

NAD (P) H Quinine Oxidoreductase (NQO1) as a Risk Modifier of Susceptibility to Chronic Myeloid Leukaemia in Sudan

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Abstract CML is the most prevalent haematological cancer among Sudanese population. Previous studies reported an association between NQO1 polymorphism and leukaemia, however these studies showed differences in the occurrence and frequency of this relationship. This study aimed to examine the association of NQO1 C609T polymorphism with the risk of CML and the clinical outcome among Philadelphia positive CML patients in Sudan. The study included 73 newly diagnosed Philadelphia positive CML patients, their NQO1 C609T genotypes (detected by PCR/RFLP) and haematological characteristics (determined by Sysmex KX-21N) were determined and compared with 60 age and sex matched normal subjects as control. When the NQO1 609CC genotype was defined as the reference, a 3.5-fold increased risk of CML for those carrying NQO1 609CT (heterozygous) genotype was observed (OR 3.461, *P* value 0.016). The frequency of NQO1 609 TT (homozygous) genotype was higher among CML patients with a 1.7 folds than control group, but with no statistical significance (OR 1.718, *P* value 0.305). The frequency of the NQO1 609CT and 609 TT genotypes combined together (mutant types) was significantly higher among CML patients with a 2.5- fold increased risk when compared with the controls (OR 2.522, *P* value 0.019). We observed a statistically significant reduction in the mean Hb level in patients with mutant genotypes than in wild type patients (*p* value 0.037), WBCs count, platelet count, basophiles count and blasts count were significantly higher in patients with mutant type when compared to those with wild type (*p* value 0.024, 0.020, 0.024 and 0.000) respectively. In conclusion, our results indicate that NQO1 C609T mutant genotypes, with low enzymatic activity, are associated with increased risk of CML and worse clinical outcome.

Keywords NQO1 polymorphism, CML, Sudan

1. Introduction

Chronic myeloid leukaemia (CML) (also known as chronic myelogenous leukemia, chronic Granulocytic leukemia and chronic myelocytic leukemia) is a clonal myeloproliferative disorder of pluripotent stem cell [1,2]. The disease accounts for around 15% of leukaemia with an incidence of 1-2 cases/100,000 population [3]. CML is the most prevalent haematological cancer among Sudanese male, and the second prevalent cancer, after breast cancer, among Sudanese female [4].

The median age, at diagnosis is 45-55 years, but all age groups including children can be affected [5]. CML has three clinical stages: a chronic phase that is characterized by an indolent onset without symptoms in 50% of patients. This is followed invariably by an accelerated phase that is

characterized by basophilia, increased peripheral blood blasts and promyelocytes and is refractory to treatment. In about 75% of patients, the accelerated-phase disease is followed by a blastic phase, which resembles acute leukaemia and causes death within 3 to 6 months [6].

CML was the first neoplasm to be associated with a specific chromosomal rearrangement, the Philadelphia chromosome, that results from a reciprocal translocations between chromosomes 9 and 22 [t (9; 22) (q34; q11)]. This translocation transposes the c-abl proto-oncogene from chromosome 9 to the breakpoint cluster region (bcr) gene on chromosome 22, and this new hybrid bcr-abl oncogene encodes a constitutively active tyrosine-kinase that promotes the growth advantage of leukemia cells through enhanced proliferation and reduced apoptosis, and generating genomic instability [7]. Although the clinical and biological aspects are well documented, little is known about individual susceptibility to CML. Polymorphic variants of several genes, diet, environmental exposure to carcinogens and individual immune system's characteristics are potential factors that increase predisposition to leukemia

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Published online at <http://journal.sapub.org/cmd>

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[8] Finding of recent studies suggest that genetic variants may affect and increase risk of CML, and among them are methylenetetrahydrofolate reductase (MTHFR) [9, 10], glutathione S-transferases (GSTs) [11, 12], and NAD(P)H: Quinone Oxidoreductase (NQO1) [13].

(NQO1) gene is located in the long arm of chromosome 16 (16q22.1), it expands approximately 20 kb with 6 exons and 5 introns 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. [14-16]. NQO1 enzyme has many functions that include protection of the cells from oxidative damage, scavenging of superoxide, stabilization of p53 and other tumor suppressors and detoxification of quinone and their derivatives. [15]

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 [17]. NQO1 enzyme activity is normal in individuals with 2 wild-type alleles (NQO1 609CC). It is variably reduced in individuals who are heterozygotes for the polymorphism (NQO1 609CT) [18]. NQO1 enzyme activity is absent in those who are homozygous for the point mutation (NQO1 609TT) [19].

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 CML and the clinical outcome among Philadelphia positive CML patients in Sudan.

2. Materials and Methods

Following informed consent 133 individuals were enrolled, 73 newly diagnosed Philadelphia positive CML patients (Diagnosis based on the haematological features and the detection of BCR-ABL transcripts) who were attended radiation and isotopes center of Khartoum (RICK); 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). 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'-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 0.5 µl from each primer). The amplified fragment was digested with 10 U *HinfI* 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 χ^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 male: female ratio was 1.5 and the median Age was 43 year, with minimum Age of 8 and maximum of 80 years. All patients were tested for the blood cell counts and NQO1 Polymorphism.

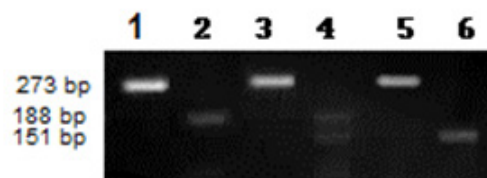


Figure 1. Analysis of *NQO1*. *NQO1* gene fragments (273 bp, lane 1,3 and 5) were amplified and digested by *HinfI* endonuclease. The digestion yielded two bands at 188 bp and 85 bp for the NQO1 609CC genotype (lanes 2), three bands at 181 bp, 151 bp, and 85 bp for NQO1 609CT genotype (lanes 4), and two bands at 151 bp and 85 bp for NQO1 609TT genotype (lane 6)

The results of blood Count for CML cases were as follows: Mean haemoglobin (Hb) level 11.1±2.1 g/dL; Mean red blood cell (RBC) count 3.6±1.4X10¹²/L; mean packed cell volume (PCV) 34.4±8.7%; Mean total white cells (TWBC) count 165.8±1.3X10³/L; Mean platelet count 402.9±313.80 X10³/L. While for the control group: Mean Hb concentration 14.7±1.2 g /dL; mean RBC count 5.3±0.5 X10¹²/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 X10³/L (table 1).

Table 2 shows the distribution of NQO1 C609T genotype frequencies between CML patients and control group. When the NQO1 609CC genotype was defined as the reference, the ORs for the CT genotype, TT genotype, and CT and TT genotypes combined together were (OR = 3.461, 95% CI: 1.265-9.472, P = 0.016), (OR = 1.718, 95% CI: 0.611-4.830, P = 0.305) and (OR = 2.522, 95% CI: 1.167-5454, P = 0.019), respectively (table 2).

Table 1. Comparison of haematological characteristics between CML patients and control subjects

Parameter	Cases	Controls	P.value
Hb mean±SD (g/dl)	11.1±2.1	14.7±1.2	0.000
RBC mean±SD (X10 ¹² /L)	3.6±1.4	5.3±0.5	0.000
PCV mean±SD (%)	34.4±8.7	45.4±3.4	0.000
TWBC mean±SD (X10 ⁹ /L)	165.81.3	6.8 ±5.3	0.000
Platelets mean±SD (X10 ⁹ /L)	402.9±313.8	234.2±52.1	0.000

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

Genotype	Cases n (%)	Controls n (%)	OR	95%CI	P value
CC	43(58.9)	47 (78.3)	referent		
CT	19 (26)	6 (10)	3.461	1.265-9.472	0.016
TT	11(15.1)	7 (11.7)	1.718	0.611-4.830	0.305
CT+TT	30(41.1)	13 (21.7)	2.522	1.167-5454	0.019

Various haematological values, including Hb level, TWBC count, platelets count, basophils count and blast count, reveal statistically significant differences between CML patients with NQO1 C609T wild type (CC) and those with mutant types (CT and TT genotypes combined together) (data were shown in table 3).

Table 3. Comparison of haematological characteristic between CML patients with wild type and those with mutant types

Parameter	Wild type (609CC)	Mutant types (609CT+TT)	P value
Hb mean ± SD (g/dl)	11.5±2.3	10.5±1.8	0.037
TWBC mean ± SD (X10 ⁹ /L)	126.3±91.5	185.9±68.7	0.024
Platelets mean ± SD (X10 ⁹ /L)	332.2±256.6	504.2±361.9	0.020
Basophils mean ± SD (%)	1.6±4.1	3.9±4.4	0.024
Blasts mean ± SD (%)	4.6±8.2	15.5±11.1	0.000

4. Discussion

Genetic polymorphisms of various kinds of genes have been recently proved to have important roles in the genesis of human malignancies [20]. Several studies have reported that individuals with NQO1 C609T mutant genotypes are at increased risk of leukemia. We examined the association between NQO1 C609T polymorphism and the risk of CML. Our study included 73 CML patients, their NQO1 C609T genotype frequencies and haematological characteristics were determined and compared with 60 age and sex matched normal subjects as control.

To determine if there is a statistically significant increase risk of CML development according to the NQO1 genotypes, we conducted logistic regression analysis, our study showed a statistically significant association between NQO1 C609T polymorphisms and the risk of CML. The frequency of the NQO1 609CC genotype was higher among controls (45.0%) when compared to CML patients (35.2%). When odds ratios

were calculated for the overall group, we observed a 3.5-fold increased risk of CML for those carrying NQO1 609CT (heterozygous) genotype (OR 3.461, P value 0.016). The frequency of NQO1 609 TT (homozygous) genotype was higher among CML patients (15.1%) than control group (11.7%), but with no statistical significance (OR 1.718, P value 0.305). The frequency of the NQO1 609CT and 609 TT genotypes combined together (mutant types) was significantly higher among CML patients (41.1%) when compared with the controls (21.7%), with a 2.5-fold increased risk of CML (OR 2.522, Pvalue 0.019). Similar findings had previously been reported [20].

When comparing the haematological values between CML patients with NQO1 C609T wild type (CC) and those with mutant types (CT and TT genotypes combined together), we observed a statistically significant reduction in the mean Hb level in patients with mutant genotypes than in wild type patients, WBCs count, platelet count, basophils count and blasts count were significantly higher in patients with mutant type when compared to those with the wild type. Higher, platelets count, basophils count and blast count are associated with worse clinical outcome in CML [21, 22], thus, in our study group, clinical outcome was worse among CML patients with NQO1 C609T mutant types (CT and TT genotypes combined together) than among patients with wild type (CC).

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 CML. However, further investigation needs to verify this hypothesis and to understand the mechanism.

5. Conclusions

In conclusion, we examined the association between NQO1 C609T polymorphism and the risk of CML. NQO1 C609T genotypes and haematological characteristics of 73 CML patients were determined and compared with 60 age and sex matched normal subjects as control. Our results indicate that NQO1 C609T mutant genotypes with low enzymatic activity are associated with increased risk of CML and worse clinical outcome.

ACKNOWLEDGEMENTS

Special thanks to the Staff of Haematology Department, Faculty of Medical Laboratory Sciences, Alneelain University and the staff radiation and isotopes center of Khartoum.

REFERENCES

- [1] Fungaro MHP, Carneiro JLV, Watanabe MAE., 2007, Stromal Cell-Derived Factor-1 Chemokine Gene Variant in

- Blood Donors and Chronic Myelogenous Leukemia Patients., *J Clin Lab Anal*, 2, 49–54.
- [2] Sawyers CL., 1999, Chronic myeloid leukemia., *N Engl J Med.*, 340, 1330-1340.
 - [3] Manero GG, Fadert S, Brien SO, Cortes, Talpaz M, Kantarjian HM., 2003, Chronic Myelogenous Leukemia: a review and update of therapeutic strategies., *Cancer*, 98, 437–457.
 - [4] Hamad HMA., 2007, Cancer initiatives in Sudan., *Annals of Oncology.*, 17s, viii32–viii36.
 - [5] Faderl S, Talpaz M, Estrov Z, Kantarjian HM., 1999, Chronic myeloid leukemia: biology and therapy., *Ann Inter Med.*, 131, 207- 219.
 - [6] Faderl S, Kantarjian HM, Talpaz M., Chronic myelogenous leukemia: update on biology and treatment. *Oncology* 1999; 13:169-184.
 - [7] Nowicki MO, Falinski R, Koptyra M, Slupianek A, et al., 2004, BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks., *Blood*, 104, 3746-3753.
 - [8] Bowen DT, Frew ME, Rollinson S, Roddam PL, et al., 2003, CYP1A1*2B (Val) allele is overrepresented in a subgroup of acute myeloid leukemia patients with poor-risk karyotype associated with NRAS mutation, but not associated with FLT3 internal tandem duplication., *Blood*, 101, 2770-2774.
 - [9] Hur M, Park JY, Cho HC, Lee KM, et al., 2006, Methylenetetrahydrofolate reductase A1298C genotypes are associated with the risks of acute lymphoblastic leukaemia and chronic myelogenous leukaemia in the Korean population., *Clin. Lab. Haematol.*, 28, 154-159.
 - [10] Moon HW, Kim TY, Oh BR, Min HC, et al., 2007, MTHFR 677CC/1298CC genotypes are highly associated with chronic myelogenous leukemia: a case-control study in Korea., *Leuk. Res.*, 31, 1213-1217.
 - [11] Hishida A, Terakura S, Emi N, Yamamoto K, et al., 2005, GSTT1 and GSTM1 deletions, NQO1 C609T polymorphism and risk of chronic myelogenous leukemia in Japanese., *Asian Pac. J. Cancer Prev.*, 6, 251-255.
 - [12] Souza CL, Barbosa CG, Neto JPM, Barreto JH, et al., 2008, Polymorphisms in the glutathione S-transferase theta and mu genes and susceptibility to myeloid leukemia in Brazilian patients., *Genet. Mol. Biol.*, 31, 39-41.
 - [13] Larson RA, Wang Y, Banerjee M, Wiemels J, Hartford C, Beau MM, Smith MT., 1999, Prevalence of the Inactivating 609C=T Polymorphism in the NAD(P)H: Quinone Oxidoreductase (NQO1) Gene in Patients With Primary and Therapy-Related Myeloid Leukemia., *Blood*, 94, 803-807.
 - [14] Iida A, Sekine A, Saito S et al., 2001, Catalog of 320 single nucleotide polymorphisms (SNPs) in 20 quinone oxidoreductase and sulfotransferase genes., *J Hum Genet.*, 46, 225-240.
 - [15] Hamajima N, Matsuo K, Iwata H et al., 2002, NAD(P)H: quinoneoxidoreductase 1 (NQO1) C609T polymorphism and the risk of eight cancers for Japanese., *Int J Clin Oncol.*, 7, 103-108.
 - [16] Krajcinovic M, Sinnett H, Richer C et al., 2002, Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia., *Int J Cancer.*, 97, 230–236.
 - [17] Gaedigk A, Tyndale RF, Jurima-Romet M et al., 1998, NAD(P)H: quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations., *Pharmacogenetics*, 8, 305–313.
 - [18] Ross D, Traver RD, Siegel D, Kuehl BL, Misra V, Rauth AM., 1996, A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): Relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity., *Br J Cancer* 74, 995.
 - [19] Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, Ross D., 1997, Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT diaphorase)., *Br J Cancer* 75, 69.
 - [20] Hishida A, Terakura S, Emi N, Yamamoto K, Murata M, Nishio K, Sekido Y, Niwa Y, Hamajima N, Naoe T., 2005, GSTT1 and GSTM1 Deletions, NQO1 C609T Polymorphism and Risk of Chronic Myelogenous Leukemia in Japanese., *Asian Pacific J Cancer Prev.*, 6, 251-255.
 - [21] Sokal JE, Baccarani M, Russo D, Tura S., 1988, Staging and prognosis in chronic myelogenous leukemia. *Semin Hematol.*, 25, 49-61.
 - [22] Baccarani M, Pileri S, Steegmann J-L, Muller M, Soverini S, Dreyling M, on behalf of the ESMO Guidelines Working Group., 2012, Chronic myeloid leukemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up., *Annals of Oncology*, 23, vii72–vii77.