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Research Article

Detection the genetic polymorphism and gene expression for apoptotic genes in patients with leukemia in Mosul city

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Abstract – Apoptosis is a highly regular process of programmed cell death to remove unwanted and damaged cells. This process occurs due to several stimulations, some of them external, such as DNA damage exposed to UV and toxin and bacteria or virus infection, and also some external stimulation, like the absence of growth hormone apoptosis. Any defect in the process of apoptosis has an association with many diseases, like cancer and autoimmune disorders. The BCL-2 and BAX genes belong to the BCL-2 family. These genes regulate the process of program cell death by inhabiting the process of apoptosis or directing the cell toward death. Leukemia is the most common type of blood cancer in children younger than 15. The characteristics by rapid or slow proliferation of cancer cells depend on the type of leukemia.The objectives of this study are the determination of the genetic variation and detection of the new mutation of BAX and BCL-2 in patients suffering from leukemia in Mosul city.According to the following study, the proportion of kids with leukemia who observed the wild genotype was (20), the proportion of mutant genotypes was (30), and the proportion of heterogeneous groups was 50 compared with the healthy control group. 70% for wild genotype, 20% for the heterogeneous, and 10% for the mutant genotype.

Keywords –Apoptosis, Gene of Apoptosis, BAX, BCL-2, PCR, Mutation, Leukemia, Gene Expression, Q-PCR

I. INTRODUCTION

Apoptosis is one of the mechanisms of the body to protect against damage and prevent accumulation of malfunctioning cells. any defect in the process of programmed cell death leads to the accumulation of the damaged cell and eventually the development of cancer and other diseases such as neurodegenerative disorders and autoimmune diseases (1). There are two main pathways mediated by the process of programmed cell death activation: the extrinsic and the interstice pathways, both of which aim to stimulate the death of the damaged cells (2) the membranes of the bcl-2 family of proteins, which divide into pro-apoptotic and anti-apoptotic membranes, have an essential role in controlling apoptosis through their membranes that either stimulate or inhibit apoptosis (3). The abnormality of the anti-apoptotic membrane of the bcl-2 family or the defect of the pro-apoptotic membrane of the bcl-2 family membrane

is commonly found in a variety of cancers (4). The bcl-2 gene belongs to the bcl-2 family and has an anti-apoptotic function located on chromosome 18. when the bcl-2 gene transfers to another different chromosome, as seen in b-cell leukemia, it leads to increased production of the bcl-2 protein, which may prevent cancer cells from dying. (5). The bax gene is located on chromosome 19. Bax proteins cause membrane depolarization. this causes the release of cytochrome c through the mitochondrial intermembrane. cytochrome c then binds with effector caspase to activate cell death (7). Micro rna is small single-strand noncoding RNA molecules consist of approximately 21–25 nucleotides. Mirnas has important role in post-transcriptional control of gene expression as well as rna silencing. (9) The main function of mirna is regular gene expression by mrna silence. Dysregulation of gene expression is the main characteristic of cancer, leading to uncontrolled cell growth and proliferation. Microrna has an essential role in the different stages of tumour progression (10). Leukemia appears to require far fewer genetic changes than any other type of tumour, leading to an increase in cancer cells as a result of genetic mutations. Researchers have been able to determine the timing of the production of genetic changes during a child's life due to the genetic simplicity of leukemia, the early age of leukemia, using genetic techniques and archived patient samples, it was determined whether the majority of recurrent genetic alterations in leukemia are formed during the embryonic period (11). Leukemia is one of the most common malignant cancers in children and adults. It is a type of malignant hematologic cancer characterized by abnormal differentiation of white blood cells, which increases the rates of cancer cells in the peripheral blood and bone marrow (12).

II. MATERIALS AND METHOD

Case study**:** This study involved a 40-person sample with an age range of 2–10 years. They were referred to alhadbaa specialized hospital from october to november 2023, depending on the clinical diagnosis. The samples were divided into two groups. The first includes patients who suffered from leukemia, and the second contains 10 samples who didn't have any health problems, and this group is considered control.

Blood sample collection and storage: 5ml of venous blood withdrawn from the kids and divide to two group the first is put in EDTA tube for DNA extraction and methylation while the second put in tube contain trizol for the purpose of RNA extraction.

DNA extraction**:** DNA was extracted from all 40 samples, including the patient and control group, using a kit supplied by transgenbiotech company.

VII. DETECTION OF THE GENETIC VARIATION OF THE APOPTOSIS GENE BY TARM-PCR.

The presence of genetic variation in the bcl-2 gene was detected at the location of bcl-2-c938a rs2279115, and the presence of genetic variation in the bax gene was detected at the location of g248a rs465878 by the tarms-pcr technique (6). the reaction was initiated by adding the following components to the contents of the premix: 4 µl of DNA template, 1 µl from each primer of the bcl-2 and bax genes, and 16 µl of d.w.

Table (1): Show The Primer of The Gene Use in The PCR Program

Primer	***Sequence***
BAX –FO	*5'-CCTGGAAGCATGCTATTTTGGGCCT-3'*
BAX –RO	*5'-ACGTGAGAGCCCCGCTGAACGT-3'*
BAX –FI	*5'-GGCATTAGAGCTGCGATTGGACTGG-3'*
BAX –RI	*5'-AGTGGCGCCGTCCAACAGCAGT-3'*
BCL-2-FO	*5- CCG GCT CCT TCA TCG TCT CC-3*
BCL-2-RO	*5- CCC AGG AGA GAG ACA GGG GAA A-3*

BCL-2-FI	*5- AATAAAACCCTCCCCCACCACCT-3*
BCL-2-RI	*5- CCCTTCTCGGCAATTTACACGC-3*

Table (2) :show the PCR program for BCL-2 and BAX gene

Stage	*Temperature*	*Time*	*Number of cycle*
Initial denaturation	*94 °C*	*5 min*	*1*
Denaturation	*94°C*	*45min*	*35*
Annealing	*67 °C for BAX, 64°C for Bcl-2*	*1min*	*1*
Extension	*72*	*1min*	*1*
Final extension	*72*	*7min*	*1*
Stop reaction	*4*	*4min*	*1*

The gene expression level of BAX and Bcl-2 genes was analysed depend on q-PCR technique and the process include several steps:

IX. RNA extraction: after the process of blood drawing from the kids 750 ml of trizol mixed well with 250 ml of the blood including the patient and control sample then use kit prepared by Transgenbiotech for the process of RNA extraction then Nano drop device use to measurement the purity of extract RNA.

X. converting the extracted mRNA molecules into cDNA molecule

the extract RNA was convert to cDNA according to the procedure of Transgenbiotech kit.

IX. q-PCR reaction: for q-PCR reaction specific housekeeping genes use with the primer of gene use and as shown the following table (3)

Table (3): the primers use in RT-PCR reaction(19)

Primer	**Sequence**
housekeeping-forward	5-GTGCTCGCTTCGGCAGCA-3
housekeeping-Reversed	5-CAAAATATGGAACGCTTC-3
Bcl-2 –RT Forward	5′ CGCATCAGGAAGGCTAGAGTT 3′
Bcl-2 –RT Reversed	5′ CAGACATTCGGAGACCACACT 3′
BAX –RT Forward	5’ -AAGCTGAGCGAGTGTCTCAAG-3’
BAX –RT Reversed	5’ -CAAAGTAGAAAAGGGCGACAAC-3’

The final reaction volume was 20 µl and as shown in table (5)

Table (4) : include the final reaction volume

Component	Volume
Ultra sybr q-PCRMIX	10 µl
RT forward primer	0.5 µl
RT Reversed primer	0.5 µl
cDNA templet	4 µl
D.W	5 µl

Table (5): the program use in q-PCR reaction

Stage	Temperature	Time
Pre denaturation	95 °C	10 min
Denaturation	95 °C	15 sec
Annealing /Extension	60 °C	1 min
Melting curve analysis	95 °C 60 °C 95 °C 60 °C	15 sec 1 min 15 sec 15 sec

RESULTS

The purity of the extracted DNA sample was measured using a biodrop, and it ranged between 1.2 and 1.8, and the concentration ranged from 75 to 100 nanograms per microliter.

Detection of the genetic variation of the BCL-2-C938A rs2279115 gene by TARM-PCR

The results of the T-ARMS-PCR reaction reveal that there is a correlation between children with leukemia and the genetic variation of the BCL-2 gene at the location rs2279115.

Table (6): shows the distribution of the percentage observed for the different genotypes of the Bcl-2 gene at the location rs2279115 between the group of healthy children and children with leukemia. The A allele is normal, and the allele C is mutant.

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
AA	8	20	14	70	P = 0.0004	0.107	0.03 to 0.36
AC	20	50	4	20	P = 0.030	4.0	1.13 to 14.08
CC	12	30	2	10	P = 0.0077	10.5	1.86 to 59.2

According to the results of the study, that the allelic percentage observed as well as the frequency of the different genotypes of the BCL-2 gene at the site rs2279115.

The results for leukemia patients showed that the frequency value of the mutant genotype CC was 30%, which is the highest percentage compared to the wild genotype AA, which is 20%. The heterogeneous AC rate was 50 % compared with healthy group, 70% for wild genotype, 20% for the heterogenotype, and 10% for the mutant genotype.

the value of the OR for the mutant genotype was 10.5 it is higher than 1.0 within the probability level p=0.0077, which is considered a risk factor for the development of the disease.

Decrease or absence the level of gene expression for BCL-2 as result of mutation nucleotide deletion or hyper methylation has significant implications on leukemia(13). one of the most important apoptosis regulator is bcl-2 gene its association with cancer development(14) .the mutation in the BCL-2 gene can disrupt apoptosis affect on cell death regulation and enhance cancer progresses(15).

Detection the genetic variation of BAX gene was detected at the location of G248A rs465878 by TARM-PCR.

The results of the T-ARMS-PCR reaction showed that there is a correlation between children with leukemia and the genetic variation of the BAX gene at the location rs465878, as the result of the PCR reaction shows the appearance of genetic variation of the BAX gene in three different genotypes GG, AA, and GA.

Table (7) shows the distribution of the percentage of observations of the different genotypes of the BAX gene at the location (rs2157719) between the group of healthy children and the patients with leukemia, where the G allele is the normal allele and the A allele is the mutant allele.

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
GG	4	10	13	65	P = 0.0001	0.05	0.01 to 0.23
GA	20	50	6	30	P = 0.14	2.3	0.74 to 7.92
AA	16	40	1	5	P = 0.0008	52.0	5.15 to 52.4

The results for leukemia patients showed that the frequency value of mutant genotype AA was 40 %, which was the highest percentage compared to the Wild genotype GG, which was10%. The heterogeneous GA rate was 50% compared to the health group. 65% for wild genotype, 30% for the heterogeneous type, and 5% for the mutant genotype.

The OR value for the mutant genotype was 52.0 and for the heterozygenous genotype was 2.3, it is higher than 1.0 within a probability level P 0.0008 , which is considered a risk factor for the development of the disease.Decreases or absence the level of expression of the BAX gene as result of nucleotide deletions, mutations, or hypermethylation have important implications in many conditions. Researchers found that mutations in the BAX gene cause a reduction in the amount of BAX protein expression, which affects the process of apoptosis regulation in tumor cells like leukemia.(8)The presence of this single

nucleotide polymorphism in BCL-2 -938 C> A and BAX248G > A in patients with leukemia has essential role its increases the ability of cancer cell to survive and affect on treatment response (6)

Measuring the gene expression level of the BAX and Bcl-2 genes

the result of the study shown in table (10) and table (11), there are change in the level of expression for apoptosis genes in the patients compared within control group.

Table (10): The table shows the values of CT and the level of gene expression for the BAX gene and the housekeeping gene for patients with leukemia compare with the control group

	CT target	CT house keeping	ΔCT target	ΔCT control	ΔΔCT	Gene expression
Control	25.77	23.62	2.15	2.15	0	1
Patients	32.98	29.94	3.046	2.15	1.072	0.47

Table (11): The table shows the values of CT and the level of gene expression for the Bcl-2 gene and the housekeeping gene for patients with leukemia compare with the control group

	CT. target	CT. house keeping	ΔCT target	ΔCT control	ΔΔCT	Gene expression
Control	25.77	21.60	4.17	4.17	0	1
Patiants	31.497	29.743	1.75	4.17	-2.286	4.8

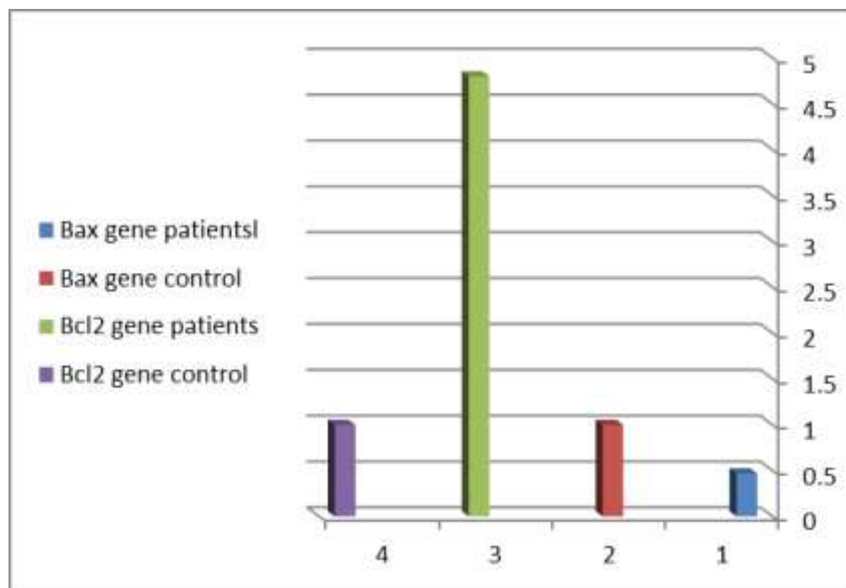

Figure (1) : shows the level of gene expression of Bcl-2 ,BAX gene in leukemia patients and the control group

The result of the study showed an increase in the level of gene expression for the Bcl-2 gene, which was 4.8 as shown in table (11), and a decrease in the level of gene expression for the BAX gene which was

0.47, as shown in table (10) ,for the kids who suffered from leukemia, compared to the level of gene expression in control samples, that was 1.0, included kids who don't have any health problems this is considered one of the main reasons for the development of the pathological condition,
so as result of increase the level of gene expression of Bcl-2 gene its can't do its function and direct the damage cell toward apoptosis(16). the abnormalities in the level of gene expression has been related to unchecked cell proliferation and resistance to apoptosis in a numbers of maligents. (17)
The change in the level of gene expression occurs for several reasons, such as different methylation patterns at the promoter region of genes and mutations of these genes. Mutation refers to a change in the DNA sequence of the cell that may be caused by mistake during cell division or exposure to a DNA damage agent like UV, or as a result of mistake during cell cycle, this is lead to synthesis defect protein enabled to perform its function.
the dysfunction in the pro-apoptotic protein like Bcl-2 and upregular of antiapoptotic protein like BAX give the cancer cells ability to evade apoptosis and further proliferation within damage or mutant DNA, thus increasing tumour progression and development. (18)

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