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Genotyping of P53 Gene Exon 4 Codon 72 in Sudanese Patients with Myelogenous Leukaemias

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Abstract:

Background: *TP53 gene, exon 4, codon 72 Arginine (Arg)/Proline (Pro) polymorphism has been reported to be associated with the risk of many types of cancers. This study aimed to examine the association of P53 codon 72 Arg/Pro polymorphism and the risk of myelogenous leukaemias.*

Study Design: *Hospital-based case-control study.*

Materials and Methods: *A total of 80 subjects were enrolled in this study, 25 with acute myeloid leukaemia (AML), 25 with chronic myeloid leukaemia (CML) and 30 healthy controls. DNA was extracted by salting out method, and codon 72 of P53 gene was genotyped by Allele-specific polymerase chain reaction.*

Results: *In all study subjects, the genotype Arg/Arg was the most frequent followed by Arg/Pro and Pro/Pro genotypes consequently. No statistically significant association was found between both AML and CML and each of Arg/Arg genotype (OR: 0.79, CI: 0.28-2.2, P.value: 0.66 for CML and OR: 1.25, CI: 0.44-3.5, P.value:0.0.66 for AM), Arg/Pro genotype (OR:0.96, CI: 0.33-2.7, P.value: 0.94 for CML and OR: 1.3, CI: 0.48-3.8, P.value:0.543 for*

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AML), and Pro/Pro genotype (OR:0.96, CI: 0.88-1.04, P.value: 1.36 for CML and OR: 0.98, CI: 0.94-1.01, P.value:0.49 for AML).However, no significant deviation from the Hardy–Weinberg equilibrium was observed in patients and controls.

Conclusion: *P53 codon 72 Arg/Pro polymorphism is not associated with the risk of myelogenous leukaemias among Sudanese patients.*

Key words: Myelogenous leukaemias, P53 polymorphism, Sudanese

Introduction

The P53 gene locates on chromosome 17p13 and contains 11 exons. [1] The P53 tumor suppressor protein is essential in the control of cell growth, apoptosis and the maintenance of genomic stability.[2] The loss of P53 function is a key event in tumorigenesis and is associated with various characteristics of tumors, including deregulation of cell cycle, genomic instability, and resistance to chemotherapy.[3,4] The P53 gene is most frequently mutated in solid tumors.[3]A common polymorphic site in the wild type P53 gene at codon 72 of exon 4 results in translation to either an arginine residue (CGC) or a proline residue (CCC).The arginine (Arg 72) allele increases the ability of P53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro 72) exhibits a lower apoptotic potential and an increased cellular arrest in G1 of the cell cycle.[5] Studies concern with the association between P53 gene polymorphism with different types of cancers showed variable results.[6,7,8,9,10 ,11 12,13]In hematological malignancies, P53 mutations has been reported by some researcher to be less frequent, while a study in Sudan showed a significant association between P53 Arg/Pro polymorphism and lymphoid leukaemias.[14,15, 16]This study aimed to examine the association between P53 gene, exon 4, codon 72 Arg/Pro polymorphism and the risk of acute and chronic myelogenous leukaemias.

Materials and methods

Study population

A total of 50 Sudanese leukemic patients attending Radiation and Isotopes Center of Khartoum (RISK) during the period from July to December 2013 were enrolled in this study, 25 patients with Acute myelogenous Leukaemia (AML) and 25 with Chronic myelogenous Leukaemia (CML). 30 healthy volunteers were enrolled as a control group.

Sample collection and DNA extraction

Venous blood sample was collected from each subject in ethylene diamine tetra acetic acid (EDTA), and genomic DNA was extracted from all samples by salting out method.

Polymerase Chain Reaction (PCR)

P53 gene, exon 4, codon 72 Arg/Pro polymorphism was detected by Allele-Specific PCR (AS-PCR).

The primer sequences used were as follow:

Pro specific primers:
Sense primer: 5' GCC AGA GGC TGCTCC CCC 3'
Antisense primer: 5' CGT GCAAGT CAC AGA CTT 3'
Arg specific primers:
Sense primer: 5' TCC CCC TTG CCG TCC CAA 3'
Antisense primer: 5' CTG GTG CAG GGG CCA CGC 3'.

A PCR reaction mixture (20 µl) was prepared for each sample. Consisted of 3 µl genomic DNA, 0.5 µl of each primer, 4 µl "5X FIREPoL" ready to load master mix (SOLIS BIODYNE, ESTONIA) and 12 µl distilled water (D.W).

Except for annealing temperature, thermocycling conditions for both Arg and Pro alleles were identical, include initial denaturation at 94^0 C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing (at 60.0°C for 30 seconds for Arg allele and 54.0°C for 30 seconds

for Pro allele), and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes.

Amplified PCR fragments and 50 base pair (bp) DNA ladder (SOLIS BIODYNE, ESTONIA) were run on 3% agarose gel containing ethidium bromide and identified under UV transilluminator using gel documentation system (SYNGENE, JAPAN).

Statistical analysis

Data of this study was collected from patients' medical files and analyzed using Statistical Package for Social Sciences (SPSS). Frequencies of Arg/Arg, Arg/Pro, And Pro/Pro genotypes were calculated, and correlation between genotypes and study groups was investigated by Chi-square test. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit X^2 test to compare the observed genotypic frequencies in normal individuals to the expected genotypic frequencies calculated from the observed allelic frequencies.

Ethical considerations

This study was approved by RICK and faculty of medical laboratory sciences, Al Neelain University, Khartoum, Sudan, and informed consent was obtained from each patient before sample collection.

Results

This study included 50 Sudanese; 25 (50%) of them with AML and 25 (50%) with CML; of those with AML, 16 (64%) were males and 9 (36%) were females, while of those with CML 14 (56%) were males and 11 (44%) were females. Further 30 healthy individuals were included as a control group. Genotyping of P53 exon 4 codon72 was performed by AS-PCR.

As shown in figures (1 &2) the size of the amplified fragment of Pro allele was 177 bp; whereas Arg allele demonstrated a 141 bp fragment.

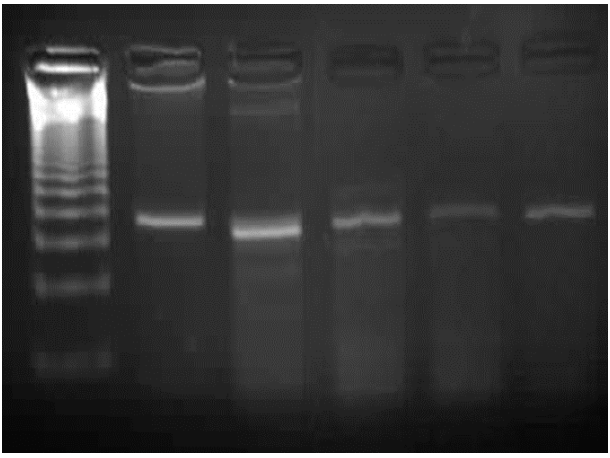

Figure (1): amplified fragment of Proline allele (171 bp)

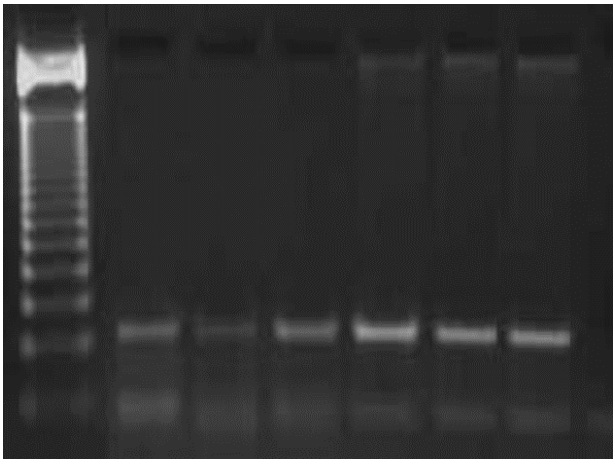

Figure (2): amplified fragment of Arginine allele (141 bp)

In both patients and control groups the genotype Arg/Arg was the most frequent, followed Arg/Pro, and Pro/Pro consequently (Table 1).

Table 1: Frequencies of P53 codon 72 genotypes in study groups

Group **Genotype**	**AML**	**CML**	**Control**
Arg/Arg	68%	68%	80%
Arg/Pro	32%	28%	17%
Pro/Pro	0%	4%	3%

No statistically significant association was found between each of the genotypes and AML or CML (Table 2),

Table 2: Association between P53 codon 72 genotypes and myeloid leukaemias

Group	AML				CML			
	O.R	CI		*P.value*	O.R	CI		*P.value*
		Lower	Upper			Lower	Upper	
Genotype								
Arg/Arg	1.25	0.44	3.5	0.66	0.76	0.28	2.2	0.66
Arg/Pro	1.3	0.48	3.8	0.543	0.96	0.33	2.7	0.94
Pro/Pro	0.98	0.94	1.01	0.49	0.96	0.88	1.04	1.36

Arg allele frequencies were 0.82 in patients with CML, 0.84 in patients with AML, and 0.88 in control group; while Pro allele frequencies were 0.18 in patients with CML, 0.16 in patients with AML, and 0.12 in control group. No deviation from the Hardy–Weinberg equilibrium was observed in patients with CML (X^2= 0.627, df =2 and P=0.79), AML (X2=0.69, df=2, and P= 0.34), and control group (X^2= 1.09, df=2, and P= 0.58).

Discussion

The functional significance of P53 polymorphisms is currently unknown. The hypothesized relationship between the codon 72 *p53* polymorphism and cancer susceptibility does not have any mechanistic basis.[17]This study is a case- control study conducted to examine the association of P53 codon 72 polymorphism with myelogenous leukaemias among Sudanese patients. The results showed that, Arg/Arg genotype was the

most frequent among all patients and control groups, followed by Arg/Pro and Pro/Pro consequently. There was no a significant association between the Arg/Arg, Arg/Pro and Pro/Pro genotypes and both AML and CML. This finding was disagree with the finding of Dunna *et al* who conducted case control study included 141 acute myeloid leukaemia (AML) and 245 control samples and conducted that, the Arg/Arg genotype was the most common in both patients and controls followed by Arg/Pro and Pro/Pro; also they found a significant correlation between Arg/Arg genotype and AML.[12]Many studies conducted on patient's different types of malignancies showed variable results; Pro/Pro genotype has been reported to be associated with increased susceptibility to stomach cancer and lung cancer.[6,18] Presence of Arg/Arg genotype has been associated with increased susceptibility to cervical cancer and ovarian cancer.[9,10] Presence of Arg/Pro genotype has been associated with increased susceptibility to breast cancer and acute and chronic lymphoid leukaemias.[8]Variations in these studies could be due to ethnic variations or due to differences in the nature of these diseases. Furthermore, Huda *et al* studied the association of P53codon 72 polymorphism with different type of cancer in Sudan; and concluded that, P53 Arg/Pro polymorphism has different pattern of frequency in different types of cancer among Sudanese patients.[19] In the present study, Arg allele frequencies were 0.82 in patients with CML, 0.84 in patients with AML, and 0.88 in control group; while Pro allele frequencies were 0.18 in patients with CML, 0.16 in patients with AML, and 0.12 in control group. In addition, no deviation from Hardy-Weinberg equilibrium was observed in all patients and control groups. These findings indicate that, P53 codon 72 Arg/Pro polymorphism is not associated with either acute or chronic myeloid leukaemias.

Conclusion

P53 codon 72 Arg/Pro polymorphism is not associated with the risk of either acute or chronic myeloid leukaemias among Sudanese.

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