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Impact of XRCC genes polymorphisms on leukemic patients

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ABSTRACT

Several single nucleotide polymorphisms (SNPs) are found to be linked with the risks of many different cancer diseases. XRCC (X-ray cross complementing) genes as DNA repair genes have effective role in numerous Cancer disease. The goal of this study was to evaluate the link between SNPs of XRCC1, XRCC2, XRCC3 and XRCC4 genes and leukemia disease in our study in Egypt. The genes polymorphisms were genotyped in 48 Leukemic Egyptian patient and 48 healthy one by (RFLP–PCR and tetra ARMS techniques) in a case-control study. For the XRCC1 gene, there is high significant difference in genotypes and alleles distribution between control and patient groups. But for the the other three genes XRCC2, XRCC3, and XRCC4, *there* were no significant difference between neither genotypes nor alleles frequency distribution between control and diseased groups (p = 0.323, 0.940, and 0.208 respectively). Our data suggest that only XRCC1 gene polymorphism may play an important role in leukemia susceptibility among Egyptians.

Introduction

It is very known that increasing number of white blood cells more than normal range (4-11) × 10^3 cells/ ml of blood is the main feature of leukemia diseases [1]. Body cells are normally unprotected from environmental mutagenic attack of the different physical and chemical carcinogenic factors. Radiation, chemical carcinogens, and fast foods represent the majority cause for producing different free radicals which lead to direct DNA damage. Insufficiency of DNA repair may lead to deletions and/or transformations of serious genes that participate in carcinogenesis mechanism [2]. There are various mechanisms to repair the DNA injury and preserve it in typical state. Some of these mechanisms is responsible for single strand breaks and others for double strand breaks [3].

A huge number of DNA repair genes are identified and a lot of them have different variants in humans among different population [4]. The danger of repair genes mutations is due to the risk of the protein function alteration and deficiency in DNA repair ability that may lead to genetic mechanism variability and carcinogenesis [5,6]. A huge number of researchers have presented strong associations between variations (SNPs) of DNA repair genes and DNA destruction and danger of different forms of human malignancy growth [7-9].

X-ray repair cross-complementation group 1 (XRCC1) is a very significant protein in DNA repairing system which uses base excision repair (BER) mechanism [10]. It plays a major role in the active healing of DNA destruction caused by alkylating agents, active oxygen, and ionization [11]. The XRCC1 gene with 17 exons is sited on chromosome 19q13.2- 13.3 [12]. This gene was identified to have 300 single polymorphisms cited in the dbSNP databank (http://www.ncbi.nlm.nih.gov/SNP), Arg194Trp SNP (exon 6, C to T substitution, and rs1799782) is one of the most frequent SNP in XRCC1 gene [13]. While, science didn't completely approve the functional impacts of these polymorphisms in XRCC1, the latest investigations demonstrated that amino acid modification at conserved areas may change its task [14]. Kinetic repair of any protein may decrease as a result of this protein biochemistry alteration.

Homologous recombination repair (HRR) is another DNA repair pathway which involves at least 16 protein components, containing XRCC2 and XRCC3 proteins [15,16]. One of the most significant SNPs in XRCC2 is located at chromosome 7q36.1 exon 3 and gives rise to a substitution from arginine to histidine at the codon 188 (31479G/A, rs#3218536). It was found to be associated with some cancer types [17].

Another important polymorphism in XRCC3 is located in exon 7. It results in an amino acid replacement at codon 241 (Thr241Met) that may disturb the protein job or its contact with other proteins involved in DNA injury and repair [18]. XRCC3 variant allele has been linked

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with increased danger of different types of cancer [19-22].
XRCC4 gene is an important component of non-homologous end-joining mechanism of DNA double strand breaks [23]. The disruption of *XRCC4* may reduce proliferation and chromosomal instability as shown in a study for an animal model [24].
Several inconsistent studies have linked polymorphisms in both XRCC3 and XRCC4 to lung cancer risk, including rs861539, rs6869366 (G-1394T), and rs1799796 [25-27].
Since XRCC1, XRCC2, XRCC3 and XRCC4 play important role in DNA repair, we studied the functional polymorphisms of them in association with Leukemia risk in an Egyptian population.

Subjects and methods

The current study included 96 age matched volunteers divided into two groups (48 controls and 48 of different leukemia patients). Samples were collected between January 2012 and September 2012 from oncology clinics of Tanta cancer institute Tanta University. The detailed history of the volunteers was taken with the consents of them. For each case, history, full clinical examination, routine laboratory investigations and specific laboratory investigations (detection of XRCC1 gene polymorphism (rs1799782) and XRCC3 (rs861539) were done. The patients included in this work were chosen to have family history of malignancy, pure leukemia with no metastasis or other malignancy, and with no chronic diseases as diabetes or hypertension.
The patients were evaluated according to the type of disease, their sex and age. The clinical pathological features of the patients were summarized in **Table 1**. The healthy people were chosen with no signs or symptoms of leukemia. They were randomly selected from Alborg laboratory in Tanta. The study protocol was approved by the ethical committee of Zagazig University, and informed consent for the experimental use of specimens was obtained from all participants.

Data collection

The clinical archive files of different leukemic patients were available as a source for their data. Also, the volunteers fulfilled a questionnaire about their medical and familial histories in particular. Leukemic patients completed the questionnaire at the time of clinic meeting while controls were interviewed at the laboratory at the time of enrollment.

Blood collection and biochemical assay

3 ml of blood sample was withdrawn from every case carefully and was collected in an EDTA containing tubes for isolation of DNA.

DNA Isolation

DNA was isolated from EDTA blood using a spin column technique according to the protocol TIAN amp Genomic DNA Kit; TIANGEN BIOTECH (BEIJING) CO.LTD. The quality of the genomic DNA was tested using agarose gel electrophoresis. DNA was stored at -20 C till the time of use.

Genotyping for XRCC1 (Arg194Trp) (C>T) rs#1799782

Part of extracted DNA was used for the detection of XRCC1 Arg194Trp polymorphism using tetra-primer amplification refractory mutation system (t-ARMS-PCR). This method is rapid, simple, and economical for SNP analysis based on allele-specific primers. In this method four primers are necessary to amplify a larger fragment from template DNA comprising the SNP and two smaller fragments representing each of the two allele-specific products.
The genomic sequences of genes were taken from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). For this polymorphism, we used two external primers (Forward outer and Reverse outer) and two allele-specific internal primers that were designed in opposite orientation (Forward inner and Reverse inner) for detection of each allele. The allele-specific amplicons have different lengths and can be separated easily by standard gel electrophoresis. PCR reaction was done in a final volume of 20 µl containing 10 µl (2x PCR Master mix solution i-Taq TM) containing (i-Taq TM DNA Polymrase (5u/µl) 2.5 U,dNTPs 2.5 mm each , PCR reaction buffer 1x ,(gel loading buffer 1x) + 5µ of working primer soln (1 of the outer primers: 10 internal primers)+ 5µ of DNA sample.
The tetra primer ARMS-PCR primer sequences, the annealing temperature, and the amplicon sizes are listed in **Table 2.**
The cycling conditions for PCR program were 6 min at 95^{o}C for 5 s for activation followed by 35 cycles of 95^{o}C for 30 s for denaturation, 63^{o}C for 30 s for annealing, 72^{o}C for 30s for elongation and a final cycle 72^{o}C for 10min for final elongation. PCR products were separated by standard electrophoresis on 2% agarose gel with ethidium bromide.

Genotyping for XRCC2 G>A (Arg188His) rs#3218536

All samples were analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) for detection of XRCC2 (Arg188His) polymorphism using Hph I restriction enzyme (NEW ENGLAND BioLabs).
PCR reaction was done in a final volume of 20 µl containing 10 µl (2x PCR Master mix solution i-Taq TM) containing (i-Taq TM DNA Polymerase (5u/µl) 2.5 U, dNTPs 2.5 mm each, PCR reaction buffer 1x), (gel loading buffer 1x) , 5µ of working primer soln, and 5µ of DNA sample. The primer sequences, the annealing temperature, and the amplicon sizes are listed in **Table 2**.
The Thermal cycling was performed as following: initial activation at 95°C for 5 min, followed by 35 amplification cycles of three steps consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 10 min.
5 µl of each PCR product with 290 bp were digested with 1µl of Hph I (NEW ENGLAND BioLabs Inc) at a temperature of 37°C for 24h for detecting SNP of XRCC2.

The choosing of the restriction enzyme was done by using one of the web programs for restriction enzyme choosing (http://www.insilicase.co.uk/Default.aspx) which help to choose the suitable restriction enzyme by inputting the sequence around the variant to the program for choosing the suitable restriction enzyme.
Restriction products were subjected to electrophoresis in 2 % agarose gel with ethidium bromide (1 mg/ml) for visualization under ultraviolet light and three genotypes were detected: GG (280 bp), AA (144-146 bp) and GA(280-144-146bp) **Fig 1**.

Genotyping for XRCC3 (Thr241Met) (C>T) rs #861539

Another part of extracted DNA was used for the detection of XRCC3 Thr241Met polymorphism using (RFLP-PCR). PCR reaction was done in a final volume of 20 µl containing 10 µl (2x PCR Master mix solution i-Taq TM) containing (i-Taq TM DNA Polymrase (5u/µl) 2.5 U, dNTPs 2.5 mm each , PCR reaction buffer 1x, (gel loading buffer 1x), 5µl of working primer soln, and 5µ of DNA sample. The primer sequences, the annealing temperature, and the amplicon sizes are listed in **Table 2**.

Table 1: The clinical-pathological features of the patients

Group	Mean ± SD Age	SE	Range	Sex	Percent	Type of disease	percent
Control	27.92 ± 7.10	1.02	16—45	26 male	54.2%		
				22 female	45.8%		
Patient	39.58 ± 21.00	3.03	5 – 80	24 male	50%	ALL	27.1%
						AML	4.2%
				24 female	50%	CLL	20.8%
						CML	47.9%

Leukemic patients hematological parameters				
	Mean ALL	**Mean AML**	**Mean CLL**	**Mean CML**
WBC /cumm	50110	66570	46540	35680
Lymphocytes /cumm	25670	30100	27680	24930
Hb gm%	7.2	4.7	5.9	5.4
Platelets /cumm	87655	70890	98770	77840

Table 2: Designed primers used in the study for all XRCC genes

SNP	Primer sequence	Restriction enzyme	Annealing temperature (°C)	Fragments' length
XRCC1 Arg194Trp	**FO: 5'-CGTCCCAGGTAAGCTGTAC-3' RO: 5'-CACTCCTATCTATGGGACACAG-3' FI: 5'-CGGGGGCTCTCTTCTTCATCC-3 RI: 5'-CACCTGGGGATGTCTTGTTGATACA-3'**	-	**63°C for 30 sec**	**Outer: 471 Arg: 297 Trp: 219**
XRCC2 Arg188His	**F: 5'-TGT AGT CAC CCA TCT CTC TGC-3' R: 5'-AGT TGC TGC CAT GCC TTA CA-3'. PCR product: 290 bp**	**Hph I**	**55°C for 30 sec**	**GG: 280 -10 AA 144-146 GA: 280-144-146**
XRCC3 Thr241Met	**F: GCCTGGTGGTCATCGACTC R: GCTTCCGCATCCTGGCTAAA PCR product :211bp**	**NcoI**	**60°C for 30 sec**	**CC= 211 TT= 97-114 CT= 211-114-97**
XRCC4 G-1394T	**F: 5'-GAT GCG AAC TCA AAG ATA CTG A-3' R: 5'-TGT AAA GCC AGT ACT CAA ACT-3' PCR product :300bp**	**HincII**	**53°C for 30 sec**	**GG= 100-200 TT= 300 GT= 300-200-100**

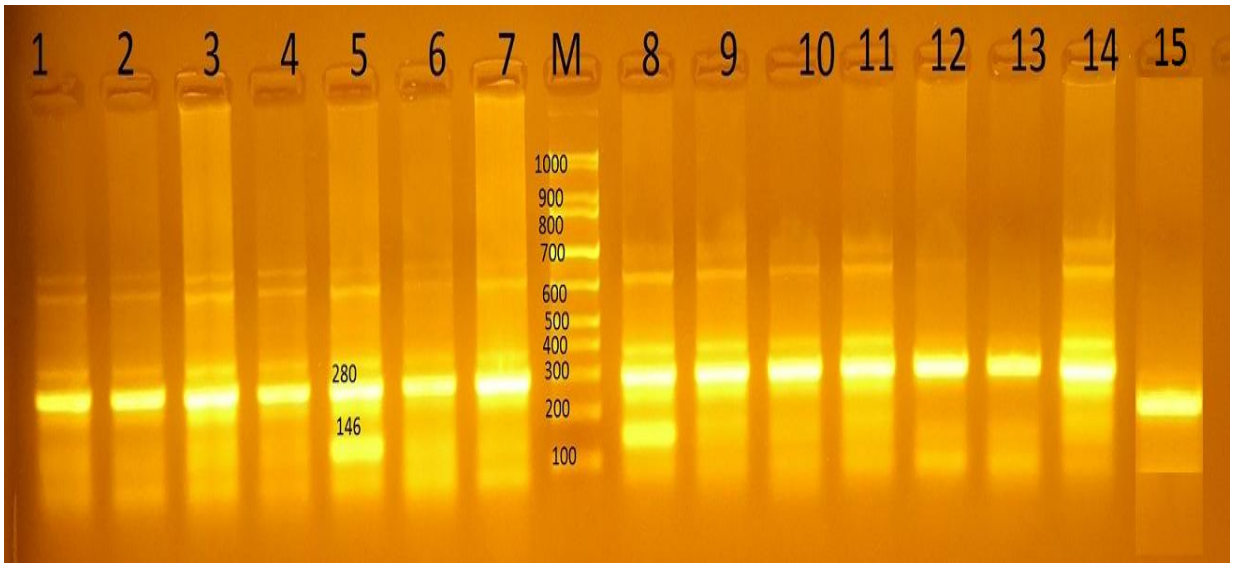

Fig. 1: Representative agarose gel electrophoresis results for XRCC2 (R188H) Polymorphism in Leukemic patients by the RFLP–PCR method. Lane M marker (100 bp), lanes 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 13 and 14 are (GG) genotype, lanes 5 and 8 are (GA) genotype, and lane 15 is (AA) genotype.

The cycling conditions for PCR program were 5 min at 95^{o}C for activation followed by 35 cycles of 94^{o}C for 30 s for denaturation, 60^{o}C for 30 s for annealing, 72^{o}C for 30s for elongation and a final cycle 72^{o}C for 10min for final elongation.

5 µl of PCR products of 211 bp was digested overnight with the restriction enzyme NcoI (NEW ENGLAND BioLabs).

The choosing of the restriction enzyme was done by using one of the web programs for restriction enzyme choosing (http://www.insilicase.co.uk/Default.aspx) which help to choose the suitable restriction enzyme by inputting the sequence around the variant to the program for choosing the suitable restriction enzyme.

Restriction products were subjected to electrophoresis in 2 % agarose gel with ethidium bromide (1 mg/ml) for visualization under ultraviolet light and three genotypes were detected: wild homozygous (211 bp), mutant homozygous (97-114 bp) and heterozygous (211-114-97bp**) Fig 2**.

Genotyping of XRCC4: G-1394T (rs 6869366)

All samples were analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) for detection of XRCC4 (G-1394T) polymorphism using HincII (NEW ENGLAND BioLabs).

PCR for all polymorphisms were done in a final volume of 20 µl containing 10 µl of 2x PCR Master mix solution i-Taq TM containing {i-Taq TM DNA Polymrase (5u/µl), 2.5 U,dNTPs 2.5 mm each , PCR reaction buffer 1x ,gel loading buffer 1x} + 5µ of working primer soln.+ 5µ of DNA sample. The primer sequences, the annealing temperature, and the amplicon sizes are listed in **Table 2**.

The Thermal cycling was performed as following: initial activation at 95°C for 5 min, followed by 35 amplification cycles of three steps consisting of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72^{o}C for 30 s, and a final extension at 72^{o}C for 10 min.

5 µl of each PCR product of 300 bp was digested with 1µl of HincII at a temperature of 37°C for 24h for detecting SNP of XRCC4.

The choosing of the restriction enzyme was done by using one of the web programs for restriction enzyme choosing (http://www.insilicase.co.uk/Default.aspx) which help to choose the suitable restriction enzyme by inputting the sequence around the variant to the program for choosing the suitable restriction enzyme.

Restriction products were subjected to electrophoresis in 2 % agarose gel with ethidium bromide (1 mg/ml) for visualization under ultraviolet light and three genotypes were detected: GG (100-200 bp), TT (300 bp) and GT(300-200-100bp**) Fig 3**.

Statistical analysis

All data were analyzed statistically using Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 23 software). Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Categorical variables are expressed as frequencies and percentages. The independent t test and one way ANOVA were used to compare quantitative data. Correlation & regression analysis were used to study the relation between numerical variables, Chi-square was used to examine the relationship between categorical variables. Odds ratio test was studied under 5 models (allelic- homozygote-heterozygote-dominant-recessive) with confidence interval 95%, also HardyWeinberg equilibrium test used for categorical variables. P-value <0.05 was considered significant difference and P-value <0.001was considered highly significant difference. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 23).

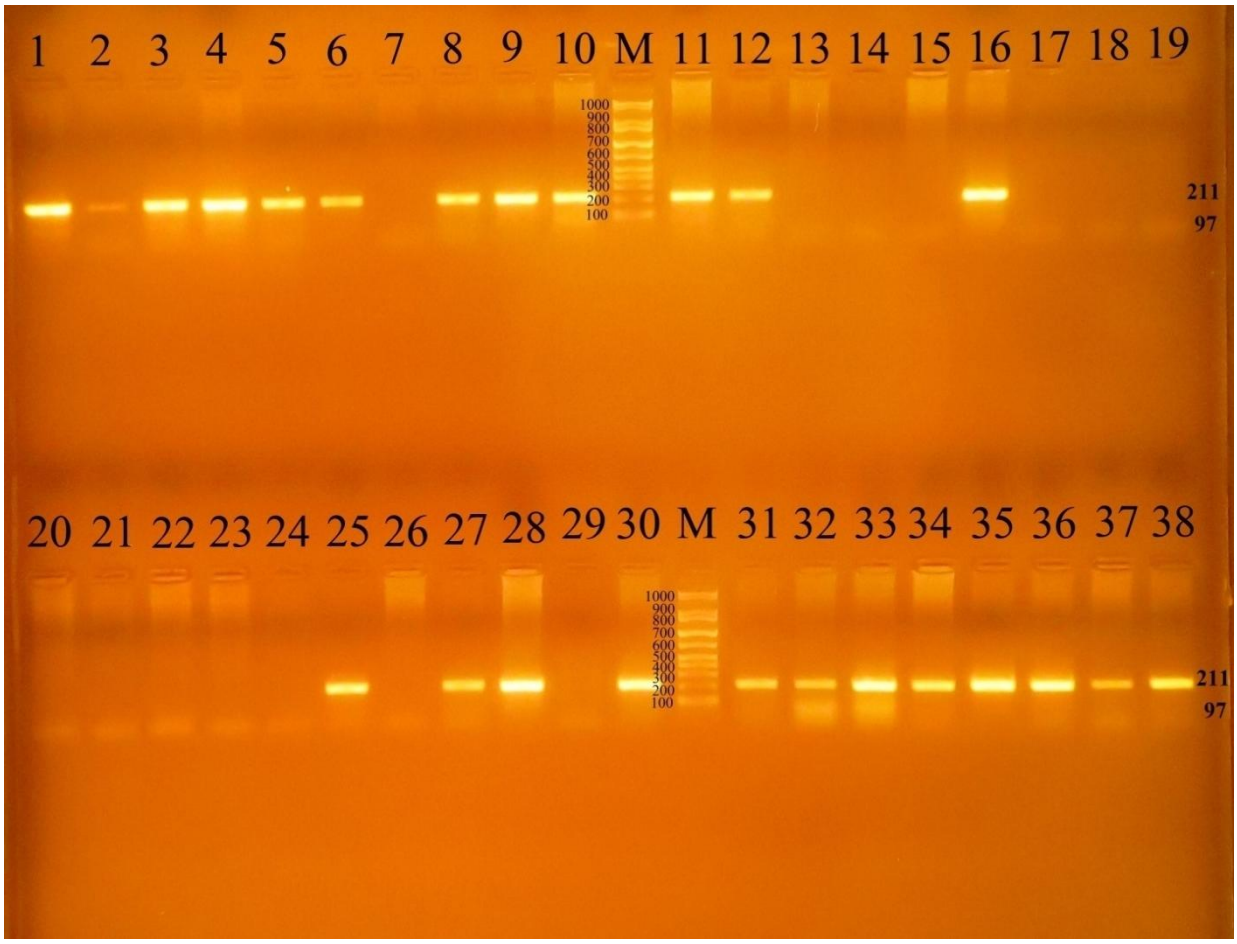

Fig. 2: Representative agarose gel electrophoresis results for XRCC3 (C/T) Polymorphism in Leukemic patients by the RFLP–PCR method. Lane M marker (100 bp), lanes 1→6, 8, 9, 11, 12, 16, 25, 27, 28, 30, 31 and 34→38 are (CC) genotype,and lanes 10, 32 and 33 are (CT) genotype.

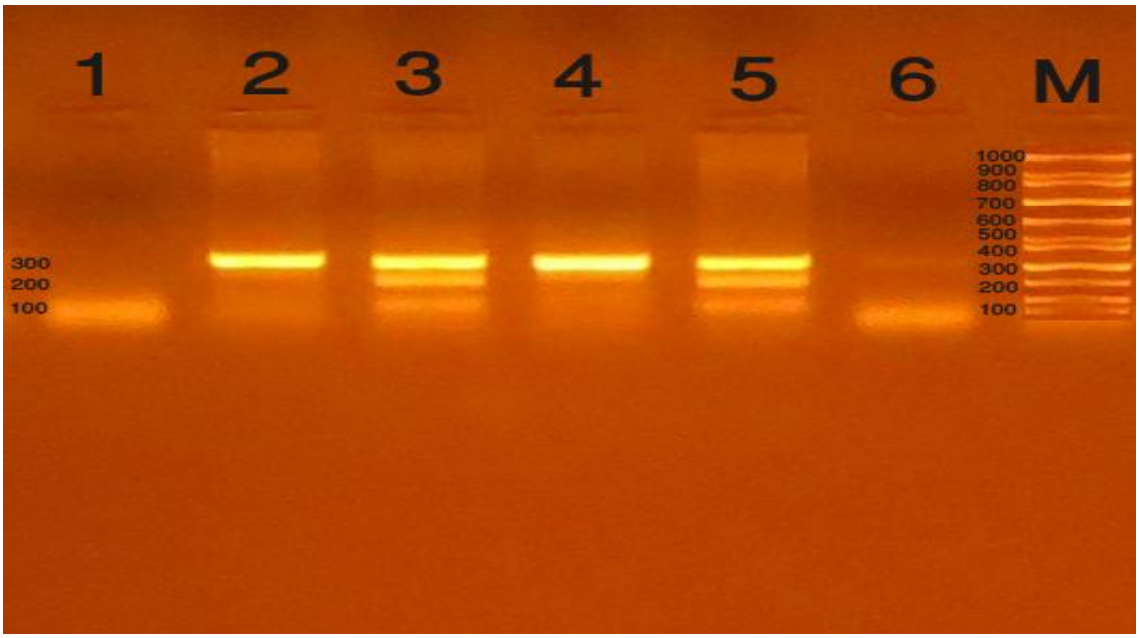

Fig. 3: Representative agarose gel electrophoresis results for XRCC4 (**G-1394T**) Polymorphism in Leukemic patients by the RFLP–PCR method. Lane M marker (100 bp), lanes 2, and 4 are (TT) genotype, lanes 5 and 10 are (GG) genotype, lanes 7 and 9 are heterozygote (GT) genotype.

Results

XRCC1 (Arg194Trp) polymorphism and Leukemia disease risk

The genotype frequencies of homozygous (CC), heterozygous (CT), and homozygous mutated (TT) were 4.2, 58.3, and 37.5% in patients with Leukemia respectively; and 64.6, 18.8, and 16.7% in controls, respectively. Generally there was a significant difference in the genotypes frequencies of the XRCC1 polymorphism between control and leukemic patient (P = 0.000**) (**Table 3)**.

The frequency of C allele was 33.33 % in leukemic patients and 73.96 % in controls **(Table 3)**. Regarding the risk of development of leukemia the CC wild type genotype and C wild type allele were taken as references. These data suggested that the C allele was high significantly associated with an decreased risk of leukemia in all the genetic models (p< 0.05) except the heterozygote model (OR: 1.3827% CI: (0.451- 4.244) (P= 0.570) which was associated with increased risk for leukemia. **(Table 4)**.

XRCC2 (Arg188His) polymorphism and Leukemia disease risk

The genotype frequencies of homozygous (GG), heterozygous (GA), and homozygous mutated (AA) were 43.8, 52.1, and 4.2% in patients with Leukemia respectively; and 29.2, 66.6, and 4.2% in controls, respectively. Generally there was not asignificant difference in the genotypes frequencies of the XRCC2 polymorphism between controls and leukemic patients (P = 0.323) **(Table 5)**.

The frequency of A allele was 30.21% in leukemic patients and 36.46% in controls **(Table 5).** Regarding the risk of development of leukemia the GG wild type genotype and G wild type allele were taken as references. These data suggested that the A allele was not significantly associated with an increased risk of Leukemia (OR: 0.7214% CI: (0.3957 – 1.3151) (P = 0.286) (**Table 6)**.

Table 3: Distribution of XRCC1 genotype and allele frequencies in leukemia disease patients and control subjects.

Genotype frequencies								
XRCC1	**CC**		**CT**		**TT**		**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**	**N**	**%**		
Control group	31	64.6	9	18.8	8	16.7	39.088	0.000**
Patient group	2	4.2	28	58.3	18	37.5		
Allele frequencies								
XRCC1	**C**		**T**				**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**				
Control group	71	73.96	25	26.04			31.857	0.000**
Patient group	32	33.33	64	66.67				

Table 4: Association between XRCC1 (Arg194Trp) polymorphism and Leukemia disease risk.

XRCC1	**Test of association**			
Comparison	**Odds Ratio**	**C.I (95%)**	**Pearson Chi square**	**p-value**
Homozygote comparisons (CC vs. TT)	0.0287	0.005 – 0.150	25.898	0.000**
Heterozygote comparison (CT vs. TT)	1.3827	0.451- 4.244	0.3219	0.570
Dominant model (CC/CT vs. TT)	0.3333	0.128 – 0.869	5.274	0.022*
Recessive model (CC vs. TT/CT)	0.0238	0.005 – 0.111	38.834	0.000**
Allele contrast (C vs. T)	0.1761	0.094 – 0.328	31.856	0.000**

Table 5: Distribution of XRCC2 genotype and allele frequencies in leukemia disease patients and control subjects.

Genotype frequencies								
XRCC2	**GG**		**GA**		**AA**		**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**	**N**	**%**		
Control group	14	29.2	32	66.6	2	4.2	2.260	0.323
Patient group	21	43.8	25	52.1	2	4.2		
Allele frequencies								
XRCC2	**C**		**T**				**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**				
Control group	60	62.5	36	36.46			1.140	0.360
Patient group	67	69.79	29	30.21				

Table 6: Association between XRCC2 (Arg188His) polymorphism and leukemia disease risk.

XRCC2	**Test of association**			
Comparison	**Odds Ratio**	**C.I (95%)**	**Pearson Chi square**	**p-value**
Homozygote comparisons (AA vs. GG)	0.6667	0.0838 – 5.3011	0.1484	0.700
Heterozygote comparison (AG vs. GG)	0.5208	0.2215- 1.2247	2.259	0.133
Dominant model (AA/AG vs. GG)	0.5294	0.2276 – 1.2341	2.203	0.138
Recessive model (AA vs. GG/AG)	1	1.350 – 7.4050	0.0001	1.00
Allele contrast (A vs. G)	0.7214	0.3957 – 1.3151	1.139	0.286

XRCC3 (Thr241Met) polymorphism and Leukemia disease risk (Table 7)

The genotype frequencies of homozygous (CC), heterozygous (CT), and homozygous mutated (TT) were 87.5, 8.3, and 4.2 % in patients with leukemia, respectively; and 85.4, 10.4, and 4.2 % in controls, respectively. Generally, there was not a significant difference in the genotypes frequencies of the XRCC3 (Thr241Met) polymorphism between controls and leukemia patients (P = 0.940), **Table 7**.

The frequency of C allele was 91.67 % in leukemic patients and 90.62 % in controls (Table 7). Regarding the risk of development of leukemia the CC wild type genotype and C wild type allele were taken as references. These data suggested that the neither TT genotype nor T allele were associated with an increased risk of leukemia under any genetic model (p> 0.05), **Table 8**.

XRCC4 (G-1394T) polymorphism and Leukemia disease risk

The genotype frequencies of homozygous (GG), heterozygous (GT), and homozygous mutated (TT) were 8.3, 60.4, and 31.3 % in patients with leukemia, respectively; and 4.2, 77.1, and 18.8 % in controls, respectively. Generally, there was not a significant difference in the genotypes frequencies of the XRCC4 (G-1394T) polymorphism between control and leukemia (P = 0.208), **Table 9**.

The frequency of G allele was 38.54% in leukemic patients and 42.71% in controls in **(Table 9).** Regarding the risk of development of Leukemia the GG wild type genotype and G wild type allele were taken as references. These data suggested that the T allele was not significantly associated with an increased risk of Leukemia (OR: 0.8413% CI: (0.4726 – 1.4974) (P =0.5567), **Table 10**.

Discussion

Leukemia is a dangerous disease that starts in the bone marrow. The bone marrow stem cells mature into different kinds of blood cells which either grow old or get damaged and replaced by new cells. However, the leukemic person bone marrow makes irregular white blood

cells called leukemia cells. These odd cells don't die when they must do. They do uncontrolled proliferation without death. They crowd out normal blood cells preventing them from doing their job [1].

Table 7: Distribution of XRCC3 genotype and allele frequencies in leukemia disease patients and control subjects.

Genotype frequencies								
XRCC3	**CC**		**CT**		**TT**		**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**	**N**	**%**		
Control group	41	85.4	5	10.4	2	4.2	0.123	0.940
Patient group	42	87.5	4	8.3	2	4.2		
Allele frequencies								
XRCC3	**C**		**T**				**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**				
Control group	87	90.625	9	9.375			0.651	0.799
Patient group	88	91.67	8	8.33				

Table 8: Association between XRCC3 (Thr241Met) polymorphism and leukemia disease risk.

XRCC3	**Test of association**			
Comparison	**Odds Ratio**	**C.I (95%)**	**Pearson Chi square**	**p-value**
Homozygote comparisons (CC vs. TT)	1.0244	0.1377 – 7.6200	0.0006	0.981
Heterozygote comparison (CT vs. TT)	0.8000	0.0775 – 8.4741	0.0344	0.853
Dominant model (CC/CT vs. TT)	1	0.1350 – 7.4050	0.0001	1.00
Recessive model (CC vs. CT/TT)	1.1951	0.3701 – 3.8594	0.089	0.766
Allele contrast (C vs. T)	1.1379	0.4197 – 3.0853	0.0645	0.799

Table 9: Distribution of XRCC4 genotype and allele frequencies in leukemia disease patients and control subjects.

Genotype frequencies								
XRCC4	**GG**		**GT**		**TT**		**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**	**N**	**%**		
Control group	2	4.2	37	77.1	9	18.8	3.136	0.208
Patient group	4	8.3	29	60.4	15	31.3		
Allele frequencies								
XRCC4	**G**		**T**				**Pearson Chi square**	**p-value**
	N	**%**	**N**	**%**				
Control group	41	42.71	55	57.29			0.345	0.659
Patient group	37	38.54	59	61.46				

Table 10: Association between XRCC4 (G-1394T) polymorphism and leukemia disease risk.

XRCC4	Test of association			
Comparison	**Odds Ratio**	**C.I (95%)**	**Pearson Chi square**	**p-value**
Homozygote comparisons (GG vs. TT)	0.8413	0.4726 – 1.4974	0.0359	0.8497
Heterozygote comparison (GT vs. TT)	0.4703	0.1803 – 1.2267	2.4265	0.1193
Dominant model (GG/GT vs. TT)	0.5077	0.1968 – 1.3095	2	0.1573
Recessive model (GG vs. GT/TT)	0.4783	0.0834 – 2.7440	0.7111	0.3991
Allele contrast (G vs. T)	0.8413	0.4726 – 1.4974	0.3445	0.5567

Different types of leukemia can be occurring according to the type of WBCs that is affected. Lymphoblastic and myeloblastic leukemia are two different types of leukemia affecting lymphoid and myeloid cells, respectively [28].

Moreover, the age of the person is important factor for determining the specific type of leukemia. So that, four common types of leukemia are present including Chronic lymphocytic leukemia (CLL), Chronic myeloid leukemia (CML), Acute lymphocytic (lymphoblastic) leukemia (ALL), Acute myeloid leukemia (AML) [29].

Association between cancer incidence and defect of repair capacity because of mutations or polymorphisms has been proved by many studies [30].

One of the most important categories of DNA repair genes is X-Ray Repair Cross-Complementing genes that are proved to be associated with several types of cancer [31]. This study aimed to study on the relation between four of XRCC genes (XRCC1 (Arg194Trp), XRCC2 (Arg188His), XRCC3 (Thr241Met), and XRCC4 (G-1394T)) and leukemia incidence in Egypt in our research.

Several studies discussed the relation between (XRCC1, XRCC3, and XRCC4) genes and leukemia incidence in different population other than Egyptians. However, to the best of our knowledge, this is the first study to collect the relation between these three genes and leukemia in Egyptian population. Moreover, no previous reports for relation between XRCC2 gene and leukemia all over the world. Our study is the first study to give results about this polymorphism with leukemia disease.

According to our research suggestion, the alteration of the ordinary expression and/or the protein function of the XRCC genes may be explained by the allele variations of these genes. These changes may be silent, and cause no obvious pathological change in the human body. However, they cause lower capacity of the repair system in case of hard DNA damage which may be a risk for carcinogenesis.

Modern life styles, pollution and environmental conditions can increase the risk for carcinogenesis because of genomic instability. In turn, the need for fixing this DNA damage will increase too. In those persons whose DNA repair systems cannot do at its normal effectiveness, the genetic deficiencies will increase the number of odd cells, and thus raise the danger of cancer occurrence as leukemia. This fact may also clarify chromosomal translocation happening in up to 75% of children with leukemia [32,33].

XRCC1 is an important protein using BER pathway [34-36]. Several valid SNPs in the XRCC1 have been proved to be associated with several types of cancer [37]. The functional impacts of these polymorphisms in XRCC1 are still unknown. However, it is proposed that amino acid modification at preserved regions may change its function [38]. This change in protein leads to the assumption that variant alleles may decrease kinetics repair, thereby resulting in leukemia.

Several studies discussed the correlation between XRCC1 polymorphisms and leukemia risk but the results were contradictory and inconclusive. our results are in agreement with the studies that approved the direct relation between XRCC1 gene and leukemia [39-51]. However, other reports did not accept this gene as risk factor for leukemia [52-57].

Zhang and his team [58] reported in their Meta-analysis that XRCC1 (Arg194Trp) may influence the susceptibilities of some leukemia types. This is in agreement with our results showing a significant difference in the genotypes frequencies of the XRCC1 **(**Arg194Trp**)** polymorphism between control and leukemic patient (P = 0.000).

XRCC3, a Rad-51-related protein, is elaborated in repairing DNA double-strand breaks and maintaining chromosomal integrity by homologous recombination repair (HRR) [59-61].

Several genetic epidemiologic studies made a research focus on the role of XRCC3 gene polymorphisms as a risk factor for cancer such as breast cancer, colorectal, bladder, and leukemia [62-65].

A study in Turkish population revealed that no statistical association between leukemia and XRCC3 Thr241Met polymorphisms [66]. This result agrees with our results that showed no significant association between this

polymorphism and leukemia. However, another study in Romanian population revealed that the XRCC3 Thr241Met polymorphism may be a genetic risk factor for leukemia [67].

Although the meta-analysis studies usually give informative and conclusive results, but there was great difference in the outcome of Yan group meta-analysis (2014) [68] and Qin team meta-analysis [69]. The first suggests no association between XRCC3 Thr241Met polymorphism and leukemia risk in the overall populations. However, the second proposed an association between this polymorphism and leukemia incidence

We suggest that absence of association between leukemia risk and this substitution from threonine to methionine is due to both are of the Cβ branched type. In addition, methionine role in protein function is limited due to its sulfur atom which can bind to methyl group or any metal. So we suggest that the substitution from threonine to methionine may decrease efficiency of protein function but not to change it.

XRCC4 is a specific protein for repairing DNA double-strand breaks using non-homologous end-joining repair pathway. We investigated one common polymorphic variant of XRCC4 G-1394T (rs6869366) with the risk of leukemia incidence in Egyptian population.

Recent genetic studies suggest the link between XRCC4 gene variations and several types of cancer [70-72]. To our knowledge, there is only one study for Taiwan leukemic children investigated the role of XRCC4 (G-1394T) in 266 leukemic children [73]. They reported for the first time the association between G-1394T of the XRCC4 gene with childhood leukemia (p=0.0022). However, they disagree with our results in which we showed no significant association between this polymorphism and leukemia risk in adult Egyptian patients. The difference between our result and their results may be due to the age of the patients. In fact, the substitution from G to T nucleotide is synonymous polymorphism and so doesn't change amino acid and doesn't affect protein structure. Moreover, this polymorphism is positioned upstream of the gene and so doesn't distress protein function.

XRCC2 protein is a one of the RecA/Rad51 family that participates in homologous recombination repair system. Several recent studies approved the link between variants of XRCC2 gene and cancer [74-76]. XRCC2 variant is known as potential cancer risk factors, especially Arg188His (rs3218536) polymorphism [77-79]. However, many of these studies have low statistical power and the results remained under debate.

For leukemia, no studies have been made to investigate the association between this polymorphism and leukemia incidence. Most of studies investigated the association between leukemia risk and the RAD51 gene, a homologous gene for XRCC2 gene [80-82]. However all of these studies were inconsistent and still under debate.

Our study found no statistical association between XRCC2 Arg188His and leukemia risk in our research in Egypt. This is probably because the substitution from Arginin to Histidine amino acid alters the protein structure but didn't affect its function because both of them are polar and hydrophilic. Moreover, they are both frequent in protein active or binding site.

In conclusion, XRCC1 show significant association with leukemia incidence in Egypt in our study. Controversially, XRCC2, XRCC3, and XRCC4 polymorphisms have not significant association with leukemia disease incidence in Egypt. Ethnic differences are one of the most important factors for allele frequency differences. However, in spite of these conclusions it should be kept in mind that Well-designed studies with larger sample sizes, more ethnic groups, and more clinic types should be considered to further clarify the association. These results can be valuable as a pilot study for the comparison of the similar studies in different locations in Egypt and also in other countries. On the other hand, the number of cases in our study is limited and would need to be supported with additional studies with larger number of Egyptian leukemic patients.

Conflict of interest

Authors declare no conflict of interest.

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