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ASSOCIATION OF HUMAN TELOMERASE REVERSE TRANSCRIPTASE (HTERT) MNS16A POLYMORPHISM WITH HAEMATOLOGICAL FINDINGS LYMPHOID LEUKAEMIA IN SUDANESE PATIENTS

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ABSTRACT
Recently A minisatlite tanden repeat (MNS16A) located in the dounstream of the human telomerase reverse transcriptase (hTERT) gene has been identified and reported to have effect on the telomerase activity and Htert expression. The purpose of this study was to investigate the association between hTERT tandem repeat variants and hematological findings in lymphoid leukaemias. A total of 56 Sudanese patients with lymphoid leukaemias. 36 CLL and 20 ALL and 50 healthy volunteers acontrol group were enrolled in this study. Five (ml) of venous blood sample was collected from each subject and poured into ethylene diamine tetra acetic acid (EDTA) container for Complete blood count using automated Haematolgy analyzer and molecular analysis. DNA was extracted from anticoagulated blood sample using salting out method and the hTERT MNS16A variants were detected using allele specific polymerase chain reaction (PCR). The results showed that, there were 2 alleles in lymphoid leukemia's patients and also in the control group, these are 271bp and 302bp. Accordingly three genotypes were reported in CLL and ALL patients 271/271, 271/302 and 302/302, while only one genotype was reported in the control group 271/302 ; and there was no statistically significant association between hTERT MNS16A genotypic variants and difference in mean haematological findings of patients with lymphoid leukaemias.

KEYWORDS: minisatlite tanden, dounstream, leukaemias.

INTRODUCTION
Lymphocytic leukemias (also known as lymphoid or lymphoblastic leukemia) start in the cells that become lymphocytes. Tow main types of lymphoid leukemia; Chronic lymphocytic leukaemia (CLL) and cute lymphoblastic leukemia (ALL).

CLL is the most common form of leukaemia, mainly affects an older age group; 90% of patients are over the age of 50; it rare to occur below 40 years of age. It common in men than women.[1] A cute lymphoblastic leukemia (ALL) is the most common cancer in children, representing 23% of cancer diagnosis among children younger than 15 years of age. ALL has abimodal age distribution, peaking in children between 3and 5years of age and again in persons older than 65 years. Pediatric ALL occurs slightly more often in boys than girls and in white children more often than in black children.[1]

Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.[2]

The length of telomeric DNA ranges between 1 and 12 kb. It has a unique structure and serves as a binding site for a number of specific DNA-binding proteins. Telomere length 50-200 bps shortens with each cell division. Because DNA polymerase cannot initiate synthesis from the end of lagging strand DNA. This incomplete replication is known as "end replication problem".[4]

Researchers are interested in the possible effects of telomeres and telomerase in carcinogenesis, due to the relation between the telomeres and telomerase in the cell cycle .Telomere length alterations have been observed in many types of cancer and telomerase activation is believed to be crucial in most immortal and cancer

cells.[3,5] Hematopoietic stem cells (HSCs) depends not only on telomerase but also telomere binding proteins, the proliferative capacity of the cells, and some other determinants.[6,7]

Telomerase is composed of three essential components: catalytic protein (human telomerase reverse transcriptase- hTERT), the functional RNA component (human telomeric RNA- hTR), and telomerase associated protein 1(TEP1). The genes coding these proteins were mapped to 14q11.2, 5p15.33, and 3q26, respectively.[8, 9] Telomerase activity has been reported to be up regulated in response to cytokine-induced proliferation and cell cycle activation in primitive HSCs, while progressive down regulation is seen in more mature clones.[10]

Telomere length and telomerase expression alterations have been reported in various kinds of haematological malignancies and some of them appear to have prognostic significance[11 , 12] The aim of this study was to investigate the association between hTERT tandem repeat variants and hematological findings in lymphoid leukaemias.

MATERIALS AND METHODS

Study population and Sample collection

A total of 56 Sudanese patients with lymphoid leukaemias. 36 CLL and 20 ALL attended to radiation and isotopes center of Khartoum (RICK), Sudan, during the period from December 2014 to february 2015 were enrolled
in this study; ALL their age ranged between 4-25 years,CLL age ranged between45 -85years , In addition 50 apparently healthy volunteers were also recruited to participate in this study as a control group.

After informed consent Five (ml) of venous blood was collected from each subject in ethylene diamine tetra acetic acid (E.D.T.A) blood tube.

- Complete blood count will be performed for all patients and control subjects using automated Haematolgy analyzer.

DNA Extraction

DNA was extracted from E.D.T.A anticoagulated blood samples using salting out method.

Analysis of hTERT tandem repeat variants

The hTERT MNS16A variants were detected by Allele-specific polymerase chain reaction (TECHNE, TC412, UK). Two microliter (µl) of DNA was amplified in a total volume of 20µL containing 0.5µl of each forward primer (5'- AGGATTCTGATCTCTGAAGGGTG-3') and reverse primer (5'-TCTGCCTGAGGAAGGACGTAT -3'), 4µl Matser mix (GoTaq® Green Master Mix, Promega, USA) and 13µl sterile distilled water. The cycling conditions include initial denaturation at 95°C for 5 minutes, 35 cycles each consist of 95°C for 30 seconds, 64°C for 45 seconds, and 72°C for 1 minute, then final extension at 72°C for 10 minutes.

Four µl of the PCR product (ready to load) was electrophoresed on 2% agarose gel stained with ethedium bromide and then demonstrated by gel documentation system.

Statistical analysis

Data of this study was analyzed by statistical package for social sciences (SPSS). Correlation between hTERT tandem repeat variants and qualitative variables was tested by cross-tabulation and chi-square test. Means of hematological findings in patients with the three genotypic variants were compared by ANOVA test.

Ethical considerations

This study was approved by the faculty of medical laboratory science, Al Neelain University and informed consent was taken from each subject before sample collection.

RESULTS

A total of 56 patients attended to the RICK, Sudan, diagnosed with lymphoid leukemia's were enrolled in this study; 20 ALL patient their age ranged between 4-25 years (Mean±SD: 10.9±4.8).36 CLL patients their age ranged between 45 – 85years . 50 apparently healthy volunteers as a control group 35(70%) of them were males and 15(30%) of them were were females.

The results showed that, there were 2 alleles in lymphoid leukemia's patients and also in the control group, these are 271bp and 302bp. Accordingly three genotypes were reported in CLL and ALL patients 271/271, 271/302 and 302/302, while only one genotype was reported in the control group 271/302.

The mean of Platelet count in study subjects with CLL was 155.9 +/- 80 SD(; *P.value* : 0.4). There was no statistically significant association between PLT count and the genotype.

The mean of total white blood cell count in study subjects with CLL was 83.1+/- 71SD (; *P.value* : 0.35). There was no statistically significant association between TWBC count and the genotype.

The mean of red blood cell count in study subjects with CLL was 3.3+/- 1.1 SD(; *P.value* : 0.22). There was no statistically significant association between RBC count and the genotype.

The mean of hemoglobin measure in study subjects with CLL was 9.4 +/- 3.3SD (; *P.value* : 0.22). There was no statistically significant association between HB measure and the genotype .

The mean of packet cell volume measure in study subjects with CLL was32+/- 8.1SD (; *P.value* : 0.52).

There was no statistically significant association between PCV measure and the genotype.

The mean of mean corpuscular volume in study subjects with CLL was 90.3 +/- 9.1SD (; *P.value* : 0.34). There was no statistically significant association between MCV measure and the genotype.

The mean of mean corpuscular hemoglobin in study subjects with CLL was 28.7+/- 2.7SD (; *P.value*: 0.64). There was no statistically significant association between MCH measure and the genotype.

The mean of mean corpuscular hemoglobin concentration in study subjects with CLL was 31.2 +/-1.5 SD (; *P.value*: 0.35). There was no statistically significant association between MCHC measure and the genotype.

The mean of Platelet count in study subjects with ALL was 67.3+/- 49.9 SD(; *P.value* : 033). There was no statistically significant association between PLT count and the genotype.

The mean of total white blood cell count in study subjects with ALL was51.7 +/-45.5 SD (; *P.value* : 0.27). There was no statistically significant association between TWBC count and the genotype.
The mean of red blood cell count in study subjects with ALL was 2.5+/-0.8 SD(; *P.value* : 0.39). There was no statistically significant association between RBC count and the genotype.

The mean of hemoglobin measure in study subjects with ALL was 7.4+/-2.9 SD (; *P.value* : 0.33). There was no statistically significant association between HB measure and the genotype.

The mean of packet cell volume measure in study subjects with ALL was23.6+/-8.9 SD (; *P.value* : 0.33). There was no statistically significant association between PCV measure and the genotype.

The mean of mean corpuscular volume in study subjects with CLL was85. 1 +/-6.8 SD (; *P.value* : 0.33). There was no statistically significant association between MCV measure and the genotype.

The mean of mean corpuscular hemoglobin in study subjects with ALL was30.3 +/-4.2 SD (; *P.value* : 0.33). There was no statistically significant association between MCH measure and the genotype.

The mean of mean corpuscular hemoglobin concentration in study subjects with ALL was 26.5+/-4.3 SD (; *P.value*: 0.73). There was no statistically significant association between MCHC measure and the genotype.

HAEMATOLGICAL FINDING	CLL			ALL		
	Mean	**SD**	***P.value***	**Mean**	**SD**	***P.value***
PLT	155.9	80.1	0.4	67.35	49.9	0.33
MCHC	31.29	1.54	0.35	32.5	4.33	0.64
MCH	28.7	2.7	0.64	30.3	4.29	0.33
MCV	90.3	9.1	0.34	85.1	6.89	0.33
PCV	32	8.1	0.52	23.65	8.98	0.33
HB	9.46	3.3	0.22	7.4	2.97	0.33
RBC	3.36	1.1	0.22	2.55	0.84	0.39
WBC	83.18	71.1	0.35	51.7	45.59	0.27

DISCUSSION

In the current study we investigated the association of hTERT tandem repeat variants among Sudanese patients with CLL and ALL . The results revealed the presence of three hTERT tandem repeat genotypic variants: 271/271, 271/302 and 302/302; this based on the presence of two alleles 271bp and 302bp. All the control subjects were found to have the genotype 271/302.

The present study showed that, there was no statistically significant difference in mean haematological findings of patients with the three genotypic variants , meaning that there was no relation between hTERT genotypes and haematological finding .

CONCLUSION

The hTERT MNS16A 302\271 variant was no significantly associated with haematological findings in lymphoid leukaemias.

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