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Genetic Polymorphism of DNA Repair Gene (OGG1) In Iraqi Acute Myeloid Leukemia Patients

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Abstract:

Base excision repair genes are one of the most defense mechanism against mutagenic substrates. Genetic variation in these genes might be reduced the DNA repair capacities and may increase the susceptibility to many cancers like (Acute myeloid leukemia-AML). Among these genes (8-Oxoguanine DNA glycosylase-OGG1) which may modify the individual risk of developing diseases like (AML) in which the bone marrow makes abnormal myoblasts, red blood and platelets. This is the first study in Iraqi population, which aimed to find the association of (OGG1) polymorphism (ser326cys) to the susceptibility to (AML) in (68 patients and 60 healthily as control) by using (Polymerase Chain Reaction-PCR) – (Restriction Fragment Length Polymorphism-RFLP) assay.

Our results showed that the most common genotype was (CG) in both patients and controls (67.65% , 60.00%) respectively with no significant different. Also the same result found according to gender (69.44% , 65.63%) in male and female respectively. Most patients were in (30-50) years groups and with genotype (CG) (68.75%) which was highly significant than other groups. From this study it can be concluded that (OGG1) polymorphism may associated with a risk of (AML).

Key word: ***OGG1 , polymorphism , AML , PCR – RFLP.***

Introduction:

Cancer is a disease of multicellular organisms whose basis is abnormal, unregulated cell proliferation, often accompanied by abnormal accumulation of mutations [1]. Changes in many genes are required to transform a normal cell into a cancer cell; the transformation of a normal cell into a cancerous one is a multistep process. Mutations accumulate over time and over cell generations, and the susceptibility to cancer increases rapidly with age [2]. Leukemia is a cancer of the white blood cells and bone marrow. The bone marrow is the spongy inner part of bones where blood cells are made. Although leukemia starts in the bone marrow, it can spread to the blood, lymph nodes, spleen, liver, central nervous system and other organs. It does not usually form a solid mass or tumor [3]. Leukemia is characterized by uncontrolled growth of blood cells. Leukemia can be either acute or chronic. Chronic leukemia progresses more slowly than acute leukemia, which requires immediate treatment. Leukemia is also classified as lymphocytic or myelogenous.

Lymphocytic leukemia refers to abnormal cell growth in the marrow cells that become lymphocytes, a type of white blood cell that plays a role in the immune system. In myelogenous leukemia, abnormal cell growth occurs in the marrow cells that mature into red blood cells, white blood cells, and platelets. In acute leukemia, immature, functionless cells accumulate in the marrow and blood. The marrow often can no longer produce enough normal red and white blood cells and platelets [4].

(Acute myeloid leukemia- AML) also known as acute myelogenous leukemia or acute nonlymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal

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blood cells. Older people are more likely to develop AML than younger adults or children [5].

And its incidence increases with age. The real reason are still not known but like other cancer AML is a complex disease caused by the combination effect of environmental such as chemical exposure, ionizing radiation and genetic factors [6]. According to Iraqi cancer Registry 2010, leukemia ranks the 4th cancer among the commonest ten cancers. AML is slightly more common in men, with a male-to-female ratio of 1:3 [7].The malignant cell in AML is the myeloblast. In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells; a normal myeloblast will gradually mature into a mature white blood cell. However, in AML, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation. [8].

The clinical signs and symptoms of AML result from the fact that, as the leukemic clone of cells grows, it tends to displace or interfere with the development of normal blood cells in the bone marrow. [9].This leads to neutropenia, anemia, and thrombocytopenia. The symptoms of AML are in turn often due to the low numbers of these normal blood elements [10].

Some people with certain types of cancer have inherited DNA mutations from a parent. These changes increase their risk for the disease [11]. Many studies reported that DNA damage by (reactive oxygen species-ROS) in hematopoietic precursor cells influence the carcinogenesis to several cancers including acute leukemia [12]. Oxidative damage to DNA is associated with mutations that activate oncogenes or inactivate tumor suppressor genes [13]. The repair of DNA damage is under genetic control. DNA repair genes may play a key role in maintaining genome integrity and preventing cancer development [14]. One of the most important of the DNA repair system is (base excision repair -BER), and the key component of BER pathway is human 8-oxoguanine DNA glycosylase 1 (OGG1) which catalyzes the removal of 8 – Oxoguanine glycosylase. Studies on the genetic structure of OGG1 detected the presence of several polymorphisms within this locus [15]. Among them, OGG1 Ser326Cys polymorphism is of particular interest, since it may play a major role in different kinds of cancers. The amino acid substitution from serine to cysteine in codon 326 is the result of a C/G substitution at position 1245 in the 1-specific exon 7 ofOGG1[16].

This first case in the Iraqi people was designed to provide more information about the association between the polymorphisms of OGG1 on AML risk. We have found important results as regards the association of OGG1 polymorphisms and patients of AML.

Material and Methods:

The study was included 68 patientswith AML (37 male, 31 female) and 60 healthy people (32 male, 28 female). Their age rang was (16-68) years. Blood samples were taken from the Baghdad Teaching Hospital (Department of Hematology) and from (Al Mustansiriya University) / The National Center for Research and Treatment of Blood Diseases , for the period from November 2014 until February 2015.

Collection of Blood Samples:

Five milliliters of blood of each patient as well healthy human were obtained by vein puncture using 5 ml disposable syringes. The blood sample was put into EDTA tube, this blood was mixed gently and put on shaker for (5 min) then all blood samples were placed in a cooled – box under sterile circumstances and this tube was stored in the freezer (-20C°) until DNA extraction ..

Genomic DNA Extraction and Genotyping:

Genomic DNA was isolated from 2 ml whole blood collected in EDTA tubes using the Genomic DNA Purification Kits (Promega , USA).The quality of DNA in all sample were determined using (0.8%)

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agarose gel electrophoresis (Figure 1). the (Single Nucleotide Polymorphisms -SNPs) of OGG1 gene (Ser326Cys) were determined using (Polymerase Chain Reaction-PCR) – (Restriction Fragment Length Polymorphism-RFLP) assay. according to the method described by [17]. PCR amplifications were detected in a total volume of 30 µL as follow table (1)

(Table 1)The materials used in the enzyme polymerase chain reaction

Component	Reaction size
Go Taq Hot start Green Master Mix	15 µl
Template DNA	5 µL
Primers	1F 1R
Deionized Water	8 µL

The forward primerof OGG1 was F : 5'-ACTGTCACTAGTCTCACCAG -3'and reverse primer was R : 3'-TGAATTCGGAAGGTGCTTGGGGAAT -5.

Thermal cycling condi¬tions were as follows: an initial denaturation step at 94°C for 5min, 30 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and a final extension step at 72°C for 7 min.

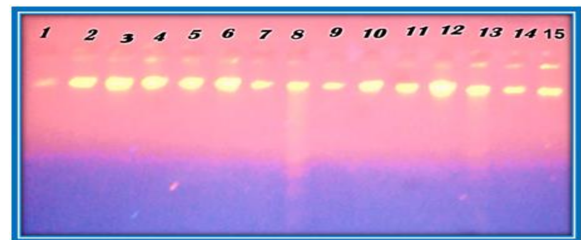
The resulting 200-bp fragment, generated by PCR, was separated on a 2% agarose gel and visualized by ethidium bromide staining to confirm its presence (Figure 2). After amplification, PCR products was digested with (Fun4HI) restriction enzyme (Promega\U.SA).The mixture was incubated at 37 °C for 24 hour using an incubator. The digestion products separated on a 3% agarose gel staining by ethidium bromide and visualized. The revealed one of three possibilities; a single band at (200 base pairs) indicating the presence of a homozygote CC allele (wild type), the presence of two fragments (100 and 200 base pairs) indicating the presence of a CG heterozygote mutant allele, and lastly band (100 base pairs) indicating the presence of an GG homozygote mutant allele (Figure 3).

StatisticalAnalysis:

The Statistical Analysis System- SAS (2012) [18] was used to effect of different factors in study parameters. (Least significant difference –LSD) test was used to significant compare between means and Chi-square test was used to significant between percentage in this study.

Results :

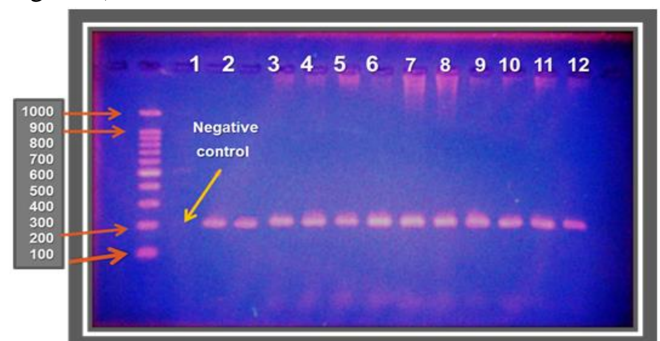
A total of (128) blood samples of (68 patient, and 60 controls) were enrolled in this study . Genomic DNA were extracted from blood and all samples observed bound in agarose when used gel electrophoresis as shown in figure (1)



(Figure 1) illustrates the stages of extraction of the total DNA samples studied

DNA bounds extracted from blood samples on the (0.8%) gel electrophoresis,stained with ethidium promide.

Gel electrophoresis of amplified DNA products showed the band of OGG1 gene at level 200 bp .(Figure 2)



(Figure 2): Agarose gel electrophoresis of PCR product (200 bp) of the OGG1 gene. The PCR product resolved by 2% agarose gel electrophoresis (70 volt/ an hour and half)

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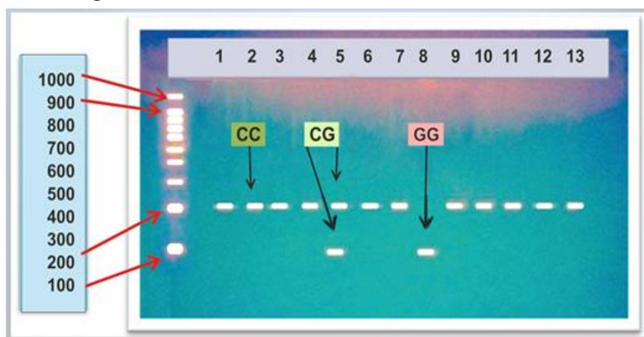
Products of amplified DNA were digested with products of amplified DNA were digested with (Fun4 H1) enzyme and the samples were then run in 3% agarose by gel electrophoresis. Homozygous wild type genotype CC was indicated by 200 bp band while the presence of (100 bp) and (200 bp) band were indicated the heterozygous mutate type CG.The homozygous mutate type (GG) exhibited the band with (100 bp) band(Figure 3)



(Figure 3): Photograph of the PCR products of the OGG1 gene after (Fun4HI) enzyme digestion and on a 3% agarose gel.

The result of OGG1 gene polymorphism distribution in both patients and controls was showed in table (2):

(Table 2):Genotype distribution of OGG1 polymorphism in AML patients and control

Group	Total	Polymorphism			P-value
		CC	CG	GG	
Patients	68	21(30.88%)	46(67.65%)	1 (1.47%)	0.0026 **
Control	60	23(38.33%)	36(60.00%)	1 (1.67%)	0.0018 **
P-value	---	0.052 *	0.263 NS	0.972 NS	---

** (P<0.01), NS: Non-significant.

The percentage of CC genotype was (38.33%) in controls and (30.88%) in patients with AML with significant difference (P<0.01).

The percentage of heterozygous and homozygous mutant were (60.00%) and (67.65%) , (1.67%) and (1.47%) respectively in controls and patients that not statistically significant. The association between the OGG1 polymorphism and gender in AML group was showed in (table 3)

(Table 3) the distribution of genotypes of the gene OGG1 by gender in AML patients

Gender	Total	Polymorphism			P-value
		CC	CG	GG	
Male	36	10(27.78%)	25(69.44%)	1(2.78%)	0.0001 **
Female	32	11(34.38%)	21(65.63%)	0(0.00%)	0.0012 **
P-value	---	0.036 *	0.391 NS	0.613NS	

* (P<0.05), ** (P<0.01), NS: Non-significant.

In male,the genotype heterozygote (CG) was 25 (69.44%), with highly significant differences between (CG) and (CC, GG) = 10 (27.70%), 1 (2.78%) respectively, (P<0.0001). In females also found that the genotype heterozygote (CG) was the most presence where = 21 (65.63%), which showed significant differences (P < 0.0012) for both (CC) 11 (34.38%) and (GG) 0 (0.00%).

The difference was not significant when compared between males and females in the distribution of polymorphism ratio of the two genetic types (CG) and (GG) patients, but the wild-type genotype (CC) was significantly (P <0.05) between males and females.
The relationship between OGG1 polymorphism and the age group in AML group was showed in table (4)

(Table 4) the distribution of genotypes of the gene OGG1 by age group in AML patients

Age group (y)	Total	Polymorphism			P-value
		CC	CG	GG	
Less of 30	17	4(23.53%)	13(76.47%)	0(0.00%)	0.0001 **
30-50	32	9(28.13%)	22(68.75%)	1(3.13%)	0.00636 **
More than 50	19	8(42.11%)	11(57.89%)	0(0.00%)	0.00581 **
P-value	---	0.0144 **	0.0152 **	0.418 NS	---

** (P<0.01), NS: Non-significant.

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Table (4) illustrated the distribution of genotypes of the gene by age groups in patients, the heterozygote pattern (CG) was the most presence (32) in the age groups (50-30), and the proportion of genotypes (CC, GC and GG) respectively where (23.53%, 76.47%, 0.00%) in the category age less than (30 years old), while the proportion of genotypes (CC, CG, GG) and respectively (28.13%, 68.75%, 3.13%) in the (50-30 years), and the percentage (42.11%, 57.89%, .00 %) in the age group (the largest of 50 years) and it was the difference of moral high (P <0.01) for these ages and all categories.

Discussion:

There are several studies indicate that many types of cancer arise because of (DNA)interactions with free radicals. The dangerous of free radicals (Reactive Oxygen Species-ROS) appear in possibility of interaction with the (DNA), which may lead to unwanted shifts in cell. where intervention of free radicals in the reactions of oxidation a quick reactions with food components.

Or with the organism's cells, which then lead to produce abnormal components to be responsible for stimulating tumor cells. Usually, gets damage in cells if increased the amount of free radicals in the cells more than the amount of anti-oxidant compounds (Antioxidant defense). And consequently to occurrence of mutation, which then may lead to activated (Oncogene), or to convert the anti-tumor genes (Tumor suppressor gene) from an effective case to the inefficient state, and may cause destruction to the cell, or transformation into a cancer cell.In spite of that oxygen molecules (O2) it is important for all organ systems, but later studies have shown that the presence of free radicals, is a product natural to the metabolism of the cells [19].That can destroy the cells to the extent that their impact can be fatal if the body fails to neutralize them . Where the damage of oxidation of DNA in particular, is one of the serious harm caused, and it is because what affected the DNA and the body failed to repair the damage .This partial contribution to the promotion of diseases like carcinogens [20]. In order to prevent this damage, possess organs of the body defensive mechanisms against (ROS). The most important of these mechanisms is to repair the damage genes (DNA repairs systems) and the most important one of them is the (Base Excision Repair-BER) repair genes

One of these genes is (Oxoguanine glycosylase-OGG1).This gene is located in chromosome (3) in short-arm on the site (25). In many human cancers, participate (OGG1) in the reform (8-oxoguanine) a damaged base formed as a result of (ROS) and the failure to remove (8- oxoguanine) may lead to mutation, which stimulates the gene (OGG1) on fission bond (glycosidic) between base that are repaired and sugar left the base demilitarized purine and pyrimidine apurinic / apyrimidinic (AP sites) in DNA. There are several studies indicate that there are several sites for multiple genotypes present in the gene (OGG1) and the most study is the location (ser326cys) associated with the biological effectiveness of this gene also this site plays a major role in various diseases.

This mutation (C / G) substitution Center (1245) in Exon (7) gene (OGG1) had occurred as a result of the replacement of the amino acid serine with cysteine in the code (326), which led to a reduction of the activity of DNA and associated allele OGG1 (326 Cys) with a higher risk of certain cancers, including lung [21].In recent years, experienced an increase in the incidence of several types of cancer, including acute myeloid leukemia disease (AML). and many studies suggest that the main cause of these diseases might be the overlap of the individual with genetic and environmental factors surrounding it. Li et al in (2013) [22] that as well as the overlap between the genetic factors of the individual and the factors environment, there is an overlap between the genes with each other. As the polymorphism of the genotype of the gene (XRCC1) on site (399) differed as to the wild style (Arg / Arg) it was allele (Gn1) the seriousness of the

throat cancer agent. The gene (APE1) showed a decrease in its impact as a risk factor for the disease rate, and that there was no difference significantly between patterns. The (OGG1) at the site (ser326cys) did not show any significant differences between the three patterns of (CC,CG,GG) although the mutant allele (GG) factor was not dangerous of the infection throat cancer.On the other hand, the increasing free radicals and the rate of progress in age and repeated infectious diseases for people, form factors that would increase the power of carcinogens and weaken the power of repair systems. So that leads to the difference of the effectiveness of the people for the prevention of carcinogens [22].The different in results many due to the sample size and this may be insufficient for statistical power to detect the effect of OGG1 gene on AML.

Conclusion:

This is the first study to determine the correlation of acute myeloid leukemia with OGG1 genetic polymorphism in the population of Iraq. Our results suggest that :The more genetic patterns of gene OGG1 in Iraqi society is heterogeneous pattern (CG).

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