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Original Research Article

NAD (P) H Quinine Oxidoreductase (NQO1) as a Risk Modifier of Susceptibility to Polycythaemia Vera in Sudan

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Abstract

Polycythaemia Vera (PV) is a clonal disorder characterized by overproduction of mature red blood cells in the bone marrow. Myeloid and megakaryocytic elements are also often increased. Although JAK2 gene mutations are the primary genetic abnormality in PV that has a direct association with the pathogenesis of the disease, many other genetic abnormalities have been reported to be a risk factor in PV.The aim of this study was to examine the association of NQO1 C609T polymorphism with the risk of PV and the clinical outcome among PV patients in Sudan. The study included 48 PV patients, their NQO1 C609T genotypes (PCR/RFLP) and haematological characteristics (Sysmex KX-21N) were determined and compared with 50 age and sex matched normal subjects as control. When the NQO1 609CC genotype was defined as the reference, a 3.1-fold increased risk of PV for those carrying NQO1 609CT (heterozygous) genotype was observed (OR 3.071, P value 0.028).No significant differences were observed in the mean Hb level, RBCS count and PCV between patients with mutant genotypes and those with wild type, this finding indicated that there is no association between NQO1 C609T polymorphism and the clinical outcome of PV. In conclusion, our results indicate that NQO1 C609T mutant genotypes with low enzymatic activity are associated with increased risk of PV.

Keywords:NQO1; Polycythaemia Vera; Sudan

1.Introduction

Polycythaemia Vera (PV) is a clonal disorder characterized by overproduction of mature red blood cells in the bone marrow[1]. Myeloid and megakaryocytic elements are also often increased[2]. Genetic and environmental factors have been implicated in rare cases. Familial PV has been associated with mutation of the erythropoietin (EPO) receptor[3]. An increased number of cases have been reported in survivors of the atomic bomb explosion in Hiroshima during World War II. The disorder typically occurs in the sixth or seventh decade of life. It occurs more commonly in men than in women[4].

The primary defect involves a pluripotent stem cell capable of differentiating into red blood cells, granulocytes, and platelets[5]. Clonality has been demonstrated through glucose-6-phosphate dehydrogenase studies as well as restriction fragment length polymorphism of the active X chromosome[6]. Erythroid precursors in PV are exquisitely sensitive to erythropoietin, which leads to increased red blood cell production. Precursors in PV are also more responsive to cytokines such as interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor, and steel factor.

The abnormal proliferation of PV is due to constitutive activation of the JAK-STAT pathway, with the majority of patients (>90%) harboring the V617F mutation. A similar JAK2 exon 12 mutation is found in the few patients lacking the V617F mutation[5].

Although JAK2 gene mutations are the primary genetic abnormality in the PV that has a direct association with the pathogenesis of the disease, many other genetic abnormalities have been reported to be a risk factor in PV example TET2, NFE2 and NQO1 genes[7-9].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[10-12]. 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[11].

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[13].

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 PV patients in Sudan.

2. Materials and Methods

Following informed consent, 108 individuals were enrolled, 48 polycythaemia vera patients (Diagnosis based on the haematological features) who were attended Fedeal hospital in Khartoum state; and 60 apparently healthy subjects as controls. 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). NQO1 fragment Was Amplified using the forward primer: 5`-AGTGGCATTCTGCATTTCTGTG-3` and reverse primer: 5`-GATGGACTTGCCCAAGTGATG-3`. The amplification was carried out in thermo-cycler (Techne) 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 0.5µl 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) version 22 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).

3. Results

The study included 27 male and 21 female. There median Age was 43 year, with minimum Age of 18 and maximum of 75 years. All patients were tested for the red blood cell counts and NQO1 Polymorphism. The results of blood Count for polycythaemia vera cases were as follows: Mean haemoglobin (Hb) level 15.5±2.3 g/dl; mean red blood cell (RBC) count 6.5±0.8X10¹²/L; mean packed cell volume (PCV) 50.6±5.8%. While for the control group: Mean Hb concentration 14.6±1.2 g /dl; mean RBC count 5.3±0.5 X10¹²/L; mean PCV 45.1±3.4 %.

Table 1: Comparison of haematological characteristics between polycythaemia vera patients and control subjects

Parameter	PV Cases	Controls	Pvalue
Hbmean±SD (g/dl)	15.5±2.3	14.6±1.2	0.015
RBC mean±SD ($X10^{12}$/L)	6.5±0.8	5.3±0.5	0.000
PCV mean±SD (%)	50.6±5.8	45.1±3.4	0.000

Table 2 shows the distribution of NQO1 C609T genotype frequencies between polycythaemia vera patients and control group. When the NQO1 609CC genotype was defined as the reference, the OR for the mutant genotype (CT genotype) was 3.071(95% CI: 1.131-8.342, P = 0.028).

Table 2: Comparison of NQO1 C609T Polymorphism Frequencies in Cases and Controls

Genotype	PV cases N (%)	Controls N(%)	OR	95% CI	P value
CC	32(66.7)	42 84.0)	referent		
CT	16(33.3)	7 (14)	3.071	1.131-8.342	0.028

Hb level, RBC count and PCV revealed no significant differences between PV patients with NQO1 C609T wild type (CC) and those with mutant types (CT), (table 3).

Table 3: Comparison of haematological characteristics between polycythaemia vera patients

Parameter	Wild type (609CC)	Mutant types (609CT)	P value
Hbmean±SD (g/dl)	15.5±2.4	15.5±2.0	0.92
RBC mean±SD ($X10^{12}$/L)	6.5±0.9	6.4±0.7	0.54
PCV mean±SD (%)	50.0 ±5.4	51.7± 6.5	0.37

4. Discussion

Genetic polymorphisms of various kinds of genes have been recently proved to have important roles in the genesis of haematological neoplasm[14]. Our study included 48 PV patients, their NQO1 C609T genotype frequencies and haematological characteristics were determined and compared with 50 age and sex matched normal subjects as control.

To study the association of NQO1 genotypes and PV; we conducted logistic regression analysis; our study showed a statistically significant association between NQO1 C609T polymorphisms and PV. The frequency of the NQO1 609CC genotype was higher among controls (84.0%) when compared to PV patients (66.7%). When odds ratios were calculated for the overall group, we observed about three-fold increased risk of PV for those carrying NQO1 609CT (heterozygous) genotype (OR 3.71, P value 0.028 95%CI 1.131-8.342). Gross-Davis *et al* reported an association between NQO1 polymorphism and increased risk of Myeloproliferative Neoplasms. The majority of their study group were PV patients (24/27) (15).

When comparing the haematological values between PV patients with NQO1 C609T wild type (CC) and those with mutant types (CT), we observed no statistically significant differences in the mean Hb level, RBCS count and PCV between patients with mutant genotypes and those with wild type, this finding indicated that there is no association between NQO1 C609T polymorphism and the clinical outcome of PV.

Reduced detoxifying power for toxic quinone and free radicals and/or the decreased stability of p53 resulting from the NQO1 inactivating polymorphism may influence the susceptibility to PV. However, further investigation needs to verify this hypothesis and to understand the mechanism.

5. Conclusion

In conclusion, our results indicate that NQO1 C609T mutant genotypes with low enzymatic activity are associated with increased risk of PV.

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