ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
Volume 4, Issue 3, May-June, 2015, 22-24

Research Article

NAD (P) H: QUINONE OXIDOREDUCTASE 1(NQO1) POLYMORPHISMIN SUDANESE PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

[1] Hiba M. Haneen, [2] Mohamed M. Babekerand [2*], Mahdi H. A. Abdalla

[1]Faculty of Medical Laboratory Sciences, Alneelain University, Sudan

[2]Department of Haematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan

Received 30 April 2015; Accepted 15 May 2015

ABSTRACT

Primary or essential thrombocythaemia (ET) is a clonal haemopoietic disorder, it is one of myeloproliferative neoplasm (MPN)that manifests primarily as a marked increase in platelets count with spontaneous aggregation of abnormal functionally platelets.Many genetic polymorphisms are considered as a risk factor in ET. The aim of this study was to examine the association of NQO1 C609T polymorphism with the risk of ET in Sudan. The study included 60 ETpatients, their NQO1 C609T genotypes (PCR/RFLP) and haematological characteristics (Sysmex KX-21N) were determined and compared with 60 age and sex matched normal subjects as control. No significant association was observed between NQO1 mutant genotypes (CT and TT combined together) and the risk of ET (OR= 0.405, 95% CI: 0.131-1.247, P = 0.115). The present study included a relative small sample of patients. Future studies, with large sample size are required to confirm our finding.

Keywords: Essential thrombocythaemia, NQO1 polymorphism, Sudan.

INTRODUCTION:

Primary or essential thrombocythaemia (ET) is a clonal haemopoietic disorder, it is one of myeloproliferative neoplasm (MPN) that manifests primarily as a marked increase in platelets count with spontaneous aggregation of abnormal functionally platelets (1). This disease usually affect middle aged to elderly people with an average age of 50-60 years with an incidence of 2-3 per 100,000 per year , but there is a significant incidence in younger patients (2,3).Multiple factors are believed to contribute to the pathogenesis of thrombosis in ET, advanced age and history of thrombosis are the 2 major risk factors (4).

Many genetic polymorphisms such as JAK (V617F), P53, MPL, TET2 and NQO1 are considered as a risk factor in ET(5).NQO1 gene is located in the long arm of chromosome 16 (16q22.1), it expands approximately 20 kb with 6 exons and 5 entrons that code for NQO1 protein, a flavoenzyme mainly cytosolic enzyme formed of 273 amino acid residues, that plays necessary role in the protection against exogenous and endogenous quinone by catalyzing two and four electron reduction of these substrates, such as hydroquinone (6-8). NQO1 enzyme has many functions that include protection of the cells from oxidative damage, scavenging of superoxide, stabilization of p53 and other tumour suppressors and detoxification of quinone and their derivatives (7).

The polymorphism occurs at exon 6 at nucleotide 609 (C-T) in the human NQO1 gene results in a proline to serine substitution at position 187 in the amino acid structure of the NQO1 protein (9). Previous studies reported an association between NQO1 polymorphism and leukaemia; however these studies showed differences in the occurrence and frequency of this relationship. The aim of this study was to examine the association of NQO1 C609T polymorphism with the risk of PV and the clinical outcome among E patients in Sudan.

MATERIALS AND METHODS:

Following informed consent 120 subjects were enrolled, 60 ET patients (Diagnosis based on the haematological feature) who were attended radiation and isotopes centre of Khartoum (RICK); and 60 age and sex matched normal subjects as control.

Two ml of EDTA anticoagulated blood was collected from each subject for haematological and molecular analysis. Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. Blood cell count was performed by automated cell counter (Sysmex KX-21N). DNA was extracted by DNA extraction kit (analytik Jena Biometra, Germany), according to manufacturer's instructions. NQO1 fragment Was Amplified using the forward primer: 5`-

*Corresponding author: Mahdi H. A. Abdalla | E-mail: mahdi_razig@hotmail.com

AGTGGCATTCTGCATTTCTGTG-3` and reverse primer: 5`-GATGGACTTGCCCAAGTGATG-3`. The amplification was carried out in thermo-cycler (Techno TC-412, UK) with initial denaturation step for 8 minute at 95 °C Followed by 35 Cycles consisting of 3 steps: Denaturing step at 94 °C for 30 second, Annealing step at 56 °C For 1 minute and extension steps at 72 °C for 40 minute with final Extension step at 72 °C for 10 Minutes.

The PCR reactions was performed in a final volume of 20 µl containing (4 µl premixed ready to use 5x FIREPol master mix (Solis BioDyne,Russian), 12.0µl DNAase free DW, 3 µl genomic DNA and 0.5 µl from each primer). The amplified fragment was digested with 10 U Hinf1 endonuclease (New England Bio lab, UK) over night and was visualized on agarose gel electrophoresis.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient´s data was performed using t-test. Comparison of frequency distribution between groups was made by means of the X^2 test. All tests are two-sided and P-value less than 0.05 have been considered as statistically significant. Crude odds ratios (OR) were also calculated and given with 95% confidence intervals (CI).

RESULTS:

The male: female ratio was 1.4,and the medianage was 50 years, with minimum age of 22 and maximum of 80 years. All patients and control were tested for the blood cell count and NQO1 polymorphism.

The results of blood Count for ET cases were as follows: Mean haemoglobin (Hb) level 12.2± 2.8 g/dL; Mean red blood cell (RBC) count 5.1±1.4X10^{12}/L; mean packed cell volume (PCV) 39.1±9.8 %; Mean total white cells (TWBC) count 15.8±11.6 X10^9/L; Mean platelet count 1213.7±518.7X10^9/L. While for the control group: Mean Hb concentration 14.7±1.2 g /dl; mean RBC count 5.3±0.5 X10^{12}/L; mean PCV 45.4±3.4 % Mean total white cells (TWBC) count 6.8 ±5.3 X109/L; Mean platelet count 234.2±52.1 X10^3/L (table 1).

Table 1: Blood count data between ET patients and control.

Parameters	Cases	Control	P value
Mean RBCs ±SD	5.1±1.4 X10^6/ul	5.3±.54 X10^6/ul	0.464
Mean HB±SD	12.2±2.8g/dl	14.6±1.2g/ dl	0.000
Mean PCV±SD	39.1±9.8%	52.6±55.8 %	0.069
MeanPlatlets± SD	1213.7±518.7X10^3/ul	234.2±52.1 X10^3/ul	0.000
Mean TWBC±SD	15.8±11.8 X10^3/ul	6.8±5.3 X10^3/ul	0.000

Table 2 shows the distribution of NQO1 C609T genotype frequencies between ET patients and control group. No significant association was observed between NQO1 mutant genotypes (CT and TT combined together) and the risk of ET(OR= 0.405, 95% CI: 0.131-1.247, P = 0.115)

Table 2: NQO1 C609T Polymorphism Frequencies in Cases and Controls

Genotype	Cases n (%)	Controls n (%)
CC	55(91.7)	49 (81.7)
CT	2(3.3)	6(10)
TT	3(5)	5(8.3)
CT+TT	5 (8.3)	11 (18.3)

DISCUSSION:

We examined the association of NQO1 C609T polymorphism and the risk of ET. Our study included 60 ET patients, their NQO1 C609T genotype frequencies and haematological characteristics were determined and compared with 60 age and sex matched normal subjects as control. We observed a low frequency of NQO1 mutant genotypes (8%) among the study group. Our study revealed no significant association between NQO1 C609T mutant genotypes and the risk of ET.Low impact of NQO1 C609T polymorphism as a risk factor in ETsuggesting that the phenotypes resulting from NQO1proteins have no contribution in the pathogenesis of ET. The present study included a relative small sample of patients. Future studies, with large sample size are required to confirm our finding.

CONCLUSION:

In conclusion, we examined the association of NQO1 C609T polymorphism and the risk of ET. Our results indicated low frequency of NQO1 C609T mutant

genotypes with low impact in the risk of ETamong the study group.

REFERENCES:

1. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 2008; **22:** 14–22.
2. Finazzi G, Xu M, Barbui T, Hoffman R, Hoffman R, Benz EJ JrShattil SJ, et al. Essential thrombo cythemia.*Hematology: Basic Principles and Practice.* 2009; 5th Ed Philadelphia, PA Churchill Livingstone 1149-1166.
3. Kuhij, RidellB Epidemiology of the myeloproliferative disorder. Essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis. Pathol Biol. 2001;49:164-166.
4. Barbui T, Barosi G, Birgegard G, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet.*JClinOncol.* 2011;29: 761 -70.
5. Chaligné R1, Tonetti C, Besancenot R, Roy L, Marty C, Mossuz P, Kiladjian JJ, Socié G, Bordessoule D, Le Bousse-Kerdilès MC, Vainchenker W, Giraudier S. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. Leukemia. 2008;22:1557-66.
6. Hamajima N, Matsuo K, Iwata H et al., NAD(P)H: quinoneoxidoreductase 1 (NQO1) C609T polymer-phism and the risk of eight cancers for Japanese., Int J ClinOncol., 2002; 7:103-108.
7. Krajinovic M, Sinnett H, Richer C et al., Role ofNQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia., Int J Cancer., 2002;97:230–236.
8. Gaedigk A, Tyndale RF, Jurima-Romet M et al., NAD(P)H:quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations., Pharmacogenetics, 1998; 8:305–313.
9. Bernarda Lozi et al, Analysis of the C609T Polymorphism of NQO1Gene in South Croatian Patients with Hematological Malignancies , Coll. Antropol. 2011;35:385–388.