ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
Volume 4, Issue 4, July-August, 2015, 107-110

Research Article

DETECTION OF GENETIC POLYMORPHISM OF NAD (P) H: QUINONE OXIDOREDUCTASE 1 (NQO1) AMONG SUDANESE PATIENTS WITH ACUTE LYMPHOID LEUKAEMIA

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Received 08 August 2015; Accepted 24 August 2015

ABSTRACT

Polymorphic variations of several genes associated with dietary effects and exposure to environmental carcinogens may influence susceptibility to leukemia development. This study aimed to examine the association of NQO1 C609T polymorphism with the risk of acute lymphoid leukaemia (ALL) and the clinical outcome among ALL patients in Sudan. Seventy five newly diagnosed ALL patients were enrolled in this study, their NQO1 C609T genotypes (detected by PCR/RFLP) and haematological feature were determined and compared with 75 age and sex matched normal subjects as control. The study revealed a 2.9-fold increased risk of ALL for those carrying NQO1 609CT (heterozygous) genotype (OR 2.878, *P* value 0.040). The frequency of NQO1 609 TT (homozygous) genotype was slightly higher among ALL patients with a 1.1 folds than control group, but with no statistical significance (OR 1.096, *P* value 0.869). We observed a statistically significant reduction in the mean Hb level and RBCs count in patients with mutant genotypes than in wild type patients (*p* value 0.000 and 0.003), WBCs count was significantly higher in patients with mutant type when compared to those with the wild type (*p* value 0.000). In conclusion, our results indicate that NQO1 C609T mutant genotypes, with low enzymatic activity, are associated with increased risk of ALL and worse haematological features.

Key words: Acute lymphoid leukaemia, NQO1 polymorphism, Sudan.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is heterogeneous group of hematologic malignancies that arise from clonal proliferation of immature lymphoid cells in the bone marrow, peripheral blood and other organs. (1). ALL is the most common paediatric malignancy. It represents 25% of all childhood cancers and approximately 75% of all cases of childhood leukaemia (2). A sharp peak of ALL incidence is observed at 2–5 years of age (3). There has been a gradual Increase in the incidence of ALL in the past 25 years (4).Many genetic polymorphisms (e.g. TEL-AML1 fusion (5) E2A-PBX (PBX1) fusion (6) BCR-ABL fusion (P185) (7) MLL-AF4 fusion (8) IGH-MYC fusion (9) TCR-RBTN2 fusion (10) NQO1) are considered to be a risk factor in ALL and are associated with the disease outcome. (NQO1) gene is located in the long arm of chromosome 16 (16q22.1), it expands approximately 20 kb with 5 entron and 6 exons 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, NQO1 protects cells from oxidative damage [11, 12, 13].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. Derivatives The polymorphism at position 609 in exon6 and (C- T) in the human NQO1 gene results in a proline to serine substitution at position codone 187 in the amino acid structure of the NQO1 protein, resulting in loss of the enzyme activity. [14]Polymorphisms occurring for antioxidant enzyme such as NQO1 have been associated with an increase risk to develop malignant . The lymphoid cells, similar to others, can be affected by oxidative damage and failure of tumor suppressor genes

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function which lead to failure of normal cellular program death. The aim of this study was to examine the association of NQO1 C609T polymorphism with the risk of ALL and the clinical outcome among ALL patients in Sudan.

MATERALS AND METHODS

The study included 75 ALL newly diagnosed patients and 75 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 (Sysmex-Kx21) at Radiation and Isotopes Center- Khartoum (RICK) and Fedail Hospital after immediate performance of CBC, the blood samples were frozen for subsequence molecular analysis.

Molecular analysis was performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. The DNA was isolated by salting out method; (in brief) 300 ul of whole blood was lysedusingred cell lysis buffer Containing (8.3NH4CL, 1gmKHCO3, 1.8ml5% EDTA and1litre of distilled water) . the pelette was lysed by WBCs lysis buffer containing(1.576 Tris-HCL, 1.088 gm EDTA, 0.0292 gm NaCL, 2%SDS, and100 ml distilled water) .High molecular concentration of 6 M sodium chloride containing(35gm of NaCL added to 1 litre of distilled water) was added consebtively to separate the protein fraction. Finally, ice cold ethanol was added to get the DNA fibre which were separated and resuspended in TE buffer containing (2.42 Tris base, 0.57ml acetic acid , 50ul EDTA (0.01M) and100ml distilled water) and stored at -20 c° until used.

The quality of genomic DNA was determined by electrophoresis on1% agarose gel stained with ethidium bromide. NQO1 fragment was amplified using the forward and reverse primers (Show in table 1). The amplification was carried out in thermocycler (Techne) with initial denaturation step for 8 minute at 95 c° followed by 35 cycles consisting of 3 steps: Denaturing at 94 c° for 30 second, annealing at 56c° for 1 minute and extension at 72 c° for 40 minute with final extention at 72 c° for 10 minute. The PCR reactions was performed in a final volume of 20 µl containing (4ul premixed ready to use 5x firepol - master mix (Solis Bio Dyne) , 0 .5ul from each primer , 12 ul DNase free water and 3ul DNA.

The amplified fragment was digested at 37c° over night with 10 U Hinf1 endonuclease (Promega-USA) and 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 the t-test and person correlation test. Results with P value < 0.05 were considered statistically significant.

RESULT

The male: female ratio was 1.7and the median Age was 9 year, with minimum Age of 1 and maximum of 33 years. All patients were tested for the blood cell counts and NQO1 Polymorphism. The results of blood Count for ALL cases were as follows: Mean haemoglobin (Hb) level 10.5±1.9 g/dL; Mean red blood cell (RBC) count 3.8±0.8X10^{12}/L; mean packed cell volume (PCV) 32.7±5.9%; Mean total white cells (TWBC) count 154.9±169.5X109/L; Mean platelet count 92.4±74.9X10^3/L.While for the control group: Mean Hb concentration 13.8±1.3 g /dL; mean RBC count 4.8±0.4X10^{12}/L; mean PCV 40.7±3.8 % Mean total white cells (TWBC) count 7.1±1.8 X109/L; Mean platelet count 254.0±69.4X10^3/L

Table 1: Comparison of haematological characteristic between ALL patients and control subjects.

Parameter	Cases	Controls	P.value
Hbmean±SD (g/dl)	10.5±1.9 g/dL	13.8±1.3 g /dL	0.003
RBC mean±SD(X1012/L)	3.8 ± 0.8X10^{12}/L	4.8±0.4X10^{12}/L	0.000
PCV mean±SD (%)	32.7±5.9%	40.7±3.8 %	0.004
TWBCmean±SD (X109/L)	154.9±169.5X109/L	7.1±1.8 X109/L	0.000
Platelets mean±SD (X109/L)	92.4± 74.9X10^3/L	254.0±69.4X10^3/L	0.086

Table 2 shows the distribution of *NQO1*C609T genotype frequencies between ALL patients and control group. When the NQO1 609CCgenotype was defined as the reference, the ORs for the CT genotype and TT genotype, (OR = 2.878, 95% CI, 1.051- 7.876,P = 0.040) and (OR = 1.096, 95% CI:0.368-3.262, P = 0.869), respectively.

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

Genotype	ALL patients n (%)	Controls n (%)	OR	95%CI	P value
CC	49 (65.3)	47 (78.3)	Referent		
CT	18 (24)	6 (10)	2.878	1.051-7.876	0.040
TT	8 (10.6)	7 (11.7)	1.096	0.368-3.262	0.869

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

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

Parameter	Wild type (609CC)	Mutant type (609CT+TT)	P value
Hbmean±SD (g/dl)	11.2±1.4	9.3±2.3	0.000
TWBC mean±SD (X10^9/L)	66.1±61.6	322.2±182.3	0.000
Platelets mean±SD (X10^9/L)	121.8±75.6	37.0± 27.6	0.332
RBCs mean±SD (X10^{12}/L)	3.9±0.7	3.3±0.9	0.003
Blasts mean ±SD (%)	33.5±17.2	71.9±18.5	0.307

DISCUSSION:

Our study included 75 newly diagnosed ALL patients, their NQO1 C609T genotype frequencies and haematological characteristics were determined and compared with 75 ages and sex matched normal subjects as control. The study showed a statistically significant association between NQO1 C609T polymorphisms and the risk of ALL. The frequency of the NQO1 609CC genotype was higher among controls (78.3%) when compared to ALL patients (65.3%). When odds ratios were calculated for the overall group, we observed a 2.9-fold increased risk of ALL for those carrying NQO1 609CT (heterozygous) genotype (OR 2.878, P value 0.040). The frequency of NQO1 609 TT (homozygous) genotype was slightly lower among ALL patients (10.6%) than control group (11.7%), but with no statistical significance (OR 1.096, *P* value 0.869). Similar findings had previously been reported.

We observed a statistically significant reduction in the mean Hb level and RBCs count in patients with mutant genotypes than in wild type patients (*p* value 0.000 and 0.003), WBCs count was significantly higher in patients with mutant type when compared to those with the wild type (*p* value 0.000).

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

CONCLUSION:

In conclusion, we examined the association between NQO1 C609T polymorphism and the risk of ALL. Our results indicate that NQO1 C609T mutant genotypes with low enzymatic activity are associated with increased risk of ALL and worse haematological feature.

ACKNOWLEDGEMENTS

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

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