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Associations of NQO1 C609T Polymorphism with Acute Lymphoblastic Leukemia Risk in Sudanese in Khartoum State

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Abstract

Background: *NAD (P) H: Quinone Oxidoreducatase (NQO1) it is enzyme protects cells against oxidative stress and toxic quinines; hence prevents cells against mutagenicity, free radicals effects and toxic oxygen metabolites. Mutations of NQO1 decrease the level of NQO1 activity which is associated with an increased risk of cancer development. Several previous studies focus in the role of NQO1 as risk factor for ALL.*

Objectives: *This study aimed to investigate the association of NQO1 C609T polymorphism with the development of acute lymphoblastic leukaemia (ALL) and hematological parameters among ALL patients in Sudan.*

Methods: *Fifty newly diagnosed ALL patients were enrolled in this study, compared with fifty healthy volunteers age and sex matched*

as control. NQO1 C609T genotypes detected by PCR/RFLP and haematological parameters were determined by using automated cell counter analyzer (Sysmex KX21N).

Results: *This case control study, conducted at flowcytometer center in the period from October 2016 to October 2017 to detect gene mutations associated with ALL. This study included 50 patients and 50 healthy volunteers as control, matching in age and sex; 29(58%) of the patients were males while 21(42%) were females, (M:F=1.4:1).Their age range from 1 to 65 years, with mean age (15.33). C609T polymorphism revealed statistical significant association increased risk for ALL; for heterogenisity (CT) of NQO1(C609T) OR 5.351, P value 0.0245; 5.35-fold increased risk of ALL, while for NQO1 609 TT homozygous mutant genotype with a 1.1 folds risk but with no statistical significance (OR 1.189,95%CI 0.2780-5.088, P value 0.8151). Mean WBCs count, mean RBCs count, mean Plts count and mean Hb level showed no statistically significant in patients with mutant genotypes than in wild type patients (P .value= 0.081, 0. 0.817, 0.216 and 0.993) respectively.*

Conclusion: *This study concluded that NQO1 C609T polymorphism was found statistical significant association between (CT) variant and increased risk for ALL, while (TT) variant statistical insignificance. Hematological parameters were insignificant in (CT/TT) compared with wild type (CC).*

Key words: NQO1, polymorphism, ALL**.**

1- INTRODUCTION

NAD (P) H: quinone oxidoreducatase (NQO1), also known as DT-diaphorase, is a flavo enzyme detoxifies quinines derived from the oxidation of phenolic metabolites of benzene by catalyzing two or four electron reductions of these substrates. It can protect cells against oxidative damage by preventing redox cycling and the generation of free radicals. Single-nucleotide polymorphisms (SNPs) are the most common type of variant in

the human genome [1]**.** The NQO1 act as pro oxidant agent due to it is contribution in the formation of reactive oxygen species[2]. Many single nucleotide polymorphisms (SNPs) have been investigated in NQO1. NQO1*2(C609T) polymorphism (proline-to-serine; Pro187Ser) substitution in exon 6 lead to low activity, destabilizes and inactivates the enzyme with heterogeneous variant (CT), while homogenous mutant genotype (TT) cause complete lack of enzyme activity [3,4].

2- MATERIALS AND METHODS

The study included 50 ALL patients and 50 controls. Under informed consent, 5 ml peripheral blood samples were collected into tubes with EDTA from each subject. Complete blood count was performed by automated cell counter analyzer (Sysmex-Kx21) at flowcytometer center Khartoum –Sudan and Sharq Alneel Hospital. Molecular analysis was performed at the research lab in Sudan University of Science and Technology College of Medical Laboratory Sciences. Genomic DNA was extracted by DNA extraction kit (analytic Jena Biometra, Germany), according to manufacturer's instructions.

The quality of genomic DNA was determined by electrophoresis on 2% agarose gel stained with ethidium bromide. NQO1 fragment was amplified using the forward and reverse primers (5-ATTCTCTAGTGTGCCTGAG-3, 5-AATCCTGCCTGGAAGTTTAG-3). The amplification was carried out in thermocycler (Healtorce.K690, China), with initial denaturation at 95 ^{o}C for 5 minutes, followed by 35 cycles; denaturation at 95 ^{o}C for 1 minute, annealing at 60 ^{o}C for 45 seconds, extension 72 ^{o}C for 1 minute, final extension 72 ^{o}C for 5 minutes, then, digestion of the PCR products for the NQO1 C609T polymorphism using HinfI (New England Biolabs- England).

2-1 Statistical analysis

Data collected, checked and analyzed using statistical package for social science (SPSS) software version 25. Mean ± STD, frequencies, chi- square, t-test was obtained. Results with *P. value* ≤ 0.05 were considered statistically significant, odd ratio calculated with GraphPad Prism6 programme version 6.07.
College of Medical laboratory Sciences Research Board- Sudan University of Science and Technology approved this study; written informed consent was obtained from all patients and healthy control individuals who participated in this study.

3- RESULTS

This study involved 50 patients and 50 healthy volunteers as control, matching in age and sex; male represent 29(58%) and 21(42%) females, M: F = 1.4:1 aged between 1 to 65 years, with mean age (15.33).

All 100 DNA samples (from patients and controls) were successfully genotyped using the PCR-RFLP; NQO1 C609T genotypes for the case and control group were displayed as tables.

Table 1: Comparison of NQO1 C609T Polymorphism Frequencies in Cases and Controls.

Genotype	Case Frequency	Control Frequency	*P. value*	OR(95%CI)
CC	37(74%)	44(88%)		
CT	9(18%)	2(4%)	0.0245	5.351 (1.087-26.34)
TT	4(8%)	4(8%)		
Total	50(100%)	50(100%)	0.8151	1.189(0.2780-5.088)

Table 2: Comparison of NQO1 C609T Polymorphism wild type and mutant type in cases and controls.

NQO1 609 mutant	Case	Control	*P. value*	OR(95%CI)
Wild type (CC)	37(74%)	44(88%)	0.0744	2.577(0.8911-7.450)
Mutant types (CT/TT)	13(26%)	6(12%)		
Total	50(100%)	50(100%)		

Table 3: Comparison of NQO1 C609T Polymorphism and hematological parameters among study subjects

Parameters	Wild type (CC) Mean ± SD	Mutant (CT+TT) Mean ± SD	*P. value*
TWBCs ($X10^9$/L)	47.4840 ± 127.34341	21.6126 ± 16.76586	0.081
RBCs ($X10^{12}$/L)	3.6170 ± 1.09246	3.3700 ± .92792	0.321
PLTs ($X10^9$/L)	214.0247 ± 171.65124	147.7895 ±150.76051	0.104
Hb (g/dl)	10.2346 ± 2.77025	9.1316 ± 2.33810	0.084

4- DISCUSSION

NQO1 C609T polymorphism is associated with the elevated risk of childhood ALL , hererozygosity was higher in patients when compared with controls, the odds ratio was 5351 ; however, this difference was statistically significant (95% CI: 1.087-26.34, p= 0.0.0245), but homozygosity of mutatant genotype was insignificant with OR 1.189 (95% CI: 0.2780-5.088, p= 0.8151) and alleles also was insignificantly associated with risk of ALL (OR 2.577 (95% CI: 0.8911-7.450, p= 0.0744), this finding was consolidated by review and meta- analysis done by Jeffrey and his colleagues 2008, they showed that the NQO1 C609T variant have no strong association with childhood ALL or AML but may be associated with mixed lineage leukemia–positive childhood leukemia ALL [5]. Another study also support this result, done by Abdelaziz and Alqatary in Dammam in Saudi Arabia 2013, they found no statistically significant association between the NQO1 C609T polymorphism and risk of childhood ALL (CT/TT versus

CC; OR, 0.95; 95% CI, 0.55- 1.64) [6]. Another study done by Sirma, 2004 in Turkey did not support the role of the NQO1 C609T polymorphism in the increased risk of pediatric acute leukemia [7]. On the other hand this finding were disagree with previous study were carried in Sudan by Abdalla and Kobar 2015, they found 2.9- fold increased risk of ALL for those carrying NQO1 609CT heterozygous genotype (OR 2.878, P value 0.040) [8]. Another family based study with similar finding by Infante-Rivard *et al.,* (2007) in Canada suggested that the NQO1 C609T variant was associated with the risk of developing childhood ALL (OR 1.39 , 95% CI: 1.07, 1.79) [9]. Another French-Canadian study by Krajinovic *et al*.,(2002) showed that children carrying at least 1 mutant allele of the NQO1 C609T polymorphism had an increased risk of developing ALL [10], the association between the NQO1 C609T polymorphism and the ALL risk was also analyzed by Li and Zhou (2014), they found that this polymorphism increased the ALL risk, also they compare between the alleles and was found that the NQO1 C609T polymorphism increased the ALL risk [11]. A study done in American population by Smith *et al.,* (2002) was also indicate that the NQO1 C609T polymorphism is associated with the elevated risk of childhood ALL (OR = 2.47, 95% CI: 1.08, 5.68) [12], as well as Italian study done by Lanciotti *et al.,* 2005, (OR = 5.55, 95% CI: 1.81, 16.98) [13]. While in Brazilian children, the NQO1 and myeloperoxidase (MPO) polymorphisms were shown to have a protective function against leukaemogenesis [14]. Lack of agreement between these studies might be due to differences in the duration of the exposure to the NQO1 substrates and small sample sizes, ethnic groups, nutrition and life style as well as the demographic stratification that exists in these kinds of studies.

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