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Original article

Polymorphisms in the NADPH quinone dehydrogenase 1 (*NQ01*) gene in Saudi patients with acute myeloid leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is a hematological malignancy that contains hereditary subgroups. AML can arise as a result of a previous myeloid malignancy. AML's molecular pathogenesis is not yet complete. NADPH quinone dehydrogenase 1 (NQ01), which is linked to AML, is one of a few handfuls of genes that have a role in AML pathogenesis. The goal of this study was to look at the roles of C609T and C465T polymorphisms in the *NQ01* gene in the development of AML in Saudis. In this experimental study, 100 AML patients and 100 healthy controls were chosen. For the C609T and C465T PCR products, restriction enzymes were utilized in this study. AML cases and controls were investigated for genotype and allele frequencies. The average age of AML patients and control subjects was 39.9 ± 12.06 years. AML patients were 61% male and 39% female, while controls were 54% male and 46% female. The C609T and C465T polymorphisms in the *NQ01* gene were not linked with any of the genotypes ($p > 0.05$). According to the findings of this investigation, the C609T and C465T polymorphisms play no effect in AML patients in Saudi Arabia.

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1. Introduction

One of the most pressing issues in oncohematology is acute leukemia (AL). Proliferation of immature leukocytes, which account for more than 20% of bone marrow cells or peripheral blood cells, is the primary diagnostic criterion (Al-Tamimi et al., 2022). Accelerated maturation of immature leukemic blasts disrupts normal blood cell formation in patients with AML, a diverse disease. Overall, AML patients face a bleak prognosis due to the fact that resistance, disease relapse, and toxicity are some of the toughest barriers to overcome during treatment (Al-Juaimlani et al., 2023). The biology of the disease, in addition to age and co-morbidities, significantly affects the prognosis of patients with AML. Cancer-causing mutations in FLT3, NPM1, KIT, CEBPA, and TP53 are just a few of the genes that have been shown to convey predictive information thanks to targeted sequencing (Albalawi et al., 2023) and allo-HCT (Zia et al., 2020). While induction chemotherapy is successful in treating most individuals with AML, recurrence and refractory illness remain significant obstacles (Rasool et al., 2015). Most patients with AML achieve cytomorphologic remission, however around half of those who do experience relapse following first treatment (Xabregas et al., 2022). While environmental variables like previous chemotherapy or chemical exposure may play a role in some cases, genetic variances such chromosomal abnormalities or single gene mutations account for the vast majority. Identification of these genetic anomalies is crucial for risk assessment and therapy planning (Kampen et al., 2013). Around 0.5%-1.0% of adult individuals with AML had the translocation t (6;9), and this anomaly has been linked to a poor response to treatment. Activating FLT3 mutations were investigated in 55 patients in a recent global *meta*-analysis (Fernandes et al., 2022; Al-Khatib et al., 2023).

Genetic polymorphisms play a significant role in AML, particularly single nucleotide polymorphism (SNP), which contributes to immune system modification and is linked to both tumors and cancers, and previous research has shown an association between cancer risk and specific SNPs (AlKhulaifi et al., 2022; Khan et al., 2015). Several SNPs were studied in relation to AML, and NADPH quinone dehydrogenase 1 (NQ01) was one of them that revealed

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a link. It is an enzyme that helps the body get rid of PAH and lessens the effects of oxidative stress on the blood cells that make up the immune system. A C-to-T mutation at nucleotide 609 in the *NQO1* gene has been related to a polymorphism that causes decreased enzyme activity. There is a lack of information about the association between the TT genotype (present in 5–25% of the population) and Acute Lymphoblastic Leukemia risk (Alshammary et al., 2023). NQ01 carriers with low or absent activity are more likely to develop cancer from exposure to certain quinones (such as benzene metabolites) and nitroaromatic chemicals. At least two sequence alterations in the DNA have been linked to the wide range of NQO1 activity: a C to T transition at position 465 and another C to T transition at position 609, both of which result in either reduced or absent enzyme activity (Farasani, 2023). The role of the *NQ01* gene in AML patients in Saudi Arabia has not been studied. The purpose of this case-control study was to investigate the role of C609T (rs1800566) and C465T (rs1131341) polymorphisms in the *NQ01* gene in AML patients.

2. Materials and methods

2.1. AML patients

Ethical approval and patient consent form was received for this study. In this study, 100 AML cases and 100 controls were studied (Alshammary, 2023). The inclusion and exclusion criteria and patient recruitment details was given in the previous publication (Bogari et al., 2021). Cytogenetic and histopathological studies were used to diagnose AML cases. Furthermore, cytogenetic studies were confirmed using the fluorescent in situ hybridization technique. G power (version 3.1) statistical software was used to determine the sample size at a 0.05 level of significance and an 82% power for the investigation.

2.2. Molecular analysis

A total of 2 ml of EDTA blood sample was collected from each study group and genomic DNA isolated using DNA purification kit (Al-Otaiby et al., 2021; Alshammary and Khan, 2021). The nanodrop method was used to test the purity of genomic DNA (Saif and Khan, 2022). A total of 200 Saudi subjects DNA was isolated and further used for Polymerase chain reaction (PCR) analysis with *NQ01* gene. Both C609T and C465T polymorphisms in NQ01 gene was genotyped using the following primers i.e., for C609T F-CCTCTCTGTGCTTTCTGTATCC and R- GATGGACTTGCCCAAGTGATG and for C465T F- CTAGCTTTACTCGGACCCACTC and R- GCAACAAGAGGGAAGCTCCATC. Both the SNPs were recruited as per minor allele frequency i.e., ≥5%. With a reaction volume of 50 µl, a complete set of PCR master mix, and DNA were amplified by performing PCR (Khan et al., 2019). Finally, 35 cycles of denaturation (95 °C), annealing (63 °C and 58 °C), and extension (72 °C) make up the PCR procedure. Both the initial denaturation and the subsequent elongation played a role (Yadav et al., 2016). Later, 299 bp PCR products were used to digest with *HinfI* restriction enzyme for C609T polymorphism and 464 bp of PCR product was digested with *HpaII* restriction enzyme for C465T polymorphisms in *NQO1* gene.

2.3. Statistical analysis

Power analysis was performed with G software (Version 3). Clinical data was studied using mean and standard deviation (M ± SD). The total numbers were measured using percentages (%). Hardy Weinberg equilibrium (HWE) analysis was measured in C609T and C465T polymorphisms in *NQ01* gene. Genotype and allele frequencies were measured with odds ratios, 95% confidence intervals and p values. Chi-square test was also measured. In this study, a p value less than 0.05 ($p < 0.05$) is considered significant (Zaker et al., 2011).

3. Results

3.1. Clinical details

In this experimental based study, a total of 200 subejcts were enrolled in which 100 were diagnosed with AML and remaining 100 control subjects. The enrolled ages in AML cases were in between 19 and 82 years of age and 18 to 63 years of age in the controls. The details of age and gender was shown in Table 1. The mean age of AML cases and control subejcts was confirmed to be 38.9 ± 15.1 and 39.9 ± 12.06 years and there was a significant association among the groups ($p > 0.05$). There were around 61% of males and 39% of females in AML cases and 54% of males and 46% of females in control subjects.

3.2. HWE-analysis

HWE analysis was performed in C609T and C465T polymorphisms in *NQ01* gene. Both the polymorphisms (C609T and C465T) were selected according to the minor allele frequencies. The HWE analysis was studied in C609T and C465T polymorphisms in NQ01 gene and study results confirmed both the polymorphisms was found to be in accordance after confirming with one degree of freedom (For C609T, $X^2 = 5.65$ and $p = 0.01$ and for C465T, $X^2 = 18.03$ and $p = 0.0002$). The details were showed in Table 2.

3.3. Genotype and allele frequencies for C609T polymorphism

The details of genotype and allele frequencies was showed in Table 3 for C609T polymorphism in NQ01 gene. The band size of C609T was found to be 299 bp. HinfI was the restriction enzyme used to digest the T allele or TT genotype. The CC genotype was found to be 214/85 bp, TT genotype was 151/63 bp and CT-214/151/85/63 bp. The genotype frequencies of CT and TT was found high in AML cases (26% and 8%) when compared with controls (21% and 6%) and the CC genotype was prevalent (73% in controls and 66% in cases). Both C and T allele frequencies were 79% and 21% in AML cases, respectively, and 83.5% and 16.5% in controls, respectively. None of the genotypes were found to be significantly associated with allele [OR = 0.73; 95%CI = 0.24–2.19; P = 0.24], genotype (CT vs CC) [OR = 1.36; OR = 0.70–2.66; P = 0.35] and CC vs CT + TT [OR = 1.39; 95%CI = 0.76–2.55; P = 0.28] (see Table 4).

Table 1
Information of age and gender involved in this study.

	AML cases (n = 100)	Controls (n = 100)	P-Value
Age	38.9 ± 15.1	39.9 ± 12.06	P = 0.60
Ages (Min-Max)	19–82	18–63	–
Gender-Male	61 (61%)	54 (54%)	–
Gender-Female	39 (39%)	46 (46%)	–

Table 2
HWE analysis in C609T and C465T polymorphisms in *NQ01* gene.

Polymorphisms	X^2	P value	VAF
C609T	5.65	0.01	0.17
C465T	18.03	0.0002	0.14

Table 3
Genotype and allele frequencies in C609T Polymorphism in NQ01 gene.

C609T	AML cases	Controls	X^2	ORs	95%CIs	P value
CC	66 (66%)	73 (73%)	Position	Position	Position	Position
CT	26 (26%)	21 (21%)	0.86	1.36	0.70–2.66	0.35
TT	08 (08%)	06 (06%)	0.47	1.47	0.48–4.47	0.49
CC vs CT+TT	66 (66%)	73 (73%)	1.15	1.39	0.76–2.55	0.28
CC+CT vs TT	92 (92%)	94 (94%)	0.30	0.71	0.39–1.31	0.57
C allele	158 (79%)	167 (83.5%)	Position	Position	Position	Position
T allele	42 (21%)	33 (16.5%)	1.32	0.73	0.24–2.19	0.24

Table 4
Genotype and allele frequencies in C465T Polymorphism in *NQ01* gene.

C465T	AML cases	Controls	X^2	ORs	95%CIs	P value
CC	74 (74%)	79(79%)	Position	Position	Position	Position
CT	16 (16%)	14 (14%)	0.31	1.25	0.57–2.73	0.57
TT	10 (10%)	07 (07%)	0.75	1.56	0.56–4.31	0.38
CC vs CT+TT	74 (74%)	81 (81%)	1.40	0.66	0.34–1.31	0.23
CC+CT vs TT	90 (90%)	93 (93%)	0.57	0.67	0.24–1.85	0.44
C allele	164 (82%)	172 (86%)	Position	Position	Position	Position
T allele	36 (18%)	28 (14%)	2.82	1.61	0.92–2.81	0.09

3.4. Genotype and allele frequencies for C465T polymorphism

The PCR product of C465T polymorphism was found to be 464 bp. The restriction enzyme used for C465T was HpaII and it digests the T allele or TT genotype in C465T polymorphism. The band size of CC, TT and CT was 353/111 bp, 464 bp and 464/353/111 bp. In this study, CC, CT and TT genotypes in AML cases were 74%, 16% and 10% and in controls was 79%, 14% and 7%. C and T allele frequencies were 82% and 18% in AML cases, respectively, and 86% and 14% in controls, respectively. None of the genotypes were found to be significantly associated with allele [OR = 1.61; 95%CI = 0.92–2.81; P = 0.09], genotype (CT vs CC) [OR = 1.25; OR = 0.57–2.73; P = 0.57] and CC vs CT + TT [OR = 0.66; 95%CI = 0.34–1.31; P = 0.23].

4. Discussion

Many different types of endogenous quinones are metabolized by the enzyme NQO1. When Serine is substituted for Proline at position 187, the enzyme no longer functions as intended. Aplastic anemia and leukemia are common in people who are homozygous for the 187serine allele (Yadav et al., 2018). Increased alternative splicing events result in the production of shortened mRNA lacking exon 4 in the NQO1*3 polymorphic form (C465T; C at position 465 converts to T, resulting in an Arginine substitution by Tryptophane), resulting in decreased activity. Those who are heterozygous (CT) for these variant alleles have moderate NQO1 activity, whereas those who are homozygous (TT) have almost no NQO1 activity (Lajin and Alachkar, 2013). In this study, both C609T and C465T polymorphism was studied in *NQ01* gene among Saudi patients confirmed with AML cases. This was the first study carried out in the Saudi Population and the study results showed negative association in both the polymorphisms in alleles (p = 0.24 and p = 0.09) and genotypes (p = 0.35 and p = 0.57). This study confirms as there is no role in C609T and C465T polymorphisms and AML cases in the Saudi Arabia.

The *NQO1* gene (16q22.1) was formerly known as DT-diaphorase. The *NQO1* gene encodes a cytosolic NQO1 is a 274 amino acid (30868 Da) enzyme that catalyzes the two-electron reduction of quinone molecules and protects cells from oxidative damage by preventing the formation of semi-quinone free radicals and reactive oxygen species (Jia et al., 2012). The *NQO1* gene, located in the 16q22.1 region of the chromosome, has been shown to be a multifunctional antioxidant and an incredibly versatile cytoprotector, providing further evidence that it plays a crucial role in shielding cells from the noxious effects of free radicals. NQO1 has been shown to play a protective role in apoptosis independent of its enzymatic activity, as it stabilizes the tumor suppressor protein p53 (Bolufer et al., 2007). This statement supports the current study as there was no association in any of the studied polymorphisms in *NQ01* gene.

Both C609T and C465T polymorphism was studied in different ethnicities with AML and showed all forms of association (Lajin and Alachkar, 2013; Malik et al., 2006; Voso et al., 2007; Yamaguti et al., 2009; Yang et al., 2005; Li et al., 2014; Guha et al., 2008).

Li et al. performed a *meta*-analysis study in AML with C609T polymorphism in *NQ01* gene and showed strong predictor for developing the AML (TT vs CC: [OR = 1.44; 95%CI = 1.51–1.81; and dominant model; [OR = 1.35; 95%CI = 1.09–1.68] (Li and Zhou, 2014). *NQ01* gene *meta*-analysis studies in childhood leukemia (He et al., 2017) and ALL (Pelcovits and Niroula, 2020) were conducted. He et al. conducted a *meta*-analysis study with C609T and C465T polymorphisms in the NQ01 gene in Acute leukemia risk, and the study results confirmed that C609T is a risk factor for both AML and acute lymphoblastic leukemia (He et al., 2017).

Much progress has been made in the genetic profiling of AML over the past decade. In turn, this has opened up promising prospects for developing genomically defined targeted medicines for people with AML. The standard of care for patients who carry these mutations has shifted to include the use of medications such FLT3 inhibitors and Isocitrate dehydrogenase 1 and 2 inhibitors, both of which have been studied in clinical trials. Clinical trials are also looking into different targeted medicines that attack specific mutations in AML. The American Leukemia & Lymphoma Society is funding the Beat AML trial, a multicenter study currently recruiting participants. With the expectation that this and additional trials may provide light on the clinical benefits of genomically defined targeted therapies in AM, patients are assigned to treatment based on their genomic profile (Pelcovits and Niroula, 2020).

One of the limitations of this study could be the low sample size, efficient data and missing of cytogenetics and FISH analysis.

The major strength of this study was to recruitment of All Saudi subjects towards this study.

5. Conclusion

This study concludes as both C609T and C465T polymorphism has no role in AML in the Saudi Population. This is due to AML is a rare disease with lethal malignancy and other reason could be Saudi ethnicity as it has no effect in Saudi population. Future studies should be performed with large sample size and additional cytogenetic and FISH analysis data could be useful in treating the patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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