ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 01, pp.11681-11684, January, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

AMINISATELLITE TANDEM REPEAT OF HUMAN TELOMERASE REVERSE TRANSCRIPTASE (HTERT MNS16A) IN SUDANESE PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

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ARTICLE INFO

Article History:
Received 17th October, 2014
Received in revised form
24th November, 2014
Accepted 25th December, 2014
Published online 23rd January, 2015

Key words:

Essential Thrombocythaemia,
Human telomerase reverse
Transcriptase (hTERT) and
A Minisatellite tandem Repeat (MNS16A).

ABSTRACT

Background: Essential Thrombocythaemia {E.T.} also known as essential thrombocytosis is a rare chronic blood disorder characterized by the overproduction of platelets by megakaryocytes in the bone marrow. It is one of four myeloproliferative disorders (disorders characterized by increased production of a particular line of blood cell).
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.
Objective: The purpose of this study was to determine the hTERT (MNS16A) variants among Sudanese patients with ET.
Materials and Methods: A total of 50 patients diagnosed with ET attending to the radiation and isotope center of Khartoum (RICK) Sudan, and 50 healthy volunteer as control group were enrolled in this study. For molecular analysis genomic DNA was extracted from participant`s EDTA anticoagulated blood samples by salting out method and analyzed by allele specific PCR for determination of hTERT (MNS16A) variant.
Results: A total of 50 patients diagnosed with ET attending to the (RICK) Sudan, their ages ranged between42-79 years (mean±SD: 55±15), They were correlated with 50 healthy volunteers as control group their ages ranged between 42-75 years (mean±SD: 60±8).42(84%) of patients were suffering from massive splenomegaly, four (8%) of patients were suffering from hepatomegally and also four (8%) of patient were suffering from splenohepatomegaly. The hTERT (MNS16A) genotypes271\271, 271\302 and 302\302were observed among studied patients while 271\302genotype was observed among the control subjects.
Conclusion: In summary we conclude that the (hTERTMNS16A) 271\302 variant was significantly associated increased susceptibility for ET.

INTRODUCTION

Essential Thrombocythaemia (ET) also known as essential thrombocytosis is a rare chronic blood disorder characterized by the overproduction of platelets by megakaryocytes in the bone marrow (Beer *et al*., 2009). It is one of four myeloproliferative disorders (disorders characterized by increased production of a particular line of blood cell) (Beer *et al*., 2009). It is an indolent condition. The most common symptoms are bleeding, blood clots, increased white blood cell count, reduced red blood cell count, headache, nausea, vomiting, abdominal pain, visual disturbances, dizziness, fainting, enlarged spleen and numbness in the extremities (Fu *et al*., 2013: Fu *et al*., 2012: Tefferi *et al*., 2011). A mutation in the JAK2 kinase (V617F) is present in 40–50% of ET cases, (Beer *et al.,* 2009: Vannucchi *et al*., 2010). Telomerase reverse transcriptase is a catalytic subunit of the enzyme telomerase, which together with the telomerase RNA component (TERC) comprises the most important unit of the telomerase complex (Weinrich *et al.,* 1997; Kirkpatrick and Mokbel, 2001). TERT is responsible for catalyzing the addition of nucleotides in a TTAGGG sequence to the ends of a chromosome's telomeres (Shampay and Blackburn, 1988). This addition of repetitive DNA sequences prevents degradation of the chromosomal ends following multiple rounds of replication (Poole *et al.,* 2001). Normal human cells undergo a definite number of cell divisions when grown in culture and ultimately stop dividing and undergo what is called

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replicative senescence. The number of cell divisions attained before senescence is approximately 50 divisions (Ruddon, 2003). 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 a cellular signal is eventually triggered to stop cell division (Mondello *et al*., 2004). Germline cells and to some extent stem cells and lymphocytes overcome this end replication problem and maintain cellular proliferation by expressing telomerase.

Telomerase is aribonucleoprotein complex that contains several proteins and RNA. Three human cDNA encoding the telomerase proteins complex have been identified, cloned and characterized Htert (human telomerase reverse transcriptase) and human telomerase associatedprotein1(TP1). 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 (Blackburn *et al.,* 2001). (MNS16A)-aminisatellite, is aclass of variable number tandem repeat (VNTR), is a section of DNA that consists of a short series of nucleobases (10–60 base pairs) (Minisatellite *et al*., ?). Minisatellites, which are often simply referred to as VNTRs, occur at more than 1,000 locations in the human genome. Minisatellites can sometimes be confused with the other family of VNTR, the microsatellites (also called "Short Tandem Repeats" or STRs), which are also sections of DNA but only consist of around 2–6 base pairs [(14)].Thus, minisatellites are longer in length than microsatellites. This minisatellite was shown to have promoter activity dependent on the number of tandem repeats. The structure of MNS16A was found to be characterised by two repeat elements forming a 23bp, or when separated by a CAT trinucleotide insertion, a 26bp core sequence. The sequence containing the CAT insert represents a transcription factor binding site for GATA-1. Four different variable number of tandem repeats (VNTRs) VNTR-243, VNTR-274, VNTR-302 and VNTR-333, named on the basis of their PCR fragment size, have been described (WangL *et al*., 2003)

Objective

The purpose of this study was to determine the hTERT (MNS16A) variants among Sudanese patients with ET.

MATERIALS AND METHODS

Patients and Samples

Study population

A total of 50 Sudanese patients with ET admitted to Radiation and Isotopes Center of Khartoum (RISK) during the period from March to September 2014were enrolled in this study. In addition, 50 healthy individuals were used as a control group.

Sample collection and DNA extraction

Blood samples were collected from all patients and control subjects in Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant containers and genomic DNA was extracted by salting out method.

hTERT (MNS16A) variant Analysis

hTERT(MN16A) varinat was detected using allele specific PCR (PCR-TC 412, UK). Twomicroliter (µl) of DNA was amplified in a total volume of 20µL containing 0.5µl of each sense primer(5'-AGGATTCTGATCTCTGAAGGGTG-3'),and antisense 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 of 95°C for 30 seconds (denaturation), 60°C for 45 seconds (annealing), and 72°C for 1 minute (extension) and; final extension at 72°C for 10 minutes. Four µl of the PCR product (ready to load) and 50 bp DNA ladder (SOLIS BIODYNE, ESTONIA) was electrophoresed on 2% Agarose gel, stained with ethedium bromide and then demonstrated by gel documentation system (SYNGENE, JAPAN).We observed three alleles 271/271, 302/302 and 302/271.

Statistical analysis

Data of this study was analyzed by statistical package for social sciences (SPSS), correlation between hTERT tandem repeat variants and qualitative variables were tested by cross-tabulation and chi-square test, means of age and duration were compared by anova test.

Ethical considerations

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

RESULTS

A total of 50 patients diagnosed with ET attending to the RICK, their ages ranged between42-79 years ((mean±SD: 55±15), They were correlate with 50 healthy volunteers as control group their ages ranged between 42-75 years (mean±SD: 60±8), there was 42(84%) of patients were suffering from massive splenomegaly, four (8%) of patients were suffering from hepatomegaly and four (8%) of patients were suffering from splenohepatomegaly. There was three genotypes of hTERT(MNS16A) detected among patients which are 271\271, 271\302, 302\302 while only one genotype was detected among control which is 271\302.The frequency of hTERT(MNS16A) genotype among patients showed that the 271\271 genotype was detected in 6% (3) of patients, 271\302 was found in 70% (35) of patients and the 302\302 was detected in 24% (12) of patients; while control subjects presented with only one genotype 271\271. The genotype

271\271 was detected in two males and one female of patients, 271\302 genotype was detected in 20 males and 15 females of patients while 302\302detected in seven males and five females of patients. The statistical analysis of the result showed that there is significant difference between patients genotypes and controls genotype (P.Value is 0.000), but there is insignificant difference between patients genotype with patients age, gender and duration of the disease. the result showed that the splenomegaly was detected among three patients with 271\271genotype also in 29 patients with271\302 genotype and in 10 patients with302\302 genotype, hepatomegaly detected among three patients with 271\302 genotype also in one patient with 302\302 genotype and not detected among patient with 271\271 genotype while splenohepatomegaly was detected in three patients with 271\302 genotype also in one patient with 302\302genotype and not detected among patients with 271\271 genotype, there is insignificant different between patients genotype when compared with spleno\hepatomegaly and there is insignificant difference also between treated and untreated patients when compared with patients genotype. There was statistically significant association between ET and the genotype 271\302 (OR:2.2,CI:1.9-2.5and p.value:0.00) but not with the genotypes271\271(OR:0.485,CI:-0.659-1.62 p.value:0.403) and 302\302(OR:0.432,CI:-0.115-0.979and p.value:0.120).

Table 1. The P.Value of different variables

Variable	P.Value
Pt genotype\pt genotype	0.000
Pt genotype\control genotype	0.000
Pt genotype\pt age	0.464
Pt genotype\pt duration	0.788
Treated Pt	0.87
Untreated Pt	0.77

Table 2. The odds ratio of different genotypes

Genotype	Odds ratio	Confident interval	P.Value
271\271	0.485	-0.659-1.628	0.403
271\302	2.2	1.9-2.5	0.000
302\302	0.432	-0.115-0.979	0.120

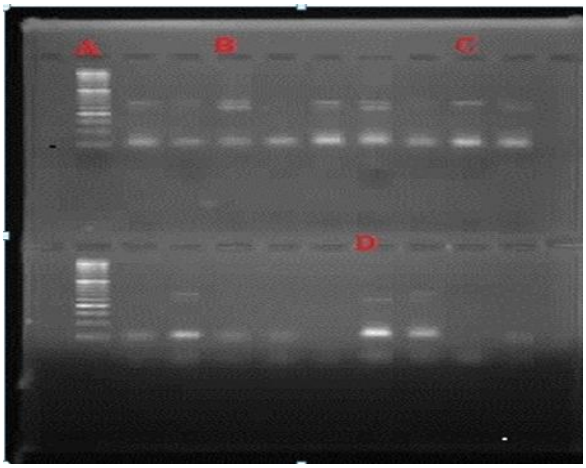

Fig. 1. Showed MNS16A genotypes which were performed using allele specific PCR (electrophoresis in 2% agarose gel). Genotype patterns: A:ladder 50bp . B :(302, 271) bp C : (302\302) and D: (271\271) bp.

DISCUSSION

Essential thrombocythemia is one of the most importance diseases, which is more commonly diagnosed at the age of 60, predominately in women. A small minority of people with ET may later develop acute leukemia or myelofibrosis, both of which can be life-threatening. Researchers have provided evidence that telomere dysfunction play an important role in cancer development.MNS16A is a polymorphic tandem repeats minisatellite of human telomerase (hTERT) gene that influences promoter activity of hTERT and thus implicates to relate with risk of several malignancies (Xia *et al.,* 2013). However, results on association between MNS16Aand cancer risk remain controversial. This study was performed to investigate the association between the hTERT(MNS16A) variant genotypes 271\271,271\302,302\302 and essential thrombocythemia in Sudanese patients. The present study showed that there were two alleles 302, 271 among Sudanese patients with ET, this finding is disagree with study done by Yan wang *et al.* (2008) which showed that there was four alleles 333,302,272,243 in non Hispanic population and also disagree with study done by Xianoping xia *et al.* (2013) which showed that there was 11 alleles of hTERT(213,240,243,271,272,274, 299,302,331,333,364) among different diseases. The current study showed that the variant genotype 302\271 of MNS16A was associated with a significantly increased risk of essential thrombocythemia (OR: 2.2, CI: 1.9-2.5and p.value:0.00).While study done by Yanwang *et al*. (2008) showed that the variant genotypes 302\271,302\243 and 243\243 of MNS16A were associated significantly with increased risk of breast cancer [OR=1.50, 95% confidence interval CI=1,15—1,96]. In the present study the long allele 271\302 was more common in ET patients and this finding is differ from finding of a study done by Yan wang *et al.* (2008) which reported that the short allele243\ 271 was more common in cancer patients and also differ from study done by Xianoping xia *et al.* (2013) which found that the short alleles had a higher relationship with the disease than the long allele. The present study agrees with study done by Yan wang *et al.* (2008) which showed that there was no assosiation between patient's hTERT genotype and their age. The overall findings of our study in comparison with other studies reflect that there was a difference in Sudanese patient's hTERT genotypes from some studies may be due to ethnic difference or technical sensitivity based difference.

Conclusion

In summary we conclude that the (hTERTMNS16A) 271\302 varient was significantly associated with ET risk. This work verified the important role of MNS16A minisatellites in ET predisposition.

Acknowledgement

By the grace of Almighty Allah and his help I completed this study, all praise to Him. My gratitude goes to Dr. Ibrahim Khider Ibrahim, my supervisor who guides me to complete this work. All appreciation to the staff of Haematology Department (Al Neelain University) who handed me a lot of favors specially Hiba -M-Haneen. Finally special thanks to patients who were so cooperative and hospitable despite their pain.

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