DNA repair gene XRCC1 polymorphisms in childhood acute lymphoblastic leukemia

Thesis

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Dedication

Love and devotion are

The sources of life.

- *My dear parents*
- *My beloved, sincere husband*
- *My sweet daughter Rawan*
- *My dear sister and brothers*
- *My dear friends*

I dedicate my work & my whole life to you

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common pediatric caner. (XRCC1) plays an important role in base excision and single-strand break repair, as a scaffold protein that brings together proteins of the DNA repair complex, and appears to be a candidate for cancer risk. However, studies on the association between polymorphisms in this protein and cancer have yielded conflicting results Genetic polymorphisms in this gene may contribute to the susceptibility to childhood ALL .The most common 3 polymorphisms are at codon 194 (Arg) to (Trp) (Arg194Trp), at codon 280 (Arg280His) and at codon 399 (Gln) (Arg399Gln).. We studied their effect on 30 ALL patients and 30 healthy controls regarding the incidence, toxicity and response to treatment using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). codon 194 Trp genetypes were found to contribute a significantly increased risk of ALL by 15 fold (OR=15.5, 95%CI=3.8-63.4,P-value<0.0001).Treatment toxicity with codon 399 polymorphism showed a favorable outcome than wild type (p-value 0.04).

Key Words:

- DNA repair gene
- XRCC1 polymorphisms in childhood acute lymphoblastic leukemia

List of Abbreviations

* **A**	adenine base
* **a PTT**	activated partial thromboplastin time
* **ACP**	acid phosphatase
* **ALL**	Acute lymphocytic leukemia or acute lymphoblastic leukemia.
* **ALL:MY+**	ALL with Myeloid Antigen Expression.
* **AML**	Acute myeloid leukemia.
* **AP**	apurinic or apyrimidinic
* **APE1**	endonuclease 1
* **Arg**	arginine
* **ATP**	adenosine triphosphate
* **BAL**	Biphenotypic acute Leukaemia.
* **BER**	base excision repair
* **bp**	base pairs
* **BRCT1**	breast cancer susceptibility protein-1
* **BRCT2**	breast cancer susceptibility protein-2
* **C**	cytosine base
* **c Ig**	cytoplasmic immunoglobulin
* **CALL**	Common acute lymphoblastic leukemia
* **CBC**	Complete blood count
* **CD**	Cluster of differentiation
* **CK2**	Cycline Kinase 2
* **CNS**	Central nervous system
* **CSF**	Cerebrospinal fluid
* **CT**	Computed tomography
* **DIC**	Disseminated intravascular coagulation
* **DNA**	Deoxyribonucleic acid
* **dNTPs**	Doxyribonucleosides triphosphates
* **dNTPs**	Doxyribonucleosides triphosphates
* **DRC**	DNA repair capability
* **DSB**	double-stranded break
* **EBV**	Epstein-Barr virus
* **EDTA**	Ethylenediamine tetra-acetic acid
* **EGIL**	European Group for the Immunological Classification of Leukemia
* **EM**	Electron microscopy
* **EMS**	Ethylmethane sulphate

* **FA**	Fanconi anemia
* **FAB**	French American British
* **FANCG**	Fanconi's anemia group G
* **FISH**	fluorescence in situ hybridization
* **G**	guanine base
* **Gln**	glutamine
* **His**	histadine
* **HLA-DR**	Human leukocytic antigen-DR
* **HNPCC**	hereditary nonpolyposis colorectal cancer
* **HR**	Homologous repair
* **HTLV-1**	Human thymic leukemia virus -1.
* **Ig**	Immunoglobulin
* **Kda**	kilo Dalton
* **LDH**	Lactate dehydrogenase
* **Lig III**	DNA ligase III
* **LN**	Lymph nodes
* **MDR**	multiple drug resistance gene
* **MLL**	mixed lineage leukemia gene
* **MLL**	mixed lineage leukemia gene
* **MMC**	Mitomycin C
* **MoAb**	Monoclonal antibodies
* **MPO**	Myeloperoxidase
* **mtDNA**	mitochondrial DNA
* **Myc**	Avian myelomytosis gene
* **NBS**	Nijmegen breakage syndrome
* **nDNA**	nuclear DNA
* **nDNA**	nuclear DNA
* **NER**	mismatch repair
* **NHEJ**	Non-homologous end joining
* **NSE**	non specific esterase
* **NSE:**	non specific esterase
* **NTD**	N- Terminal domain
* **OGG1**	8-oxoguanine DNA glyeosylase
* **PARP**	Poly (ADP-ribose) polymerase
* **PAS**	Periodic acid-schiff
* **PCNA**	proliferating cell nuclear antigen

* PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
* **Plts**	Platelets
* **PNK**	polynucleotide kinase
* **poI β**	DNA polymerase β
* **PT**	prothrombin time.
* **Rad 52**	Recombination protein Rad 52
* **RE**	Restriction enzymes
* **RNA**	Ribonucleic acid
* **ROS**	reactive oxygen species
* **RT-PCR**	reverse- transcriptase polymerase chain reaction
* **SBB**	sudan black B
* **SCE**	sister-chromatid exchange
* **SD**	Standard deviation
* **SNPs**	single nucleotide polymorphisms
* **SSB**	single-stranded break
* **T**	thymine base
* **TFIIH**	Transcription Factor IIH
* **Trp**	tryptophan
* **U**	uracil base
* **UV**	ultraviolet
* **V (D) J**	variable-diversity-joining segment rearrangement of heavy chain in immunoglobulin gene KU70 XRCC6
* **XPD**	Xeroderma pigmentosum, group D
* **XRCC1**	X-ray repair complementing defective repair in Chinese hamster cells 1

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a clonal disease of a lymphoblast and the most common malignancy of all childhood cancers ***(Bolufer et al., 2006).*** The development of this hematologic malignancy was suggested to arise by a combination of genetic susceptibility and the environmental exposure early development in fetal life and infancy ***(Sinnett et al., 2000).***

The DNA repair system is important to correct DNA damage induced by carcinogens and by antineoplastic agents and is critical for the maintenance of the integrity of genetic material and for the protection against mutations that may result in cancer. Genetic polymorphisms in this machinery may result in interindividual variations in the efficiency of this repair and may be responsible, at least in part, for greater cell susceptibility to chromosome breaks induced by genotoxic agents that would be inadequately repaired, thus becoming subject to malignant transformation. These alterations may also lead to changes in the response of cancer cells to antineoplastic agents, influencing the activity of these drugs ***(Batar et al., 2008).***

X-ray repair cross-complementing group1 (XRCC1) plays an important role in base excision and single-strand break repair, as a scaffold protein that brings together proteins of the DNA repair complex, and appears to be a candidate for cancer risk. However, studies on the association between polymorphisms in this protein and cancer have yielded conflicting results ***(Huang et al., 2009).***

Aim of the work

Aim of this study was to evaluate the effect of the polymorphisms of DNA repair gene (XRCC1) on risk of childhood acute lymphoblastic leukemia (ALL), and extended to detect its relation to the disease outcome.

Acute Lymphoblastic Leukaemia

Acute lymphoblastic leukemia (ALL) is clonal malignant disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow ***(Pui et al., 2004).*** The leukemic clone may exhibit features of either B-cell or T-cell commitment. The massive extra-medullary hematogenous spread categorizes the disease as an aggressive neoplasm but dramatic advances in its treatment over the past three decades have changed it from a universally fatal to an almost curable disease in 85% of cases ***(Settin et al., 2007).***

Incidence and Epidemiology:

There has been a gradual increase in the incidence of ALL in the past 25 years ***(Xie et al., 2003)*** and now it is considered the most common childhood malignancy representing nearly one third of all pediatric cancers, and about 80% of pediatric leukemias ***(Ziegler et al., 2005).***

Most childhood leukemias are diagnosed under the age of 8 years, with reports of peak incidences ranging from 2 years to 5 years**,** followed by falling rates during later childhood, adolescence and young adulthood. The incidence rates raise again, beginning in the sixth decade and reaching a peak in the elderly ***(Pui, 2001).***

For unexplained reasons, the incidence of ALL is substantially higher for white children than for black

children, with a nearly 3-fold higher incidence at 2 to 3 years for white children compared to black children ***(Smith et al, 2006).*** ALL occurs slightly more frequently in boys than in girls. This difference is most pronounced for T-cell ALL ***(Noriko et al., 2006).***

Pathophysiology:

There are two general mechanisms of leukemia induction. **The first** involves activation of Proto-oncogene or creation of a fusion gene with oncogenic properties in leukemias, including ALL, chromosomal translocations occur regularly. It is thought that most translocations occur before birth during fetal development ***(Greaves and Wiemels, 2003).*** These translocations may trigger oncogenes to "turn on", causing unregulated mitosis where cells divide too quickly and abnormally, resulting in leukemia. There is little indication that propensity for ALL is passed on from parents to children ***(Pui et al., 2004).***

The second mechanism involves the loss or inactivation of gene where protein products suppress leukemia (tumor suppressor genes), Loss of these genes leads to cells progressing through the proliferation cycle without the necessary brakes responsible for either repair or apoptosis of damaged DNA ***(Pui et al., 2004).*** About 50 tumor suppressor genes have been described e.g retinoblastoma and p53 gene ***(Macgregor, 2005).*** P53 tumor suppressor gene, one of the most frequently mutated genes in human