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HEMATOLOGICAL FINDINGS IN SUDANESE PATIENTS WITH LYMPHOID LEUKEMIAS WITH RELATION TO *P53* ARGININE/PROLINE POLYMORPHISM

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Abstract

Background: A common polymorphism occurs at codon 72 of *P53* gene, with two alleles encoding either Arginine (CGC) or Proline (CCC) has been reported to be associated with cancer susceptibility**.**
Objective: The aim of this study was to compare the hematological findings in Sudanese patients with lymphoid leukemias with relation to *p53* gene exon 4 codon 72 Arginine /Proline polymorphism.
Materials and methods: Genomic DNA was extracted from patients blood samples by salting out method and analyzed for determination of P53 exon 4 codon 72 genotypes using -allele specific polymerase chain reaction (AS-PCR). Complete blood count was performed using automated hematology analyzer.
Result: A total of 60 subjects were enrolled in this study, 30 with acute lymphoblastic leukaemia (ALL) and 30 with chronic lymphocytic leukaemia (CLL).
In ALL patients with Arg/Arg genotype monocytes and platelets count were found significantly lower than patients with Arg/Pro and Pro/Pro genotypes. In CLL patients PCV was found significantly lower in patients with Arg/Arg genotype than those with Arg/Pro and Pro/Pro genotypes; total white blood cell count was higher in patients with Arg/Arg followed byArg/Pro and Pro/Pro consequently but the difference was not statistically significant.
Conclusion: Arg/Arg genotype was associated with significantly low monocytes and platelet count in patients with ALL, and significantly low PCV in patients with CLL.

Keywords: P53, Codon 72 Arg/Pro Polymorphism, Lymphoid Leukemia, Haematological Findings.

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INTRODUCTION

The leukemia's are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood (Hoffbrand *et al.*, 2006). The etiology of Leukaemia as appears to be multi-factorial, including the inherited mutations in DNA, and exposure to ionizing radiation, or to chemicals like benzene or cytotoxic therapy. Exposure to these carcinogens may cause DNA damage at the level of hematopoietic progenitors and develop leukemia (Weng1 *et al.*, 2012). Acute lymphoblastic leukemia (ALL) represents a clonal proliferation of immature lymphocyte precursors. The cells may be B-cell precursors (~80 to 85% of cases) or T-cell precursors (~15 to 20% of cases). In rare cases, the lineage is unclear (Kern *et al.*, 2002). In children it is the most common malignant disease and accounts for 85% of childhood leukaemia (Provan*, et al.*, 2003). Diagnosing ALL is easy if the white cell count is increased with immature lymphoid blasts in the peripheral blood. The next essential step in the diagnosis of an acute leukemia is a bone marrow biopsy with aspiration, In many cases, the bone marrow aspiration shows a dense infiltration with leukemic blasts (>50–90%). In a few cases, the infiltrate of leukemic cells is so dense that no aspiration is possible (dry tap). Immunophenotyping is usually performed by flowcytometry on either blood or a bone marrow aspirate (Munker *et al.*, 2007). Chronic lymphocytic leukaemia (CLL) is characterized by a chronic persistent lymphocytosis. Subtypes are distinguished by morphology, immunophenotyping and cytogenetics. DNA analysis may be useful in showing a monoclonal rearrangement of either immunoglobulin or T-cell receptor genes (Hoffbrand *et al.*, 2006).

P53 tumor suppressor gene, located on short arm of chromosome 17p13, is one of the most commonly mutated genes in all types of human cancers (He *et al.,* 2011). The P53 protein is a transcription factor that regulates the expression of a wide variety of genes involved in cell cycle arrest and apoptosis in response to genotoxic or cellular stress. Growth arrest or cell death prevents damaged DNA from being replicated suggesting important role played by P53 in maintaining the integrity of the genome. Loss of functional P53 during tumorogenesis likely to represent an essential step in the switch to an angiogenic to phenotype that was displayed by aggressive tumors (Donehower *et al*., 1993). A common polymorphism occurs at codon 72 of exon 4, with two alleles encoding either arginine (CGC) or proline (CCC). The arginine (Arg 72) increases the ability of P53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro 72) exhibits a lower apoptotic potential and increased cellular arrest in G1 of the cell cycle. It has been reported to be associated with cancer susceptibility. The distribution of the three genotypes (Arg/Arg, Arg/Pro and Pro/Pro) depends largely on the ethnic composition of the studied population (Zhang *et al.,* 2003). A study done by Mohamed Ahmed *et al.* (2013) concerning with the association of P53 exon 4 codon 72 Arg/Pro polymorphism with lymphoid leukaemia among Sudanese patients showed statistically significant association between the polymorphism and both acute and chronic lymphoid leukaemia (Mohamed Ahmed *et al.*, 2013).

This study aimed to compare the hematological findings in Sudanese patients with lymphoid leukaemias with relation to *p53* gene codon 72 Arginine /Proline polymorphism.

MATERIALS AND METHODS

Study subjects

A total of 60 Sudanese leukemic patients admitted to Radiation and Isotopes Center of Khartoum (RISK) during the period from November 2014- March 2015 were enrolled in this study, 30 patients (17 male and 13 female) with ALL and 30 (21 male and 9 female) with CLL.

Sample collection and DNA extraction

Blood samples were collected from all patients in ethylene diamine tetra acetic acid (EDTA) and genomic DNA was extracted by salting out method.

Table 1. Hematological findings in CLL patients

Parameter	Genotype						P.value
	Arg/Pro		Arg/Arg		Pro/Pro		
	Mean	SD	Mean	SD	Mean	SD	
Haemoglobin (g/dl)	10.6	2.6	9.5	2.5	10.3	1.8	0.6
Haematocrit (%)	35.5	7.4	29	10.1	49.7	19.2	0.013
RBCs count ($X10^3$/µl)	3.9	1.06	3.0	1.1	3.8	1.0	0.2
T WBCs (/µl)	58.380	36641	95.500	83762	50.766	2895	0.4
Platlets ($X10^3$/µl)	205	80	146	79	193	94	0.3
Lymphocyte (%)	75	16	80	6.6	77	6.5	0.5
Neutrophil (%)	19.2	9.5	16	6.3	20	5	0.5
Monocyte (%)	4.8	5.7	2.8	2.6	2.3	0.5	0.4
Eosinophil (%)	0.4	0.8	1.0	1.2	0.6	1.1	0.5

Table 2. Hematological findings in ALL patients

Parameter	Genotype						P.value
	Arg/Pro		Arg/Arg		Pro/Pro		
	Mean	SD	Mean	SD	Mean	SD	
Haemoglobin (g/dl)	7.5	2.1	8.2	2.9	8.3	1.7	0.8
Haematocrit (%)	23	6.2	26	9	26	7	0.6
RBCs count ($X10^6$/µl)	2.5	0.7	2.8	0.8	3.1	1.2	0.5
TWBCs (/µl)	27.700	6112	21.500	3401	50.000	6297	0.6
Platlets count ($X10^3$/µl)	80	52.9	66	49	210	242	0.03
Lymphocyte (%)	42.6	20.6	33.2	17.6	27.5	9.5	0.3
Neutrophil (%)	10.5	9.5	10.6	12.6	18.2	20.9	0.5
Monocyte (%)	0.58	1.08	0.00	0.00	1.25	0.95	0.01
Eosinophile (%)	0.16	0.57	0.00	0.00	0.25	0.50	0.4
Blast (%)	44.8	15	55.7	23.4	52.5	29	0.4

Polymerase Chain Reaction (PCR)

Analysis of the P53 exon 4 codon 72 polymorphism was performed 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'.

PCR reaction mixture of 25 µl was prepared for each sample. It consists of 2 µl of genomic DNA, 1 µl of each primer, 5 µl of "5X FIREPoL" ready to load master mix (SOLIS BIODYNE, ESTONIA) and 16 µl distilled water. Thermo cycling conditions for Arg allele include initial denaturation at 94 C for 3 minutes followed by 35 cycles each consists of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Thermocycling conditions for Pro allele were similar to Arg allele except that, annealing temperature was 54°C. After amplification, PCR products and 50 bp DNA ladder (SOLIS BIODYNE, ESTONIA) were run on 2% agarose gel containing ethidium bromide and identified under UV transilluminator using gel documentation system (SYNGENE, JAPAN).

Complete Blood Count

Complete blood count (CBC) was performed by automated hematological analyzer.

RESULTS AND DISCUSSION

A previous study examined the association of P53exon 4 codon 72 Arg/Pro polymorphism and lymphoid leukemia showed a significant association between Arg/Pro genotype and the risk of both ALL and CLL (Mohamed Ahmed *al*., 2013). This study aimed to compare hematological findings in ALL and CLL patients with different P53 codon genotypes. A total of 60 Sudanese patients were enrolled in this study, 30(50%) with ALL and 30(50%) with CLL.17 (57%) of patients with ALL were males and 13(43%) was female. Of those with CLL, 21(70%) were males and 9(30 %) were females. Genotyping of P53 exon 4 codon72 was performed by AS-PCR. The size of the amplified fragment of Pro allele was 177 bp; whereas Arg allele demonstrated a 141 bp fragment.

The results showed that, total white blood cell count was fount higher in Arg/Arg genotype compared with Arg/Pro and Pro/Pro genotype in CLL patients but the difference was not statistically significant.

PCV was found higher in CLL patients with Pro/Pro genotype than those with both Arg/Arg and Arg/Pro genotypes and the difference was statistically significant (Table 1). In ALL patients, platelet count and monocyte were significantly lower in patients with Arg/Arg genotype compared with those with Pro/Pro and Arg/Pro genotypes (Table 2).

In the current study, no statistically significant difference was found in red blood cell count, hemoglobin or differential count in both CLL and ALL patients with different P53 codon 72 genotypes.

Conclusion

Arg/Arg genotype was associated with significantly low monocytes and platelet count in patients with ALL, and significantly low PCV in patients with CLL.

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