# INTERLEUKIN-12A (rs583911) POLYMORPHISM IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN IRAQ

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***Abstract***

**Background:** Acute Lymphoblastic Leukemia is a malignant disorder of lymphoid progenitor cells and the most common type of malignant neoplasms in children.

**Aim:** The present study was conducted to evaluate the role of IL-12A (rs583911) gene polymorphism in the pediatric acute lymphoblastic leukemia.

**Methods:** We have investigated single nucleotide polymorphisms of IL-12A (rs583911) gene in 120 subjects. Sixty were Acute Lymphoblastic Leukemia patients while others were apparently healthy individuals used as a controls.

**Results:** The frequencies of genotype AA was significantly more frequent in study group than in control groups, 26.7 % versus 10.0 %**,** variant allele A was more frequent in study than in control group, 43.35% versus 56.7 %.

**Conclusion**: AA genotype is mainly expressed among Acute Lymphoblastic Leukemia patients and it may considered as a risk factor for ALL.

**Key words:** Acute Lymphoblastic Leukemia, IL-12A (rs583911) , Allele, Genotype, AS-PCR.

***Introduction***

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells and the most common type of malignant neoplasms in children[(1)]. Both T-cell and B-cell precursors can give rise to ALL; B-cell ALL represents about 88%of all cases [(2)].

Its incidence peaks between the ages of 2 and 5 years; rates are lower during later childhood, adolescence and young adulthood .It is the most common leukemia in children representing 23% of cancer diagnosis among children younger than 15 years [(3)] .The incidence of the disease is higher in boys than in girls (four times for T-cell ALL), except that girls have a higher (1.5 times) incidence of leukemia in the first year of life [(4)]. The most low rates are found in developing countries, among US blacks, Israeli Jews, and Chinese and Asian Indians, whose rates may be many times lower than those in more affluent developed countries [(5)].

The pathogenesis of ALL can be related to different environmental and genetic factors: Environmental factors, such as ionizing radiation, electromagnetic field, certain diets (e.g., bioflavonoids), seem to have little association in most of the ALL cases[(6)]. However, two infection-based hypotheses, Greaves' "delayed infection" hypothesis and Kinlen's "population-mixing" hypothesis that childhood ALL arise as a consequence of an abnormal immune response in susceptible individuals to common infections, are well supported by epidemiological data [(7, 8)],while the genetic factors play an important role in the etiology of ALL and this arise from in utero chromosomal abnormalities that can lead to clonal expansion of pre-leukemic precursor cells [(9)]. Cytokines are small molecules secreted by cells in response to specific stimuli and alter the behavior of the same or other cells. Cytokines act on target cells generally within the hematopoietic system by binding to specific receptors, initiating signal trans- duction and second messenger pathways within the target cell. Production of numerous cytokines by immune cells in response to both antigen specific and non-specific stimuli is critical to the outcome of inflammatory immune responses. Many single-nucleotide polymorphisms (SNPs) were detected within cytokine gene sequences, several of these polymorphisms may be associated with differential levels of gene transcription, genetic studies have tried to correlate these cytokine polymorphisms with immune-mediated diseases. For example, associations were reported between SNPs in the TNFA promoter and rheumatoid arthritis. A number of studies reported associations between TNFA or LTA SNPs and particular cancers, including chronic lymphocytic leukemia [(10)].

Interleukin-12 (IL-12) is a pro-inflammatory cytokine that is mainly secreted by antigen-presenting cells, targets T-helper (Th) cells and natural killer cells, and stimulates the synthesis and secretion of interferon gamma (IFN-γ),which is a well established anti tumor factor. Also, there have been observed the lower serum IL-12 levels in patients with various types of cancer, this suggesting that IL-12 functions as a potent tumor suppressive factor. Biologically active IL-12 consists of two functional subunits, p35 and p40, which are encoded by the IL-12A and IL-12B genes, respectively [(11, 12)].The tumor-suppressive effect of IL-12 is well documented, thus, functional polymorphisms of the IL-12A and IL-12B genes are thought to be good genetic

candidates for cancer susceptibility. Extensive studies have explored the potential associations of IL-12A and IL-12B genetic variants with cancer risk [13, 14].The present study was conducted to evaluate the role of IL-12A (rs583911) gene polymorphism in the Acute lymphoblastic leukemia.

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## *Materials and Methods*

### *Patients*

The current study was conducted on 60patients (40 male and 20 female) attended to Central Child Teaching Hospital in Baghdad, Iraq ,for the period from first of February2018 to the first of June 2018 , the patients were diagnosed as acute lymphoblastic leukemia by hematology and pediatric consultant in the oncology unit in the hospital, Patients were interviewed directly by using an anonymous questionnaire form which covered age, sex, family history of any malignant disease and others. Another group consist of 60 apparently healthy individuals (42 male and 18 female) without any history of malignant disease were clinically considered as healthy also included in this study as a control group. Informed consent was obtained from all study subjects after explanation of the nature and possible consequences of the study.

### *Genotyping*

The genotypes of the IL-12A (rs583911) gene were determined by Allele Specific-PCR, Table (1). The PCR products were amplified using a Maxime PCR PreMix (iNtRON),then the PCR products were visualized in an ethidium bromide-stained 2% agarose gel using a UV Transilluminator.

### *Statistical analysis*

The Hardy–Weinberg equilibrium (HWE) assumption was assessed for both the patient and control groups by comparing the observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Data were presented, summarized and analyzed using two software programs. These were the Statistical Package for Social Science (SPSS) version 20 and Microsoft Office Excel 2010.Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the association between the genotypes, alleles or haplotypes and the risk of ALL. The results are presented as the mean values ± 1 standard deviation (SD), and a P value of $\leq 0.05$ was considered to indicate statistical significance.

***Results and Discussion***

***Demographic and clinical parameters***

Patients with ALL were comparable in age, gender and family history of any malignant disease with controls (Tables2, 3 and 4).

***Distribution of IL12-A rs583911 Genotypes AA, AG and GG in Control and Study Groups***

Distribution of IL12-A rs583911 polymorphism was detected by AS-PCR technique, at this locus there're three genotype GG, GA and AA figure (1).

The current study revealed that The most prevalence IL12-A rs583911 genotype was AA so it considered the wild type (reference) whereas the least frequent IL12-A rs583911 genotype was GG, therefore it regarded as the variant (mutant) genotype. Considering control group, the frequency distribution of IL12-A rs583911 genotype AA,AG and GG were 16, 30 and 14, respectively and this observed distribution did not differ significantly from the expected distribution according to Hardy Weinberg equation ($P$ = 0.444), as shown in table (5).

Considering study group, the frequency distribution of IL12-A rs583911 genotype AA, AG and GG were 6, 20 and 34, respectively and this observed distribution differed significantly from the expected distribution according to Hardy Weinberg equation ($P$= 0.001), as shown in table (6).

Indeed, genotype GG was significantly more frequent in study group than in control groups, 56.7 % versus 23.3% ($P$ = 0.001), as shown in table (6). However, there was highly significant difference in rate of allele G between study and control groups ($P$=0.002). It acts as risk factor for ALL with an Odds ratio of 2.94;95 % confidence interval of 1.71 -5.05 and the etiologic fraction of allele G as a risk factor for ALL was 0.40, as shown in table (7). Nevertheless, variant allele A was more frequent in control than in study group, 51.7% versus 26.7% respectively and may be considered as a preventive fraction (0.40).

A study by Chang *et al*, (2010) of 208 SNPs, only rs583911 of *IL12A*, which encodes a critical modulator of T-cell development, remained significant after accounting for multiple testing (odds ratio for each copy of the variant G allele = 1.52, 95% confidence interval: 1.25–1.85, p = $2.9 \times 10^{-5}$) **[15].** This result supports our finding that allelic variation exists in the gene encoding IL-12 A in children with ALL; and agree that the variant allele G may considered as a risk factor for ALL in children.

Previous studies have shown that newborns have Th2-skewed immune profiles **[16].** Furthermore, during the normal course of immune development, a shift from Th2-dominant to Th1-dominant immune profiles occurs with increasing age **[17].** It is thought that the major driving force for this immune shift is the production of IL12 by innate immune cells (e.g. dendritic cells) after exposure to microbial challenges **[18].** The IL12 protein is a heterodimer that consists of two subunits, IL12A (p35) and IL12B (p40) **[19]**. In the current study, we observed

significant variation with rs583911 of *IL12A*. Functional impact of the *IL12A* SNPs has yet to be characterized. A study by Pistiner *et al*. showed that rs2243123 of *IL12A*, a SNP in intron 2 that is 739 base pairs away from rs583911, is associated with immune sensitization to cockroach antigen [(20)]; this lends further support that the region around rs583911 may be important in either the function or the expression of IL12A and may be a promising candidate region to perform fine mapping and functional studies to determine causal variants.

## Conclusion

There was an association between IL12-A rs583911 polymorphism in the pathogenesis of pediatric ALL in Iraq. The variant allele G is considered as risk factor for Acute lymphoblastic leukemia.

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Table 1: primers sequence with orientation and the PCR product size.

| **Primers** | **Sequence (5'-3')** | | **Amplicon** |
|---|---|---|---|
| **IL12-Agene** | **Wild type R** | **CGTTGGATGAGCTTGTCTT AAGGGTTTGC** | **100bp wild type allele (A)** |
| | **Mutant type R** | **ACGTTGGATGCAAGTATAACT TCTAAAGGG** | **50 bp Mutant type allele (G)** |
| **rs583911Allele Common Forward Primer** | **TTTGCATGTTTGTTATATCCATCA** | | |

Table (2): The Patient-Control Difference in mean age.

| **Age (years)** | **Control group** ***n*** **= 60** | **Study group** ***n*** **= 60** | ***P*** * |
|---|---|---|---|
| Mean ±SD | 5.64 ±3.20 | 6.13 ±3.00 | 0.164 NS |
| Range | 2-12 | 2-14 | |
| Median (IQR) | 4.50 (5.00) | 5.5 (4.00) | |

SD: standard deviation; *n*: number of cases; *: Mann Whitney U test; NS: not significant at $P \leq 0.05$

Table (3):Comparison of Gender Frequency Distribution Between Control and Patient Groups.

| **Gender** | **Control group** ***n*** **= 60** | **Study group** ***n*** **= 60** | ***P*** * |
|---|---|---|---|
| Male, n (%) | 42 (70.0 %) | 40 (66.7 %) | 0.695 NS |
| Female, n (%) | 18 (30.0 %) | 20 (33.3 %) | |

*n*: number of cases; *: Chi-square test; NS: not significant at $P \leq 0.05$

Table (4): Association Between Disease and family history of any malignancy disease

| **Family history** | **Control group** ***n* = 60** | **Study group** ***n* = 60** | ***P* *** |
|---|---|---|---|
| Positive, *n* (%) | 0 (0.0 %) | 34 (56.7 %) | < 0.001 HS |
| Negative, *n* (%) | 60 (100.0 %) | 26 (43.3 %) | |

*n*: number of cases; *: Chi-square test; HS: highly significant at $P \leq 0.01$

Table (5): Hardy Weinberg equilibrium of IL12-A rs583911 genotype in control group.

| **Genotypes** | **Observed** | **Expected** | $\chi^2$ | ***P*** |
|---|---|---|---|---|
| AA (wild) | 16 | 25.4 | 0.587 | 0.444 NS |
| AG | 30 | 27.3 | | |
| GG (mutant) | 14 | 7.4 | | |

NS: not significant at $P \leq 0.05$

Table (6): Comparison of IL12-A rs583911 genotype between control and study groups

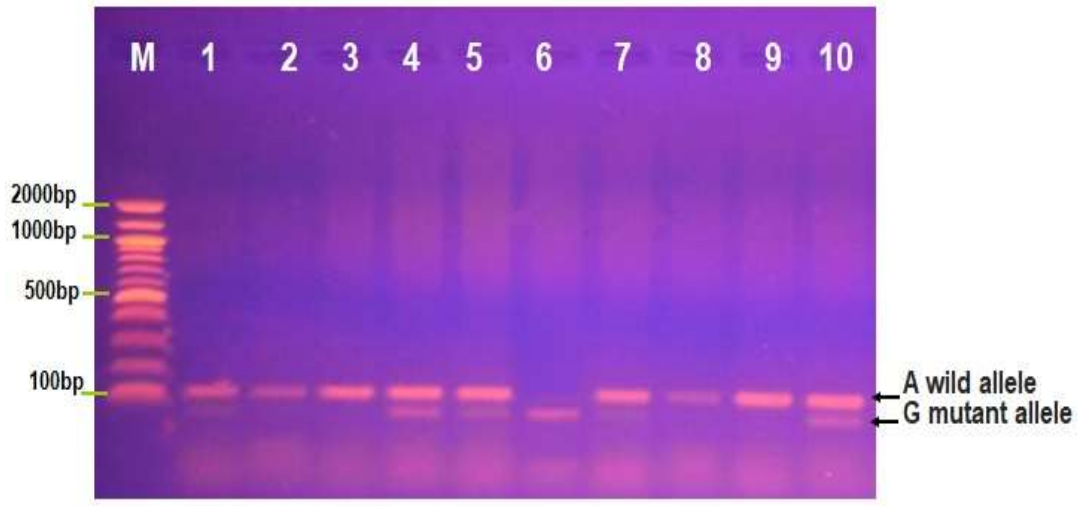
| **Genotype** | **Control group** ***n* = 60** | **Study group** ***n* = 60** | $\chi^2$ | ***P**** |
|---|---|---|---|---|
| AA | 16 (26.7 %) | 6 (10.0 %) | 14.879 | 0.001 HS |
| AG | 30 (50.0 %) | 20 (33.3 %) | | |
| GG | 14 (23.3 %) | 34 (56.7 %) | | |

*n*: number of cases; *: Chi-square test; HS: Highly significant at $P \leq 0.01$

Table (7): Comparison of IL12-A rs583911 alleles between control and study groups

| **Allele** | **Control** ***n* = 120** | **Study** ***n* = 120** | ***P*** | **OR** | **95% CI** | **EF** | **PF** |
|---|---|---|---|---|---|---|---|
| A | 62 (51.7 %) | 32(26.7 %) | 0.002 HS | 0.34 | 0.20 - 0.58 | --- | 0.40 |
| G | 58 (48.3 %) | 88 (73.3 %) | | 2.94 | 1.71 -5.05 | 0.40 | --- |

*n*: number of alleles; OR: Odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; *: Chi-square test; HS: Highly significant at $P \leq 0.01$




**Figure(1):Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL12-A rs583911 (A/G) at 2% agarose. Where M: marker (2000-100bp). Lane ( 1, 4, 5 7, and 10) as (A/G) heterozygote genotypes, and lane (2, 3, 8 and 9) as (AA) wild type homozygote genotypes, and lane (6) as (GG) mutant type homozygote genotypes. A allele product at 100bp and G allele product at 55bp AS-PCR product size.**