A minisatalleite tandem repeat of human telomerase reverse transcriptase (hTERT MNS16A) in Sudanese Patients with acute lymphoblastic leukaemia

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Abstract

Background: The catalytic subunit of the enzyme telomerase "the human telomerase reverse transcriptase (hTERT)" together with the telomerase RNA component (TERC) comprises the important unit of the telomerase complex. Recently A minisatlite tanden repeat (MNS16A) located in the dounstream of hTERT has been identified and reported to have effect on the telomerase activity and hTERT expression.

Objective: The purpose of this study was to detect the hTERT MNS16A variants among Sudanese patients with acute lymphoblastic leukaemia (ALL).

Materials and methods: A total of 48 Sudanese patients with ALL and 50 healthy volunteers acontrol group were enrolled in this study. Two and half milliliter (ml) of venous blood sample was collected from each subject and poured into ethylene diamine tetra acetic acid (EDTA) container for 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).

Results: patients' age ranged between 4-25 years, (Mean±SD: 10.9±4.8); 15(31.3%) of them were females and 33(68.7%) were males. Of the 50 healthy controls 35(70%) were males and 15 (30%) were females. Three hTERT MNS16A genotypic variants were observed among studied patients, which were 302\302 (25%), 271\271 (6.3%) and 271\302

(68.7%), while only one genotype was observed among control subjects which was 271\302. There was statistically significant association between the variant genotype 302/271 and ALL (O.R:2.2, 95% CI: 1.9 --2.5, *P.Value*: 0.00)

Conclusion: The hTERT MNS16A 302/271 variant was significantly associated with ALL.

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Introduction

Acute 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].

Childhood ALL comprises defferent biological subtypes defined by cell morphology, immunophenotype, gene expression features and genetic abnormalities, some of which are associated with disease aggressiveness, and treatment response. Progress in the treatment of childhood ALL over the last four decades has been steady, with cure rates (i.e.no evidence of disease for 10 years or more) now surpassing 80%. This advance can be attributed to three main factors : recognition of reliable prognostic factors leading to increasingly refined risk directed therapies, development of clinical trials designed to gain substantial increments in knowledge, and improvement in supportive care [2].

Normal human cells undergo a definite number of cell divisions when grown in culture and ultimately stop dividing and undergo what is called replicative senescence. The number of

cell divisions attained before senescence is approximately 50 divisions [3]. One main difference between young, replicating cells and their senescent counterpart is the length of specialized tails at the end of chromosomes called telomeres. Telomeres are specialized high-order chromatin structures that cap the ends of eukaryotic chromosomes. Telomeric DNA is composed of repetitions of the TTAGGG hexanucleotides that are bound to specific proteins called telomeric binding proteins. Every time a cell divides, 50 to 100 base pairs are lost and a cellular signal is eventually triggered to stop cell divisions [4].

Germline cells and to some extent stem cells and lymphocytes overcome this end replication problem and maintain cellular proliferation by expressing telomerase. Telomerase is a ribonucleoprotein complex that contains several proteins and RNA. Three human cDNA encoding the telomerase protein complex have been identified, cloned and characterized,human telomeric RNA (hTR), human telomerase reverse transcriptase(hTERT) and human telomerase associated protein 1(hTP1). hTERT gene expression holds promise in the diagnosis of malignancy because its expression is much stronger in immortalized cell lines and human malignancy than in normal or premalignant cells. In the last few years, telomerase had attracted considerable interest as a promising diagnostic marker in the distinction of benign from malignant lesions **[5].** HumanTERT is located at chromosome 5p15.33 and has 16 exons [6]. Recently, *Wang et al* found a polymorphic tandem repeat minisatellite (termed MNS16A) in the downstream of hTERT. MNS16A was demonstrated to have some promoter activity and may regulate the expression of antisense hTERT messenger RNA level as well as telomerase activity [7,8]. The expressions of telomerase can prevent the loss of telomeres [9–11].

This study aimed to investigate whether ther is an association between hTERT tandem repeat variants and risk of ALL or not.

Materials and Methods

Patients and samples

A total of 48 Sudanese patients with ALL attended to radiation and isotopes center of Khartoum (RICK), Sudan, during the period from June to September 2014 were enrolled

in this study; their age ranged between 4-25 years.In addition, 50 apparently healthy volunteers were also recruited to participate in this study as a control group.

After informed consent 2.5 milliliter (ml) of venous blood was collected from each subject in ethylene diamine tetra acetic acid (E.D.T.A) blood tube.

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 age and duration in patients with the three genotypic variants were compared by ANOVA test.

Results

A total of 48 patients attended to the RICK, Sudan, diagnosed with ALL were enrolled in this study; their age ranged between 4-25 years (Mean±SD: 10.9±4.8). 15(31.3%) of patients were females and 33(68.7%) were males. Of the 50 healthy volunteers (control group) 35(70%) were males and 15(30%) were females.

Two tandem repeat variants were found in patients with ALL, these are 302 bp variant, and 271 bp variant. Accordingly, patients were divided into three genotypic groups: 302/302, 302/271, and 271/271.

The result showed that hTERT 302\271 genotype was the most frequent (68.7%) among patients with ALL, followed by the genotype 302\302 (25%) and 271\271 (6.3%) consequently. The control group was found to have only one genotype (271\302).

There was statistically significant association between ALL and the genotype 271\302 (OR: 2.2, CI: 1.9-2.5, *P.value* :0.00) but not with the genotype 271\271 (OR: 0.419, CI: -0.126 0.963, *P.value* :0.130) or the genotype 302\302 (OR: .474, CI: -.669-1.6, *P.value* :0.413).

There was no statistically significant correlation between the genotypes and gender in patients with ALL (Table 1).

Table (1): Correlation of hTERT tandem repeat variants with gender

Gender / Genotype	Male	Female	*P.value*
302/302	8(66.7%)	4(33.3%)	0.50
302/271	22(52.2%)	11(47.8%)	
271/271	3(100%)	0(0%)	

The result showed that, there was no statistically significant difference in mean age and duration when compared in the three genotypic variants (Table 2).

Table (2): Comparison of the mean age and duration in patients with hTERT genotypic variants

Genotype	Age			Duration		
	Mean	SD	*P.value*	Mean	SD	*P.value*
302/302	11.17	4.80	0.636	32.8	11	0.635
302/271	11.03	4.97		28.4	14	
271/271	8.3	0.77		29.7	20	

Discussion

MNS16A, a polymorphic tandem repeats minisatellite in down-stream of hTERT gene, has been first reported to affect promoter activity in lung cancer cell lines [12]**.** In this case control study we investigated the association between the hTERT(MNS16A) genotypic variants and ALL risk in Sudanese patients.

The results of the precent study showed the presence of two alleles (302 bp and 271bp) among Sudanese patients with ALL. This finding disagree with the astudy done by wang *et al* who showed the presence of four alleles 333, 302, 272, and 243 in non Hispanic populations [13]. Also it differ from study done by xia *et al*, who showed the presence of 11 alleles (213, 240, 243, 271, 272, 274, 299, 302, 331,333, and 364) [14].

In the current study the genotypic variant 302\271 of MNS16A was associated with a significantly increased risk of ALL (OR:2.2, 95% CI: 1.9-2.5, *P.value*: 0.0). This was disagree with the finding of wang *et al* who showed that the variant genotypes 302\271, 302\243 and 243\243 of MNS16A were associated with a significantly increased risk of breast cancer (OR: 1.50, 95% 95% CI:1,15-1,96) [13].

In this study the long allele 271\302 was more common in ALL patients and this finding is also disagree with the finding reported by wang *et al* who found that the short alleles 271 and 243 were more common among cancer patients [13].

Also our result was inconsistent with that of Xia *et al* who reported that, the short allele had a higher relationship with the disease than the long allele [14].

The variations between our findings and the above mentioned studies can be related to the differences in the study populations, as both of them included patients with cancers other than haematological malignancies, while this study was conducted on ALL patients. Furthermore, variations can also be related to ethnic variations.

The present study showed that, there was no statistically significant difference in mean age of patients with the three genotypic variants , meaning that there was no relation between hTERT genotypes and age of incidence. This finding agrees with the study of Wang *et al* who also reported no relation between genotypes and age.

No statistically significant correlation was found between tandem repeat variants and gender.

Conclusion

The hTERT MNS16A 302\271 variant was significantly associated with ALL**.**

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