The Egyptian Journal of Hospital Medicine (April 2022) Vol. 87, Page 1293-1296

Impact of NQO1 C609T Polymorphism on The Outcome of Childhood Acute Lymphoblastic Leukemia from Zagazig University Hospital; Egypt

Mohamed Eissa[1], Amal Ahmed Zidan[2], Ola Aly Hossein[2], Alaa A. Omran[2]

[1]Pathology Department, College of Medicine, King Khalid University, Abha, KSA and Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

[2]Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

***Corresponding author:** Mohamed Eissa, **Mobile:** 00966599151626, **E-Mail:** eissa20002000@yahoo.com

ABSTRACT

Background: The NAD (P) H: quinone oxidoreductase (NQO1) C609T polymorphism has been widely thought to be associated with the risk of acute leukemia**.**

Objective: This case-control study aimed to assess the impact of NQO1 C609T gene polymorphism in childhood acute lymphoblastic leukemia (ALL).

Patients and Method: The study was carried out on one hundred de novo ALL children and one hundred apparently healthy children. Routine genotyping of NQO1C609T gene polymorphism by PCR-RFLP was done for all subjects.

Results: No statistically significant difference was observed between the patient group and control group as regards wild and polymorphic genotypes. However, there was a significant difference between ALL patients with wild and polymorphic genotypes regarding their immunophenotyping diagnosis (P=0.02) and FAB classification (P=0.01). There was also a significant difference between ALL patients with wild and polymorphic genotypes regarding their response to treatment. The complete remission in the wild genotype (CC) was 69.2% while in polymorphic genotypes (TT & CT) was 29.4% (P<0.05).

Conclusion: The polymorphic genotype forms of the NQO1 C609T (CT and TT) are associated with decreased response to treatment.

Keywords: NQO1, TT, CT, ALL, genotypes.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer, accounting for nearly 25% of all cancers in children and 76 percent of all leukemias in children under the age of 15, with a peak between the ages of 2 and 5 years and falling rates in later childhood, adolescence, and young adulthood **(1)**.

Childhood leukaemia, like other human malignancies, is thought to be caused by a combination of environmental stressors and inherited genetic predisposition that affects genomic stability and Deoxyribonucleic Acid (DNA) repair **(2)**. A range of inherited genetic variants, such as those influencing enzymes involved in carcinogen metabolism and detoxification and those impacting immune response modulation, may potentially contribute indirectly to the risk of leukaemia **(3)**.

To categorize leukemias into numerous particular subtypes, the current World Health Organization (WHO) classification systems included morphologic characteristics, immunophenotype, molecular genetics, and clinical data **(4)**.

Acute lymphoblastic leukemia is subdivided into distinctive subtypes by the presence or absence of specific recurrent genetic abnormalities; t (9:22), MLL rearrangement, t (12:21), hyperdiploidy, hypodiploidy and t (1:19) **(5)**. Genomic analysis has a significant impact on the diagnosis and treatment of ALL patients. Some genetic changes, such as treatment sensitivity and survival result, were enough to influence overall cancer behaviour **(6)**.

The five-year event-free survival (EFS) of pediatric ALL is currently approaches 90 percent in the developed world **(7)**. However, the results in adults ALL are away from those in children; the long-term disease-free survival rates of 30 to 40 percent have been acquired with the use of chemotherapy, as compared to 45 to 75 percent with the use of allogeneic transplantation (8).

So, allogeneic stem cell transplantation is considered, a better curative line in certain very-high-risk pediatric patients with low five-year event-free survival (EFS), such as those with BCR-ABL+ ALL or those with a poor response to induction chemotherapy **(9)**.NAD (P) H: quinone Oxidoreductase 1 (NQO1) is an enzyme utilizes reduced pyridine nucleotide cofactors Nicotinamide Adenine Dinucleotide (NADH) or Nicontinamide Adenine Dinucleotide Phosphate (NADPH) to catalyze the direct two-electron reduction of a broad range of quinones **(10)**. Polymorphisms of NQO1 gene could be influential for patient response to induction therapy and for treatment outcome in hematological malignancies **(11)**.

This case-control study was conducted to assess the impact of NQO1 C609T gene polymorphisms on the outcome of childhood acute lymphoblastic leukemia.

PATIENTS AND METHODS

This study was carried out in the Clinical and Chemical Pathology Department and Pediatric Department, Zagazig University Hospitals, Egypt. One hundred newly diagnosed pediatric ALL patients from oncology unit of pediatrics department were included in this study in addition to one hundred apparently matched healthy children. The patients were 50 males

Received: 9/10/2021
Accepted: 7/12/2021

and 50 females, their ages ranged from 6 months to 14 years.

Inclusion criteria: involved parents' consent to share in this study, diagnosed de novo ALL (at the onset of disease before receiving first induction of therapy), age < 15 years old and both sexes.

The patients were subdivided according to the immunophenotyping results into pre B-ALL, C-ALL and T-ALL. The majority of patients were C-ALL (56.7%), followed by T-ALL (23.3%) and pre B-ALL (20%).

There were no statistically significant differences between ALL patients with wild and polymorphic genotypes regarding their cytogenetic analysis & cytogenetic risk (P>0.05). All patients were treated by an induction regimen total XV regimen consisting of intravenous infusion methylprednisolone (20 mg/m^2/d) from day 1 to day 28 combined with intravenous vincristine (1.5 mg/m^2/wk) in addition to intravenous Ara-C (75 mg/m^2) from day 23 to day 28 and oral 6MP (60 mg/m^2/d) from day 22 to day 28. Genotyping of NQO1 gene polymorphism by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis was done. Whole blood genomic DNA purification QIAGEN Mini Kit was used for rapid and efficient purification of high-quality genomic DNA from whole blood samples. Two primers were used to detect the NQO1 gene polymorphism referred to as forward (F) and reverse (R). Their sequences for forward primer was 5'-CCTCTCTGTGCTTTCTGTATCC-3' and for reverse primer was 5'-GATGGACTTGCCCAAGTGATG-3'.

Ethical approval:

The study was approved by The Ethical Committee of Zagazig Faculty of Medicine. An informed consent was obtained from patients or their parents in this research. Every patient and parent received an explanation for the purpose of the study. All given data were used for the current medical research only. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

The data were collected, reviewed and then uploaded to the Statistical Package for the Social Sciences (SPSS), version 21 (SPSSVR; IBM, Armonk, NY, USA). Data were summarized using the mean, standard deviation (SD), median and range for numerical variables. The frequency, distribution and percentage were calculated for categorized variables. Chi-square (X^2) test was used for comparing categorical variables. P value ≤ 0.05 was considered significant.

RESULTS

There was a statistically significant difference between ALL patients with wild and polymorphic genotypes regarding their immunophenotyping diagnosis (P=0.02) and FAB classification (P=0.01) (Table 1).

Table (1): Comparison between wild and polymorphic genotypes with ALL immunophenotyping and FAB classification

Variables	Wild genotype (CC) (n=40)		Polymorphic genotype (CT & TT) (n=60)		χ^2	P
	No	%	No	%		
Immunophenotyping diagnosis						
Pre-B-ALL	13	32	7	11.5	8.20	**0.02***
C-ALL	10	25	46	77		
T-ALL	17	43	7	11.5		
FAB classification:						
L1	10	25	6	10	4.71	**0.01***
L2	23	57	54	90		
L3	7	18	0	0		

The results showed no significant differences between the distribution of NQO1 C609T genotypes and allele frequency in the studied groups (Table 2).

Table (2): Comparison between the distribution of NQO1 C609T genotypes and allele frequency in the studied groups

Variable	Patients (n=100)		Control (n=100)		χ^2	OR	CI	P
	No	%	No	%				
Genotype:								
CC	40	40	44	44		1	(0.2-2.03)	0.48
CT	43	43	30	30	1.46	0.64	(0.38-5.79)	
TT	17	17	26	26		1.47		
Allele:								
C(wild)	110	61.7	105	58.3	0.14	1	(0.55-2.30)	0.71
T(mutant)	69	38.3	75	41.7		1.18		

There was a statistically significant difference between ALL patients with wild and polymorphic genotypes regarding their response to the induction of treatment (P=0.03) (Table 3).

Table (3): Comparison between wild and polymorphic genotypes with response to induction therapy

Response to treatment	Wild genotype (CC) (n= 44)		Polymorphic genotype (CT & TT) (n=56)		χ^2	P
	No	%	No	%		
CR	31	70.5	16	28.5	4.69	**0.03***
NR	13	29.5	40	71.5		

Multivariate regression analysis was done to reveal the independent association of factors that may significantly affect response to treatment among the study sample. It included the age of patients, elevated WBCs count, decreased HB level, decreased platelets count, elevated ESR level, elevated LDH level, immunophenotyping, FAB classification, NQO1 genotypes and number of allele harbored by the studied patients. Multivariate regression analysis revealed that elevated WBCs count, decreased platelets count, elevated ESR level, elevated LDH level and NQO1 genotypes increase significantly slow response to treatment & non-remission rate (P<0.05) (Table 4)**.**

Table (4): Multivariate regression analysis

Variable	B	S.E.	Wald Test	P	Exp (B)	95.0% C.I.	
						Lower	Upper
Elevated WBCs count	1.78	0.15	8.79	**0.003****	3.49	2.04	7.86
Decreased Platelets count	2.1	0.26	8.02	**<0.001****	4.65	3.01	10.2
Elevated ESR level	2.09	0.34	10.12	**<0.001****	8.92	4.56	16.34
Elevated LDH level	2.11	0.39	8.09	**<0.001****	10.35	5.56	21.55
NQO1 genotypes	1.46	0.19	6.48	**0.02***	2.94	1.14	9.85

DISCUSSION

This study included one hundred de novo ALL children and one hundred healthy matched male and female children representing the same population where the cases were obtained from Zagazig University Hospital serving Zagazig city residents in in Lower Egypt from January 2020 to December 2021. This small sample was due to the general lockdown in 2020 due to COVID-19 pandemic. Acute lymphoblastic leukemia (ALL) is the most curable cancer in children [(12)].

Although significant progress has been made in the treatment of ALL, the prognosis following relapse is still poor in high-risk patients with more intensive treatment at the time of diagnosis to improve leukemia-free survival [(13)].

Most drugs used in standard protocols for ALL were developed more than 30 years ago, since that time several new drugs have been incorporated into ALL treatment protocols. However, these therapeutic strategies are also associated with increased treatment-related morbidity and mortality. Therefore, accurate study of high-risk groups at diagnosis is essential for the optimal treatment of these cases [(12)]. In spite of advanced therapeutic approaches, novel diagnostic and therapeutic tactics are still needed to ameliorate the outcome for high-risk cases [(14)].

Allogeneic stem cell transplantation is recommended for particular subsets of patients; for example, those with Ph+ and T-cell ALL with poor early response to induction therapy [(15)]. Wild-type of NQO1 may play a fundamental role in protecting cells against cancer especially on exposure to oxidative stress. It seems not only to settle the cell cycle regulator and tumour suppressor protein p53 protein and increasing its cellular half-life but also to participate to anticancer signaling pathways [(16)].

Reduced NQO1 activity in polymorphic NQO1; (C609T) polymorphism (Pro187Ser) types is associated with a predisposition to cancer. The polymorphism occurs in approximately 25% of the global human population and is particularly common in people of Chinese ethnicity [(17)].

These variants have much lower activity than the wild-type with inappropriate mobility that result in dysfunction and may predispose to cancer [(18)]. The aim of the current study was to study the role of NQO1 (C609T) gene polymorphism as a possible risk factor of childhood acute lymphoblastic leukemia by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). No statistically significant differences were observed between patients and control regarding wild genotype (CC), homozygous mutant genotype (TT) and heterozygous genotype (CT). Our results are in agreement with other results [(19)].

No statistically significant difference was also observed between the two groups regarding both (C)

and (T) alleles. Our results go hand in hand with those reported by **Ouerhani *et al.* [20]**.

On the contrary to our study, other studies reported that NQO1 genetic polymorphisms may play a role in the development of childhood ALL and NQO1 variants were associated with an increased risk of ALL. These results are not surprising because NQO1 enzyme is involved in metabolic pathways of carcinogens with imbalanced redox homeostasis [21]. The differences between our distribution of both C and T alleles and those reported in previous studies support the concept that the etiology of ALL in children is related to genetic variability at more than one gene locus and may be related to the balance between the metabolic activation and detoxification processes [22]. Lack of agreement between these studies might be due to differences in the duration of the exposure to the oxidative stress, sample sizes, duration of follow up during treatment strategy as well as the demographic stratification [17].

In the current study, regarding response to treatment of the studied patients, 69.2% of them achieved complete remission in wild-type of NQO1 (CC) while 29.4% in polymorphic types (CT and TT) with a statistically significant difference among ALL patients. This finding supports the concept that wild NQO1 genotype protect cells against toxic quinine, contribute to anticancer signaling pathways and protects cells against mutagenesis due to free radicals and oxygen metabolites.

CONCLUSION

The polymorphic genotype forms of the NQO1 C609T (CT and TT) are not associated with increased risk of pediatric ALL. However, they are associated with decreased response to treatment.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Sandler D, Ross J (1997):** Epidemiology of acute leukemia in children and adults. Semin Oncol., 24 (1): 3-16.
2. **Belson M, Kingsley B, Holmes A *et al.* (2007):** Risk factors for acute leukemia in children: a review. Environ Health Perspect., 115 (1): 138-45.
3. **Nida S, Javid B, Akbar M *et al.* (2017):** Gene variants of CYP1A1 and CYP2D6 and the risk of childhood acute lymphoblastic leukaemia; outcome of a case control study from Kashmir, India. Mol Biol Res Commun., 6 (2): 77-84.
4. **Olsen R, Chang C, Herrick J *et al.* (2008):** Acute leukemia immunohistochemistry: a systematic diagnostic approach. Arch Pathol Lab Med., 132 (3): 462-75.
5. **Taylor J, Xiao W, Abdel-Wahab O *et al.* (2017):** Diagnosis and classification of hematologic malignancies on the basis of genetics. Blood, 130 (4): 410-423.
6. **Smith J, Sheltzer J (2018):** Systematic identification of mutations and copy number alterations associated with cancer patient prognosis. Elife., 7 : 217-22.
7. **Kato M, Manabe A (2018):** Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int., 60 (1): 4-12.
8. **Faderl S, O'Brien S, Pui C *et al.* (2010):** Adult acute lymphoblastic leukemia: concepts and strategies. Cancer, 116 (5): 1165-76.
9. **Bleckmann K, Schrappe M (2016):** Advances in therapy for Philadelphia-positive acute lymphoblastic leukaemia of childhood and adolescence. Br J Haematol., 172 (6): 855-69.
10. **Morrissy S, Strom J, Purdom-Dickinson S *et al.* (2012):** NAD(P)H:quinone oxidoreductase 1 is induced by progesterone in cardiomyocytes. Cardiovasc Toxicol., 12 (2): 108-14.
11. **Whitehead T, Metayer C, Wiemels J *et al.* (2016):** Childhood Leukemia and Primary Prevention. Curr Probl Pediatr Adolesc Health Care, 46 (10): 317-352.
12. **Bhojwani D, Howard S, Pui C *et al.* (2009):** High-risk childhood acute lymphoblastic leukemia. Clin Lymphoma Myeloma, 9 : 222-30.
13. **Lu X (2013):** Therapeutic strategies for childhood high-risk acute lymphoblastic leukemia. Beijing Da Xue Xue Bao Yi Xue Ban., 45 (2): 327-32.
14. **Man L, Morris A, Keng M (2017):** New Therapeutic Strategies in Acute Lymphocytic Leukemia. Curr Hematol Malig Rep., 12 (3): 197-206.
15. **Das S, Menezes M, Bhatia S *et al.* (2015):** Gene Therapies for Cancer: Strategies, Challenges and Successes. J Cell Physiol., 230 (2): 259-71.
16. **Asher G, Lotem J, Kama R *et al.* (2002):** NQO1 stabilizes p53 through a distinct pathway. Proc Natl Acad Sci USA., 99 (5): 3099-104.
17. **Lajin B, Alachkar A (2013):** The NQO1 polymorphism C609T (Pro187Ser) and cancer susceptibility: a comprehensive meta-analysis. Br J Cancer, 109 (5): 1325-37.
18. **Pey A, Megarity C, Timson D (2019):** NAD(P)H quinone oxidoreductase (NQO1): an enzyme which needs just enough mobility, in just the right places. Biosci Rep., 39 (1): 459-64.
19. **He H, Zhai X, Liu X *et al.* (2017):** Associations of NQO1 C609T and NQO1 C465T polymorphisms with acute leukemia risk: a PRISMA-compliant meta-analysis. Onco Targets Ther., 10: 1793-1801.
20. **Ouerhani S, Cherif N, Bahri I *et al.* (2013):** Genetic polymorphisms of NQO1, CYP1A1 and TPMT and susceptibility to acute lymphoblastic leukemia in a Tunisian population. Mol Biol Rep., 40 (2): 1307-14.
21. **Lei K, Gu X, Alvarado A *et al.* (2020):** Discovery of a dual inhibitor of NQO1 and GSTP1 for treating glioblastoma. J Hematol Oncol., 13 (1): 141.
22. **Wu J, Oraee A, Doonan B *et al.* (2016):** Activation of NQO1 in NQO1*2 polymorphic human leukemic HL-60 cells by diet-derived sulforaphane. Exp Hematol Oncol., 5 (1): 27-32.