) did no found differences in HLA-G mRNA expression or levels in the plasma of patients.

In conclusion, although our study did not show a significant association between HLA-G 14 bp polymorphism or gene expression with AML or ALL in the Saudi population, further investigations including large number of subjects with more clinical data are necessary to state for this association.

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#### Conflicts of interest

The authors declare that the publishing of this paper does not include any conflicts of interest.

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