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Risk and Prognostic Value of *RAD 51* and *XRCC3* Gene Polymorphisms in Egyptian Patients with Acute Myeloid Leukemia

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ABSTRACT

Background: Genetic polymorphisms have been identified in a number of DNA repair genes and damage-detoxification genes including *RAD51* and *XRCC3* repair genes. Genetic mutation affects the structure and hence functional potency of the gene product. The decreased capacity of DNA repair might lead to mutation(s) and ultimately carcinogenesis.

Objectives: The aim of this work was to determine the prevalence of *RAD51* and *XRCC3* polymorphisms among acute myeloid leukemia (AML) patients, define their role as genetic risk factors for development of AML and their correlation with the clinical presentation, laboratory data, and treatment outcome.

Material and Methods: The study was done on 94 de novo AML patients and 50 age and gender matched controls. Samples were analyzed for prevalence of *RAD51* and *XRCC3* polymorphisms using PCR-restriction fragment length polymorphism method.

Results: Allele analysis for *RAD51-G135C* and *XRCC3* gene revealed that there was higher expression of variant alleles among AML patients compared to control (p=0.04 and 0.002 respectively). *RAD51* variant alleles were more prevalent in patients with abnormal karyotype (p=0.03). This was not the case with *XRCC3*. Patients carrying variant *XRCC3* gene did worse as regards response to treatment compared to those carrying the wild gene (p-value=0.08). Median progression free survival (PFS) for all patients was 3 months (95% CI 1.15-4.85), no statistically significant difference was found in PFS between patients with wild versus those with variant *XRCC3* or *RAD51* (p=0.16-0.19 respectively). Median overall survival (OS) for all patients was 6months (95% CI 3.54-8.46), Patients with wild *XRCC3* had a trend for longer OS compared to patients with variant *XRCC3* (p=0.08).

Conclusions: *RAD51* and *XRCC3* genes polymorphism seems to play a role in the development of AML. Their expression might have a useful predictive value on the prognosis of AML patients.

Key Words: *RAD51 – XRCC3 – AML – DNA repair.*

INTRODUCTION

Failure of DNA repair leads to genetic instability that may lead to an increased susceptibility to cancer **[1,2]**. Several genes combine to control the ability of each individual to prevent and repair DNA damage. Several genetic polymorphisms were described for damage-detoxification genes and the genes responsible for DNA repair. Polymorphisms can affect protein function, promoter activity, mRNA stability, and splice variants which may affect the ability of cells to deal with DNA damage. These polymorphisms contribute to the inter-individual variation **[3]**.

One of the most important categories of DNA damage is the double-strand breaks (DSBs) which may result into either cell apoptosis or chromosomal aberrations from loss of certain genetic material. Chemotherapeutic drugs as well as ionizing radiation in addition to endogenous agents such as reactive oxygen species, defective metabolism of telomeres or replication forks encountering a single-strand break lead to several DNA double-strand breaks **[4]**. Two main pathways are utilized for repairing of the DSBs. The first is the homologous recombination (HR) and the second less accurate pathway is the non-homologous end joining **[4,5]**. Numerou s proteins participate in the HR repair pathway including the *RAD51* protein which facilitates the initial steps **[6]**. For stability of the *RAD51* protein all through the HR process, direct interaction with *XRCC3* protein is required **[7-10]**. Lacking of the *RAD51* or its co-activators such as *XRCC3* in the cell lines leads to increased susceptibility to DNA damaging

agents **[11-13]**, and increased incidence of chromosomal aberrations **[14]**. *RAD51* and *XRCC3* play vital roles in preventing genomic instability **[15-17]**.

G/C polymorphism at position-135(G135C) in the 5'untranslated region of *RAD51* gene as well as C/T polymorphism in exon 7 of the *XRCC3* gene which results in threonine-to-methionine amino acid substitution (*XRCC3* Thr241Met), are associated with increased susceptibility to AML as reported by previous studies **[18-21]**.

This study aimed at detection of *RAD51* and *XRCC3* polymorphisms among AML patients and to assess their role as genetic risk factors for development of AML and their association with the clinical presentation, laboratory data, and treatment outcome.

PATIENTS AND METHODS

A case-control study was conducted on 94 consecutive newly diagnosed AML patients who presented to the Adult Oncology Department, National Cancer Institute (NCI), Cairo University over a period of 2 years. Fifty age and gender-matched healthy individuals from donors for bone marrow transplantation were included as controls.

The diagnosis of AML was established after clinical, morphological, cytochemical, flow cytometric, cytogenetic and molecular genetic analysis. All the cases met the AML diagnosis standards. The study was approved by the Institutional Review board (IRB) of the NCI and a written informed consent was obtained from all study subjects.

Total genomic DNA was extracted from bone marrow cells of patients with AML and Control using QIAamp DNA Mini isolation kit (QIAGEN) (Cat No.: 51304) following standard procedures according to the manufacturer's instructions.

PCR-restriction fragment length polymorphism Analysis:

The 50-ul PCR amplifications reaction consisted of 50ng of genomic DNA, 150uM, of each dNTP, 10pM of each primer (*RAD51F* (5'-TGGGAACTGCAACTCATCTGG-3') and RAD51R (5'-GCGCTCCTCTCTCCAGCAG-3'), *XRCC3-F* (5'–GGTCGAGTGACAGTCCAAAC-3') and XRCC3-R (5'–TGCAACGGCTGAGGGTCTT-3'), 1.5–2.0 mM MgCl2, and 2 units of Amplitaq Gold (PE Applied Biosystems, Warrington, United Kingdom) in 1x buffer.

Amplification was performed in a PTC-100™ Programmable Thermal Controller,after an initial heat activation step at 95°C for 10min, 35 cycles were run as follow: Denaturation at 95°C for 1min, annealing temperature for *XRCC3* at 60°C and for the *RAD51* at 52°C for 1min, extension at 72°C for 1min ending with a final extension at 72°C for 10min.

For *RAD51-G135C*, 10ul of PCR products (157bp) were digested at 37°C with 10 units of MvaI (New England Biolabs, USA) in 1x buffer supplied with the enzyme. The polymorphic C allele eliminates the MvaI restriction site, and, therefore, the digestion of polymorphic samples resulted in a single band at 157bp, whereas the wild-type allele resulted in two bands at 71bp and 87bp (Fig. 1).

For *XRCC3-Thr241Met*, 10 ul of PCR products (415 bp) were digested at 37°C overnight with 10 units of NlaIII (New England Biolabs, USA) in 1X buffer supplied with the enzyme and supplemented with 100ng/ul Bovine Serum Albumin (BSA). All XRCC3 PCR products contain an internal NlaIII site; the presence of the Met polymorphism generates an additional NlaIII site resulting in 104-bp, 141-bp, and 170-bp products for the polymorphic allele and 141-bp and 274-bp products for the wild-type threonine allele (Fig. 2).

The digested products were resolved on 3% agarose gels, stained with ethidium bromide and analyzed under UV light.

Overall survival (OS) was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Progression free survival (PFS) was defined as the time from starting therapy until documented progression or death. For patients without disease progression (DP) at the time of analysis, the date of last follow-up was considered right-censored.

Statistical methods:

Data management and analysis was performed using SPSS, version 20. Categorical

data were summarized as percentages; numerical data were summarized using means and standard deviation or medians and ranges. Chi-square test and fisher exact test were used to examine the relation between qualitative variables. Odds ratio (OR) and 95% confidence interval (CI) were calculated for risk estimation. OS and PFS were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha of 0.05 level, with a 95% confidence interval.

RESULTS

Table (1) summarizes the clinical and laboratory characteristics of the studied AML patients.

The genotype distributions in AML and control together with the adjusted odds ratio (ORs) are shown in Table (2). A statistically significant high prevalence of the variant allele of *RAD 51* was found in cases when compared with controls (p=0.04). Similarly, the *XRCC3* had a statistically significant higher expression of variant alleles among AML patients (p= 0.002).

Stratification of the AML group by age at diagnosis, gender, cytogenetic risk (Table 3) revealed no statistically significant association of *RAD51* and *XRCC3* with age, total leukocytic count and hemoglobin level. Also, the wild type of *RAD51* is significantly higher in cases with normal karyotype group (p=0.03), while *XRCC3* shows no statistically significant difference.

Seventy three patients were evaluated for response: 36 (38.3%) achieved complete remission, 37 were resistant to treatment (39.4%). 21 patients (22.3%) died before time of evaluation.

The relation between *RAD51* and *XRCC3* polymorphism and response to therapy is shown in Table (4).

Patients carrying variant *XRCC3* gene showed a trend towards worse response to treatment (p=0.08).

Median PFS for all patients was 3 months (95% CI 1.15-4.85), No statistically significant difference was found in PFS between patients with wild versus variant *XRCC3* and *RAD51* (p=0.16 and 0.19 respectively).

Median OS for all patients was 6 months (95% CI 3.54-8.46), Patients with wild *XRCC3* had longer OS compared to patients with variant *XRCC3*; however it did not reach statistical significance (p=0.08). There was no statistically significant difference in OS between patients with wild and variant *RAD51* (p=0.77).

Patients carrying both variants had a PFS of 1.1 months (95% CI 0.4-3.45) compared to 3 months (95% CI 0.76-5.24) for those carrying one or no variants, a difference that did not reach statistical significance (p=0.36).

However patients carrying both variants had a statistically significant lower OS of 1.12 months (95% CI 0.7-3.45) compared to 6 months (95% CI 2.4-9.59) for patients carrying one gene or no variant (p=0.03, Figs. 3,4).

Table (1): Clinical and laboratory characteristics of 94 acute myeloid leukemia patients.

Parameter	Findings	Parameter	Findings
Age	42.59±1.58*	FAB	
Gender:			
Male	49 (52.1)**	Mo	2 (2.3)**
Female	45 (47.9)**	M1	28 (32.6)**
Splenomegaly	26 (32.1)**	M2	30 (34.6)**
Hepatomegaly	27 (33.3)**	M3	10 (11.1)**
Lymphadenopathy	7.0 (10)**	M4	15 (17.4)**
Total leukocytic count $x10^9$/L	58.53±6.66*	M5	1 (1.2)**
Hemoglobin gm/dl	7.29±0.19*	Cytogenetics	
Platelet $x10^9$/L	93.95±20.2*	Normal karyotype	70 (74.5)**
FLT3 status No: 76		t (8:21)	8 (8.5)**
Wild	58 (76.3)**	Inv (16)	3 (3.2)**
Mutant	18 (23.7)**	t (15:17)	9 (9.6)**

* Mean ± SD, ** No (%)

Table (2): Genotype distribution of *RAD51*-G135C and *XRCC3*-Thr241Met polymorphisms in 94 AML patients and 50 controls.

Parameter	Cases		Control		OR	95.0% C.I	*p*-value
	No.	%	No.	%			
RAD 51							
Wild (G/G)	69	78.41	46	92			
Heterogenous (G/C)	13	14.77	3	6	3.12	0.99 – 9.77	0.04
Homo (C/C)	6	6.8	1	2			
XRCC3:							
Wild (Thr/Thr)	30	34.9	31	62			
Heterogenous (Thr/Met)	43	50	14	28	2.77	1.35 – 5.66	0.002
Homo (Met/Met)	13	15.1	5	10			

Table (3): Genotype distribution of *RAD51* and *XRCC3* according to demographic characteristics, and cytogenetics risk of AML patients.

Parameter	*RAD 51*		*p*-value	*XRCC3*		*p*-value
	Wild	Variants		Wild	Variants	
	69 (100%)	19 (100%)		30 (100%)	56 (100%)	
Age:						
≤60	58 (84.1)	18 (94.7)	0.45	28 (93.3)	47 (83.9)	0.32
>60	11 (15.9)	1 (5.3)		2 (6.7)	9 (16.1)	
Total leukocytic count x10^9/L:						
≤100	57 (82.6)	17 (89.5)	0.72	22 (73.3)	50 (89.3)	0.07
>100	12 (17.4)	2 (10.5)		8 (26.7)	6 (10.7)	
Hemoglobin level gm/dl:						
≤8	51 (73.9)	9 (47.4)	0.06	22 (73.3)	39 (68.6)	0.81
>8	18 (26.1)	10 (52.6)		8 (26.7)	17 (30.4)	
Cytogenetic:						
Normal Karyotype	54 (78.3)	10 (52.6)	0.03	20 (66.7)	43 (76.8)	0.22
Genetic aberration	15 (21.7)	9 (47.4)		10 (33.3)	13 (23.2)	

Table (4): Relation of *RAD51* and *XRCC3* polymorphism to response in 94 adult acute myeloid leukemia patients.

Parameter	CR		Resistant to treatment		Death		OR	95% CI	*p*-value
	No.	%	No.	%	No.	%			
RAD51:									
Wild	26	37.7	27	39.1	16	23.2	1.04	0.36-2.98	0.58
Variant	7	36.8	8	42.1	4	21.1			
XRCC3:									
Wild	15	50	11	36.7	4	13.3	2.1	0.85-5.24	0.08
Variant	18	32.1	23	41.1	15	26.8			

CR: Complete remission. OR: Odds ratio. CI: Confidence interval.

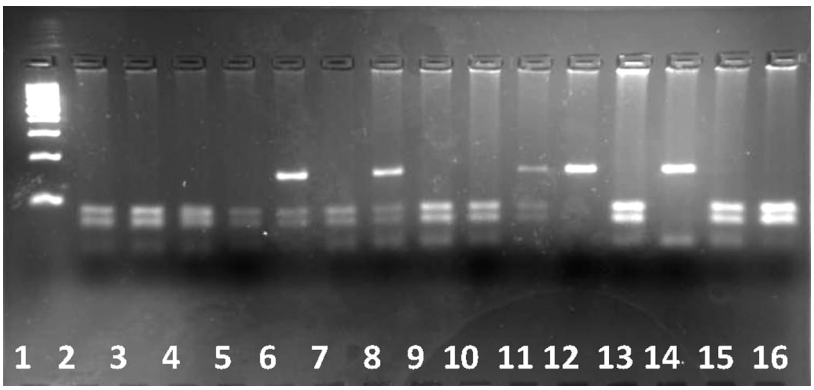

Fig. (1): Polymerase chain reaction restriction fragment length polymorphism analysis of *RAD51* gene polymorphism in AML patients.

Lane 1: Molecular weight marker 100bp with reference band 500bp.
Lanes 2, 3, 4, 5, 7, 9, 10, 13, 15 and 16: wild type: 87 and 71 bp bands.
Lanes 6, 8 and11: Heterozygous variant: 157, 87 and 71 bp bands.
Lanes 12 and 14: Homozygous variant: 157bp band.

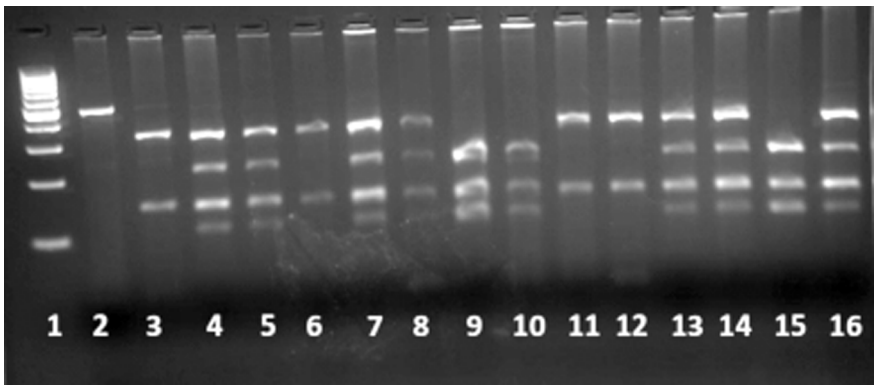

Fig. (2): Polymerase chain reaction restriction fragment length polymorphism analysis of *XRCC3* gene polymorphism in AML patients

Lane 1: Molecular weight marker 100bp with reference band 500bp.
Lane 2: Control reference uncut (415 bp band).
Lanes 3, 6, 11and 12: Wild type: 274 and 141bp bands.
Lanes 4, 5, 7, 8, 13, 14 and 16: Heterozygous variant: 274, 170, 141 and 104bp bands.
Lanes 9, 10 and 15: Homozygous variant: 170, 141 and 104bp bands.

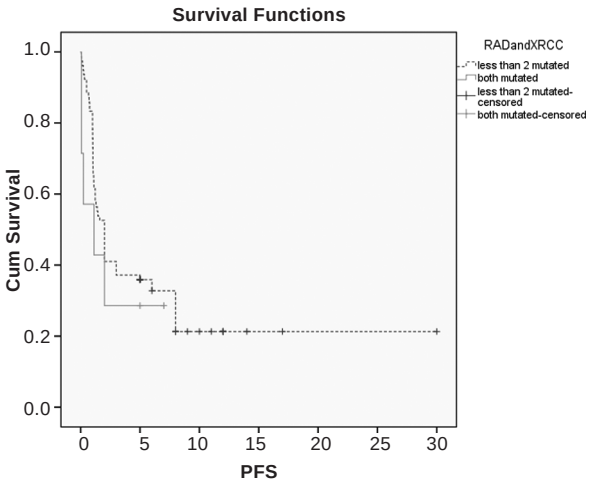

Fig. (3): Progressive free survival for patients carrying *RAD51* and *XRCC3* variants compared to those carrying one or no variant.

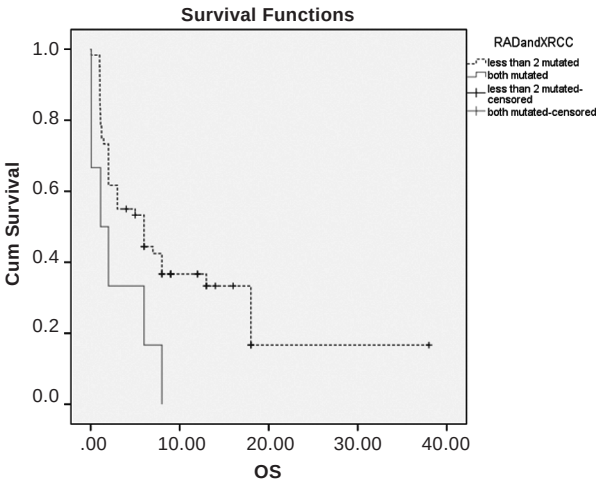

Fig. (4): Overall survival for patients carrying *RAD51* and *XRCC3* variants compared to those carrying one or no variant.

DISCUSSION

Genetic polymorphisms have been identified in a number of DNA repair genes and damage-detoxification genes including *RAD 51* and *XRCC3* repair genes.

Our findings suggest that the homologous recombination (HR) DSB DNA repair pathway may be important in the pathogenesis of AML. We have demonstrated significant higher risk for developing AML associated with the presence of polymorphisms in *RAD51* and *XRCC3* (OR=3.12 & 2.77 respectively).

We studied the distribution of polymorphisms in *RAD51* and *XRCC3* in 94 cases of AML, and 50 age and sex matched controls. Comparing the *RAD 51* polymorphism between AML patients and the control group showed that the patients had statistically significant higher expression of the variant genotype *p*= 0.04 and (OR=3.12; 95% CI 0.99-9.77). These results come in consistency with previous reports **[18,20,21]**. On the contrary, Rollinson et al. **[22]** reported that RAD51 gene polymorphism was not associated with the risk of AML.

Genotypic analysis of *XRCC3* polymorphism in AML patients revealed that 15.1% of patients expressed the homozygous variant Met/Met vs 10% in the control and 50% of patients expressed the heterozygous variant Thr/Met vs 28% of the control. These results are nearly similar to the results of Hamdy et al., **[21]** and in agreement with those of Seedhouse et al., **[18]**.

However, this was different from Voso et al., **[20]** who found that the frequency of, Met/Met and Thr/Met genotypes were 24% and 42% respectively for the patients vs. 12% and 58% respectively in the control group.

In the current study, patients with AML expressed XRCC3 variant genotype more than controls (*p*=0.002, OR 2.77and 95% CI 1.35 - 5.66). These results come in agreement with Hamdy et al., **[21]** and Voso et al. **[20]** who reported that the *XRCC3* Met allele increased the AML risk only when enzymatic activity was absent due to homozygous variant genotype, when compared with heterozygous and wild-type genotypes. On the contrary, Seedhouse et al. **[19]** reported that *XRCC3* gene polymorphism was not associated with increased risk of AML because, according to them, the sample was too small to allow conducting combined logistic regression analysis of this group.

Some studies concluded that *RAD5*1 gene polymorphism was significantly related to response to therapy, adverse effects, and prognosis of AML **[23]** and that *RAD51* gene polymorphism showed significant unfavorable outcome among AML patients **[24]**. The detection of the *RAD51* genotypes was claimed to be useful in selecting individual chemotherapy regimens for patients with AML **[23]**. However, we found no statistically significant effect of *RAD51* gene status on response to treatment (OR 1.04; 95% CI 0.36-2.97, *p*=0.58) and our results are in agreement with the another Egyptian study **[21]**.

Our patients carrying variant *XRCC3* genotype showed a trend towards worse response to treatment compared to those carrying the wild genotype (*p*=0.08). This finding is in consistency with others **[21,23]**.

In the current study, median PFS for all patients was 3ms (95% CI 1.15-4.85). No statistically significant difference was found in PFS between patients with wild versus mutated *XRCC3* (*p*=0.16) or *RAD51* (*p*=0.19).

Median OS for all patients was 6ms (95% CI 3.54-8.46), Patients with wild *XRCC3* had longer OS compared to patients with mutated *XRCC3* though not reaching statistical significance (*p*=0.08). There was no statistically significant difference in OS between patients with wild and mutant *RAD 51* (*p*=0.77).

Additionally, patients carrying both variants had a statistically significant lower OS of 1.12 months (95% CI 0.7-3.45) compared to 6 months (95% CI 2.4-9.59) for patients carrying one gene or no variant (*p*=0.03).

In Conclusion, this study highlights the importance of *RAD51* and *XRCC3* genes polymorphism as risk factors in the development of AML and its progression. Their expression might have a useful predictive value of the prognosis of AML patients.

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