EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 7/ October 2015

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

The MNS16A Tandem Repeats Minisatellite of Human Telomerase: Influence on Polycythaemia Vera susceptibility in Sudanese Patients

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Abstract:

Background: *Telomerase is a reverse transcriptase enzyme that can elongate the TTAGGG repeats* ***of*** *telomeres in cells, where it is expressed to sustain cellular immortality. The components of telomerase include RNA subunit (human telomerase RNA), a reverse transcriptase catalytic subunit, human telomerase reverse transcriptase (hTERT) and other associated proteins. A minisatellite tandem repeat (MNS16A) located in the downstream of the human telomerase reverse transcriptase (hTERT) gene; recently identified and reported to have an effect on hTERT expression and telomerase activity.*

Purpose: *The purpose of this study was to determine the hTERT (MNS16A) variants among Sudanese patients with PV.*

Materials and methods: *A total of 45 Sudanese patients with PV and 45 healthy volunteers were enrolled in this study. DNA was extracted from k3 EDTA anti coagulated blood sample GFI-1 BLOOD DNA EXTRACTION KIT. Allele specific PCR method was*

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used simultaneously to amplify hTERT (MNS16A) variant in genomic DNA. Then the data were analyzed using the SPSS.

Results: *A total of 45 Sudanese patients and 45 Healthy volunteers were enrolled in this study. Their ages ranged between 23-80 years (mean±SD: 39±1.5). 75(89.3%) of them were males and nine (10.7%) were females. The frequency of hTERT (MNS16A) genotypes for patients were 24.4 % for both 271/271 and 302/302 genotype while it was 51.2 % for 271/302 genotype, in the other hand in the control group the 271/302 genotype was percent in all subjects with frequency of 100%.*

Conclusion: *In summary we conclude that MNS16A variant (271\302) in the hTERT was significantly associated increased susceptibility to polycythaemia Vera.*

Key words: MNS16A Tandem Repeats Minisatellite, Human Telomerase, Polycythaemia Vera susceptibility, Sudanese Patients

INTRODUCTION

True polycythaemia refers to an absolute increase in total body red cell volume (or mass), which usually manifests itself as a raised haemoglobin (Hb) concentration and packed cell volume (PCV). A raised Hb (or PCV) can also be secondary to a reduction in plasma volume, without an increase in total red cell volume; this is known as apparent (or relative) polycythaemia. True polycythaemia is further subdivided into primary polycythaemia (polycythaemia vera), a clonal haematological disorder, and secondary Polycythaemia (beter known as erythrocytosis), which results from an increased erythropoietin drive, either in the presence or in the absence of hypoxia [(1).]

Telomerase is a rib nucleoprotein 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 that 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.

Telomerase activity is associated with the number of times a cell can divide playing an important role in the immortality of cell lines, such as cancer cells. The enzyme complex acts through the addition of telomeric repeats to the ends of chromosomal DNA. This generates immortal cancer cells.[2] In fact, there is a strong correlation between telomerase activity and malignant tumors or cancerous cell lines.[3] Not all types of human cancer have increased telomerase activity. 90% of cancers are characterized by increased telomerase activity. There is also evidence that telomerase activity is increased in tissues, such as germ cell lines, that are self-renewing. Normal somatic cells, on the other hand, do not have detectable telomerase activity.[4] Since the catalytic component of telomerase is its reverse transcriptase, HTERT, and the RNA component HTERC, HTERT is an important gene to investigate in terms of cancer and tumor genesis. Aminisatlite tandom repeat (MNS16A)located in the downstream of hTERT has been identified and reported to have effect on telomerase activity and hTERT expression.

MATERIALS AND METHODS

Patients and samples

From 45 patients diagnosed with polycythaemia vera three milliliter (ml) of peripheral blood was collected and pour into k3 EDTA anticoagulant container. Patients' ages ranged between 5-70 years; 54% of them were females and 46% were males. 45 age and sex matched, healthy volunteers were included as a control group.

DNA Extraction

For molecular analysis DNA was extracted from EDTA anticoagulated blood samples by (GFI-1 BLOOD DNA EXTRACTION KIT, Malaysia) according to the manufacturer's instructions.

Molecular analysis of hTERT tandem repeat variant

Allele specific polymerase chain reaction (AS-PCR) was used for the detection of hTERT (MN16A).Two microliter (µl) of DNA was amplified in a total volume of 20 µL PCR mixture containing 0.5µl of each of forward primer(5'AGGATTCTGATCTCTGAAGGGTG-3') and reverse primer (5'-TCTGCCTGAGGAAGGACGACGTAT-3'), 4µl Matser mix (GoTaq® Green Master Mix, Promega, USA) and 13µl sterile distilled water.

The cycling conditions included initial denaturation at 95°C for 5 minutes; 35 cycles each consist of 95°C for 30 seconds (denaturation), 60°C for 45 seconds (annealing), and 72°C for 1 minute (extension); final extension at 72°C for 10 minutes.

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

Data collection and analysis

Data of this study was analyzed by statistical package for social sciences (SPSS), version 21. Frequencies of HTERT (MNS16A) genotypic variants and other qualitative variables were determined; mean age of the patients with the different polymorphic variants was compared using ANOVA test, correlation between the polymorphism and qualitative variables was tested using Cross tab with Chi-square.

Ethical consideration

This study was approved by the ethical committee of the faculty of medical laboratory sciences, Al Neelain University and informed consent was obtained from each participant before sample collection.

RESULTS

A total of 45 Sudanese patients and 45 Healthy volunteers were enrolled in this study. Their ages ranged between 23-80 years (mean±SD: 39±1.5). 75(89.3%) of them were males and nine (10.7%) were females.

There were two alleles detected among patients and control subjects which are 302 bp and 271bp and accordingly there were three genotypes observed among studied patients 302/302, 302/271, and 271/271 .The frequency of hTERT (MNS16A) genotypes for patients were 24.4 % for both 271/271 and 302/302 genotype while it was 51.2 % for 271/302 genotype, in the other hand in the control group the 271/302 genotype was percent in all subjects with frequency of 100%.

The results showed that there was significant assoiation between hTERT (MNS16A) variant and patients age (P.value:0.02) and there was no statistically significant assosiation between the genotypes and gender in patients with polycythaemia vera (P.value:0.241).

DISCUSSION

Activation or upregulation of telomerase is believed to play an important step in the progression of most human malignancies such AML.hTERT(MNS16A) variants is often associated with cancer and tumour formation which considered highly significant in the development and progression, diagnosis ,prognosis and the treatment.

The percent study showed a presence of three genotypes 302/302, 302/243, 302/271 for the hTERT (MNS16A) among studied patients which is disagree with the study done by wang *et al* which reported a presence of seven genotypes 302/302, 333/333 ,272/243 ,302/243 ,302/272 ,333/302 ,243/243[5].

This study showed that the long allele 271\302 was more common in AML 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 [5]

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[6].

CONCLUSION

In summary we conclude that MNS16A variant (271\302) in the hTERT was significantly associated increased susceptibility to polycythaemia Vera.

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