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Research Article

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

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ABSTRACT

AML is a 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 AML and the clinical outcome among AML patients in Sudan. The study included 75 newly diagnosed AML patients, their NQO1 C609T genotypes (detected by PCR/RFLP) and haematological charecteristics (determined by Sysmex KX-21N) were determined and compared with 100 ages and sex matched normal subjects as control. When the NQO1 609 CC genotype was defined as the reference, a 2.4-fold increased risk of AML for those carrying NQO1 609CT (heterozygous) genotype was observed (OR 2.421, *P*value 0.012). The frequency of NQO1 609 TT (homozygous) genotype was higher among AML patients with a 1.6 folds than control group, but with no statistical significance (OR 1.634, *P* value 0.328).The frequency of the NQO1 609CTand 609 TT genotypes combined together (mutant types) was significantly higher among AML patients with a 2.4- fold increased risk when compared with the controls (OR 2.353, *Pvalue* 0.007). We observed a statistically significant reduction in the mean WBCs count in patients with mutant genotypes than in wild type patients (*p* value 0.003), but platelet count, Hb level , and RBCs count were not significantly. In conclusion, our results indicate that NQO1 C609T mutant genotypes, with low enzymatic activity, are associated with increased risk of AML.

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

INTRODUCTION

Acute myeloid leukemia (AML) (also known as acute myeloblastic leukemia, acute myelogenous leukemia, acute granulocytic leukemia, and acute non-lymphocytic leukemia) is a malignant disease of the bone marrow in which hemopoietic precursors are arrested in an early stage of development [(1)]. Mainly common in adults and slightly increase in male (2). In AML, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation (3). The result is the uncontrolled growth of an immature clone of cells, leading to the clinical entity of AML (4). Much of the diversity and heterogeneity of AML stems is because leukemic transformation can occur at a number of different steps along the differentiation pathway (5). Modern classification schemes for AML recognize the characteristics and behavior of the leukemic cell, and the leukemia sub-types may depend on the stage at which differentiation was halted. (5). Specific cytogenetic abnormalities can be found in many patients with AML; the types of chromosomal abnormalities often have prognostic significance (6). The chromosomal translocations encode abnormal fusion protein, usually transcription factors whose altered the normal cell signaling pathway "differentiation arrest" (7,8). The clinical signs and symptoms of AML result from the growth of leukemic clone cells, which tends to displace or interfere with the development of normal blood cells in the bone marrow, this leads to neutropenia, anemia, and thrombocytopenia (9). Many genetic polymorphisms (e.g. CYP3A4 (10,11), GST-M1(12,13) , GST-T1(12,13) , and NQO1 (14,15)) are considered to be a risk

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factor in AML 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 (16, 17, and 18). 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 (19). 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 function which lead to failure of normal cellular program death (20-23). The aim of this study was toexamine the association of NQO1 C609T polymorphism with the risk of AML and the clinical outcome among AML patients in Sudan.

MATERALS AND METHODS

The study included 75AML patients and 100 controls .The diagnosis of AML was based on the haematological criteria include bone marrow as significant tool to identify and manifest AML .Under informed consent, peripheral blood samples were collected into tubes with EDTA from each subject.

Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. 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.

DNA was extracted by salting out method, (in brief) 300 ul of whole blood was lysed using red 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, 1.088gmEDTA, 0.0292gmNaCL, 2%SDS, and100ml 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, at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan .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 1.2 and the median Age was 28 year, with minimum Age of 2 and maximum of 80 years. All patients were tested for the blood cell counts and NQO1 Polymorphism.

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

Parameter	Cases	Controls	P.value
Hbmean±SD (g/dl)	8.4 ±2.3	14.7±1.2	0.000
RBC mean±SD($X10^{12}$/L)	3.6±0.7L	5.3±0.4	0.062
PCV mean±SD (%)	25.7±6.9	45.4±3.4	0.000
TWBCmean±SD ($X10^9$/L)	230.7 ±220.9	6.8 ±5.3	0.000
Platelets mean±SD ($X10^9$/L)	402.9±95.0	234.2±52.1	0.014

The results of blood Count for AML cases were as follows: Mean haemoglobin (Hb) level 8.4±2.3 g/dL; Mean red blood cell (RBC) count 3.6 ± 0.7$X10^{12}$/L; mean packed cell volume (PCV) 25.7±6.9%; Mean total white cells (TWBC) count 230.7±220.9X109/L; Mean platelet count 67.9±95.0$X10^3$/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 2 shows the distribution of *NQO1*C609T genotype frequencies between AML patients and control group. When the NQO1 609 CC genotype was defined as the reference, the OR for the CT genotype, TT genotype, and CT and TT genotypes combined together were (OR = 2.421 ,95% C1: 1.214-4.830,P = 0.012), (OR 1.634,95% C1: 0.611-4.371, P = 0.328) and (OR 2.353 95% C1: 1.257-4.405, P = 0.007), respectively.

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

Genotype	AML patients n (%)	Controls n (%)	OR	95%CI	P value
CC	38 (50.7)	69 (69)	referent		
CT	28 (37.3)	21 (21)	2.421	1.214-4.830	0.012
TT	9 (12)	10 (10)	1.634	0.611-4.371	0.328
CT+TT	37 (49.3)	31 (31)	2.353	1.257-4.405	0.007

Mean TWBC count reveals statistically significant differences between AML 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 AML patients with wild type and those with mutant types

.Parameter	Wild type (609CC)	Mutant type (609CT+TT)	P value
Hb mean±SD (g/dl)	8.229 ± 2.4	8.543 ± 2.3	0.517
TWBC mean±SD ($X10^9$/L)	154.103±158.5	301.105± 249.7	0.003
Platelets mean±SD ($X10^9$/L)	79.5± 109.9	59.8± 81.9	0.773
RBCs count ±SD ($X10^{12}$/L)	3.726 ± 0.6	3.451 ± 0.7	0.377

DISCUSSION:

Genetic polymorphisms of various kinds of genes have been recently proved to have important roles in the genesis of human malignancies (18). 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 AML. Our study included 75 AML patients, their NQO1 C609T genotype frequencies and haematological characteristics were determined and compared with 100 age and sex matched normal subjects as control.

To determine if there was a statistically significant increase risk of AML 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 AML. The frequency of the NQO1 609 CC genotype was higher among controls (69%) when compared to AML patients (50.7%). When odds ratios were calculated for the overall group, we observed a 2.4-fold increased risk of AML for those carrying NQO1 609 CT (heterozygous) genotype (OR: 2.421, P value 0.012). The frequency of NQO1 609 TT (homozygous) genotype was higher among AML patients (12%) than control group (10%), but with no statistical significance (OR 1.634, *P* value 0.328).The frequency of the NQO1 609CTand 609 TT genotypes combined together (mutant types) was significantly higher among AML patients (49.3%) when compared with the controls (31%), with a 2.4- fold increased risk of AML (OR 2.353, Pvalue 0.007). Similar findings had previously been reported (24).

When comparing the studied haematological values between AML patients with NQO1 C609T wild type (CC) and those with mutant types (CT and TT genotypes combined together), we observed a statistically significant increase in the mean of WBCs count in patients with mutant genotypes than in wild type patients, platelet count, Hb level, and RBCs count were not significantly differed in patients with mutant type when compared to those with the wild type

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 AML. 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 AML .Our results indicate that NQO1 C609T mutant genotypes with low enzymatic activity are associated with increased risk of AML.

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