The Role of FUT1 Gene Expression in Acute Lymphoblastic Leukemia of Egyptian Patients: A single-Center Case-Control Study

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer globally, accounting for 25% of all cases, with a peak incidence between two and five years. The FUT1 gene is the principal precursor in ABO antigens formation on the red blood cell surface. Several studies reported the relationship between FUT1 gene expression and cancer. This study investigated the role of the FUT1 gene expression and its association with the ABO blood group phenotype in ALL patients from the Tanta cancer center, Egypt. **Methods:** A case-control study was conducted on 69 ALL Egyptian pediatric patients and 33 age-matched controls. The participants are divided based on ABO phenotyping into two main groups; O blood group patients and non-O blood group patients, with corresponding controls. 3 ml of venous blood samples on a sterile EDTA vacutainer were used for ABO phenotyping and FUT1 gene expression analysis by quantitative real-time PCR (qRT-PCR). **Results:** The FUT1 gene expression in the O blood group patients was higher than in the O blood group control (P=0.002), and in the non-O blood group patients (P<0.001). On the other hand, the relationship between the FUT1 gene expression, O blood group, and acute lymphoblastic leukemia risk using the simple and multiple logistic regression analysis showed that the crude (unadjusted) and adjusted odds ratios had no statistically significant association. **Conclusion:** FUT1 gene shows an overexpression within the O blood group phenotype; however, this overexpression is not found to be a potential risk factor for ALL in the studied Egyptian cohort.

Key Words: ALL, FUT1 gene, ABO blood group, qRT-PCR.

DOI Number: 10.14704/NQ.2022.20.12.NQ772121 **NeuroQuantology2022;20(12): 2433-2438**

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Relevant conflicts of interest/financial disclosures:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Introduction:

Acute lymphoblastic leukemia (ALL) is a heterogeneous hematologic disease characterized by the proliferation of lymphoid progenitor cells in the bone marrow, peripheral blood, and various organs **(Brown et al., 2020).** It is the most common type of childhood cancer, accounting for 25% of all cases, with a peak incidence between two and five years. Over the last several decades, ALL pediatric patients' survival outcomes and cure rates have improved significantly. However, these advances are primarily the result of advances in understanding the disease's molecular genetics and pathogenesis **(Brown et al., 2020).**

The FUT1 (H) gene is located in chromosome 19q13.3. It encodes a galactoside 2-L-fucosyltransferase enzyme that adds fucose to the precursor substrate present on the membrane of red blood cells, resulting in the formation of the H antigen. The H antigen is the primary precursor to the A and B antigens found on the membrane of the red blood cell **(Duell et al., 2015).** Its inheritance is required to express the A and B antigens on the red blood cell surface, although it is not a component of the ABO system.

Numerous reports have established a link between the FUT1 gene and cancer. Various studies have demonstrated that overexpression of the FUT1 gene promotes cancer cell proliferation and tumorigenicity, whereas silencing of the FUT1 gene has an opposite effect; for example, overexpression of the FUT1 gene promotes cell proliferation, and resistance to anticancer drugs in ovarian carcinoma **(Iwamori et al., 2005; Hao et al., 2008)**, overexpression of the FUT1 gene inhibited Notch signaling and multidrug resistance in human chronic myeloid leukemia **(Che et al., 2016).**

Additionally, the FUT1 gene regulates breast cancer growth, adhesion, migration, and cancer stem cell (CSC) properties and may be used as a therapeutic target **(Lai et al., 2019).** Furthermore, silencing the FUT1 expression inhibits tumor growth in human epithelial cell carcinomas by inhibiting the epidermal growth factor receptor (EGFR) signaling pathway **(Zhang et al., 2008) and** downregulating the human epidermal growth factor receptor 2 (HER2) signaling via EGFR silencing in gastric cancer **(Kawai et al., 2013).**

Therefore, this study aims to detect the role of the FUT1 gene expression and its association with ABO antigens in Egyptian children with acute lymphoblastic leukemia.

Methods:

The current study includes sixty-eight ALL Egyptian pediatric patients and thirty-three age-matched controls. In addition, the patients newly diagnosed with ALL who were younger than 18 years and had stable disease (no recent exacerbation) were included in the study. On the other hand, ALL patients over 18 years or with other oncological disorders and any acute or chronic condition that would preclude them from participating in the study were excluded.

Patients were chosen between November 2020 and September 2021 while undergoing treatment or receiving routine follow-ups at Tanta Cancer Center's outpatient clinic. All patients and controls underwent a comprehensive history and clinical examination; patient data, including age, family history, and general clinical examination, were obtained from Tanta Cancer Center medical records. Each case enrolled in the study signed a brief written consent. The Research Ethics Committee of Tanta University's Faculty of Medicine approved the experimental protocol under approval No.: **33659/10.**

The current study's participants are divided into two groups based on their ABO blood group phenotyping: non-O and O blood groups; each group is further subdivided into patients and a control group.

Blood sampling and processing

Peripheral venipuncture was used to obtain approximately 3 ml venous blood samples and divided into two parts: first; 1.0 mL on a sterile EDTA vacutainer for ABO phenotyping, and Second; 2.0 mL on a sterile EDTA vacutainer for genetic studies of FUT1 gene expression by quantitative real-time PCR assay (qRT-PCR).

ABO phenotyping

GRIFOLS DG GEL ABO/RH (2D) (Diagnostic Grifols, S.A., Barcelona Spain) was used for ABO phenotyping. Both forward and reverse techniques are used based on the gel technique manual.

Total RNA extraction

RNA was extracted successfully from peripheral blood samples of all groups using a total RNA isolation kit (QIAamp® RNA Blood, Catalog no. 52304), according to the manufacturer's protocol. By estimating the solution's absorbance at 260 nm, the purity and concentration of the extracted RNA were determined spectrophotometrically (RT Step1 nanodrop, Applied BioSystems. the USA). The extracted RNA was stored at -80 ºC for the reverse transcription step.

cDNA synthesis

Extracted RNA (100 ng) was reverse transcribed using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit LOTT 01068754, REF K1622,100 rxns; 10 µL of the extracted RNA was added to 1 µL of Random Hexamer primer and 1 µL RNase free water. The mixture was mixed gently, centrifuged, then incubated at 65 °C for 5 min. Each tube was then filled with the following RT master mix: 5X Reaction Buffer (4.0 ul), 10 mM dNTP Mix (2.0 ul), RiboLock RNase Inhibitor (20 U/µL) (1.0 ul), RevertAid M-MuLV RT (200 U/µL) (1.0 ul). The mixture was gently mixed and vortexed. The conditions of the thermal cycler (Bio-Rad) were as follows: incubated for 5 min at 25°C for primer annealing followed by 60 min at 42°C. Then, the reaction was terminated by heating at 70°C for 5 min. The reverse transcription reaction product is stored at -20 °C until the real-time PCR step.

Quantitative real-time PCR

Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X), LOTT 01043850, REF K0251 (Applied Biosystems® 7500 Fast Dx the Real-Time PCR Instrument) is used to perform Real-time PCR; **Table 1** contains the primer sequences for the target and reference genes.

Table1: Primer sequences of the genes included in the study.

Gene		Sequence (5'–3')	Reference
FUT1	Forward	5'-GCAGCTTCACGACTGGATGTCGGAG-3'	Taniuchi et al., 2012
	Reverse	5'-TACACCACTCCATGCCGTTGCTGGTGACCA-3'	
GAPDH	Forward	5'-ACCACAGTCCATGCCATCAC-3'	Zhou et al., 2012
	Reverse	5'-TCCACCACCCTGTTGCTGTA-3'	

The RT-PCR mixture was carried out in 20 µL total volumes for target and reference genes as the following: cDNA template (2 µL), SYBR Green master mix (10 µL), Forward primer (1 µL), Reverse primer (1 µL), and Nuclear-free water (7 µL). The PCR tubes were mixed efficiently and lay in the real-time PCR machine.

The optimized thermal analysis included two minutes of UDG pretreatment at 50°C, ten minutes of initial denaturation at 95 °C, fifty cycles of denaturation at 95 °C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72 °C for 30 seconds. Subsequently, melting curve analysis increased the temperature from 60°C to 95°C after the PCR cycles to determine the amplified products' specificity. Finally, after normalization to GAPDH gene expression, each sample detected a relative expression of the FUT1 gene. Therefore, the quantities of critical threshold cycle (CT) of the target gene (FUT1) were normalized with portions (CT) of a housekeeping gene (GAPDH) by using the 2-ΔΔCt (Yuan et al., 2006).

Statistical analysis

SPSS software, version 28 (Armonk, NY: IBM Corp USA), is used to perform the Statistical analysis. Qualitative data were described using numbers and percentages. Quantitative data were described using the median and Interquartile Range. The normality of data was assessed using the Shapiro-Wilk tests. A Mann-Whitney U test was conducted to determine the age and gene expression difference between each blood group patient and its corresponding control, as well as the O blood group and non-O blood groups of ALL patients. Also, the multiple logistic regression analysis was used to calculate the other variables' odds ratio. P-value ≤ 0.05 was considered a statistically significant result.

Results:

Demographical characteristics of the study groups

Table 2 and Figure 1 show the demographic characteristic of the participants, including sex and age in both groups. There was no statistically significant difference between the two study groups regarding sex, P= 0.990 and 0.595 for the non-O blood group and O blood group, respectively. On the other hand, there was a statistically significant difference in age between the non-O blood groups, P=0.006. The median age was significantly lower in the ALL group than in the control group. Finally, no

significant difference in age was found between the O blood groups, P= 0.499.

Table 2: A comparison between groups regarding sex and age.

Groups		Control	ALL	P-value
Non-O blood group	Male [a]	13 (65.0%)	34 (65.4%)	0.990
	Female [a]	7 (35.0%)	18 (34.6)	
	Age [b]	16 (2.0)	14.5 (8.0)	0.006**
O blood group	Male [a]	8 (61.5%)	17 (63.0%)	0.595
	Female [a]	5 (38.5%)	10 (37.0%)	
	Age [b]	10.0(8.0)	11.5(6.0)	0.499

a: values represent numbers and percentages (P-value is calculated using a Chi-square test); b: values represent the median, and parentheses represent interquartile ranges (P-value is calculated using a Mann-Whitney U-test); **: Significant value.

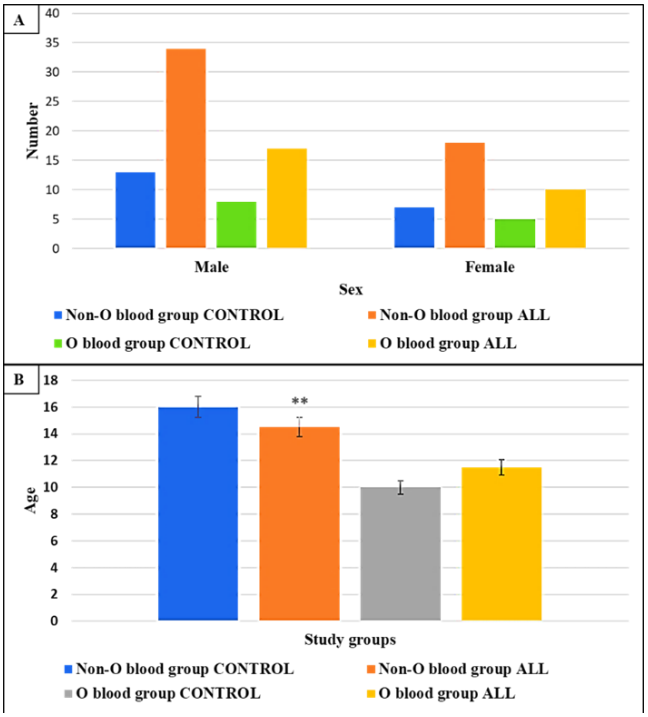

Figure 1: Histograms represent **A):** Sex distribution among different study groups. **B):** the differences between ages in the different study groups. **: Significance at P=0.006.

qRT-PCR for FUT 1 gene expression

Table 3 and Figure 2 show the FUT1 gene mRNA expression in the study groups; there was no statistically significant difference in the mRNA FUT1 gene expression between non-O blood group patients and their healthy control (P=0.599). On the other hand, FUT1 gene expression shows a statistically significant difference between O blood group patients and the healthy control; it was significantly higher in the patient groups than in the control (P=0.002). Furthermore, the FUT1 gene expression was significantly higher in O blood group patients than in non-O blood group patients, P=0.001.

Table 3: qRT-PCR data analysis for FUT1 gene relative to GAPDH housekeeping gene among the study groups.

	GAPDH CT	*FUT1* CT	ΔCT	ΔΔCT	RQ = $2^{-\Delta\Delta Ct}$	P-value
Non-O-ALL group	21.3 (2.80)	26.37 (3.08)	4.84 (1.61)	0.168 (1.61)	0.890 (1.14)	0.599
Non-O-control group	20.13 (1.66)	24.89 (1.56)	4.56 (0.655)	0.053 (0.600)	0.964 (0.434)	
O-ALL group	21.78 (3.16)	25.43 (2.60)	3.47 (1.66)	-1.553 (1.66)	2.934 (2.22)	0.002**
O-control group	20.71 (1.45)	25.55 (1.07)	4.95 (0.78)	-0.072 (0.76)	0.879 (0.64)	

Values represent the median, and values in parentheses represent interquartile ranges. P-value is calculated using a Mann-Whitney U-test, **: Significance at P=0.002.

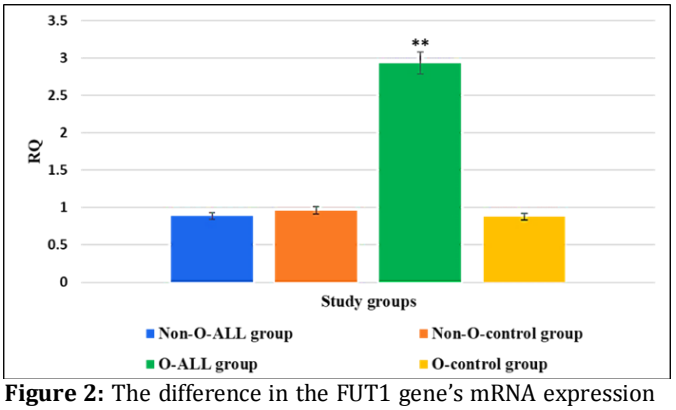

Figure 2: The difference in the FUT1 gene's mRNA expression among the study groups. **: Significance at P<0.02.

Additionally, Figure 3 illustrates the amplification plot and the melting curve of FUT1 gene expression. Finally, the melting curve was done to validate the expected PCR product's specific generation and avoid primer dimmers.

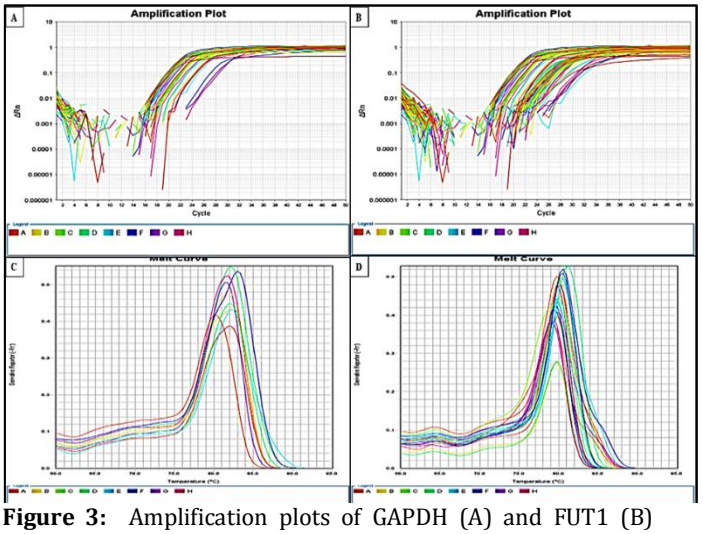

Figure 3: Amplification plots of GAPDH (A) and FUT1 (B) genes; melting curves of GAPDH (C) and FUT1 (D) genes.

Association of FUT1 gene expression with ALL risk

Here, simple and multiple logistic regression analysis was used to analyze the relationship between the FUT1 gene expression, O blood group, and acute lymphoblastic leukemia risk. The crude (unadjusted) and adjusted odds ratios are illustrated in **Table 5.** No statistically significant association was found.

Table 5: Crude and adjusted odds ratio (95% confidence interval) from logistic regression analysis identifying the association between plasma FUT1 gene expression, O blood group, and ALL.

Predictor variable	Crude odds ratio	Adjusted odd ratio
RQ = $2^{-\Delta\Delta CT}$	1.541 (0.995-2.386)	1.513 (0.983-2.330)
Non-O blood group (ref)	1.00	1.00
O blood group	1.560 (0.653-3.729)	1.465 (0.602-3.567)

ref= reference category; crude odds ratios measure the association between the two variables without controlling for any other variables; adjusted odds ratios measure the association between the two variables while controlling for other variables in the model. (Multiple logistic regression analysis, SSPS).

Discussion:

The FUT1 gene and its output, the -(1,2) fucosyltransferase enzyme, play an essential role in tumorigenesis. However, to the best of our knowledge, the precise nature of this mindset is unknown at the moment. Numerous studies have established a link between increased FUT1 gene and alpha-2-L-fucosyltransferase expression and solid tumor progression; for example, **Sun et al.,** 1995 discovered a link between increased FUT1 and alpha-2-L-fucosyltransferase expression and malignant progression of human colon adenocarcinoma. In the present study, the FUT1 gene expression showed a higher significant expression in O blood group patients than in its healthy control; its expression was upregulated by 2.934 -fold when compared with the control group, and this overexpression of the gene was statistically significant (P= 0.002). In line with these results, overexpression of the FUT1 gene in RMG-I cells derived from human ovarian carcinoma increased alpha1,2-fucosyltransferase activity and altered glycolipid composition, promoting malignant cell proliferation and tumorigenesis and resistance to anticancer drugs such as 5-fluorouracil and carboplatin (3, 4). In addition, colon cancer was found to be associated with the FUT1 gene. For example, FUT1 gene overexpression in HT-29/M3 colon cancer cells results in an increase in H type II and Ley, Leb, and a decrease in Lex, altering the glycosylation of MUC1 and MUC5AC gastric apomucins and decreasing their interaction with E-selectin, resulting in increased invasive and metastatic capacity **(Mathieu et al., 2004; Mejías-Luque et al., 2007).**

On the other hand, our data show no statistically significant difference in the FUT1 gene expression between non-O blood group patients than its healthy control, P = 0.599. In addition, the FUT1 gene expression of O blood group patients show a statistically higher significant expression when compared to non-O blood group patient, P < 0.001. The FUT1 gene regulates LAMP-1 and LAMP-2 fucosylation in breast cancer, affecting the autophagic flux rate via mTOR signaling and autolysosome formation. Additionally, it plays a role in synthesizing Globo H and fucosyl-lactosylceramide **(Tan et al., 2016).** Furthermore, the FUT1 gene regulates cancer stem cell features such as cell proliferation, epithelial-mesenchymal transition, carcinogenesis, and metastasis **(Lai et al., 2019).** Moreover, overexpression of FUTI in the BxPC-3 cell line resulted in a significant drop in sialyl-Lewis antigen production, which is associated with an inhibition of E-selectin sticking properties and a decrease in the gastrointestinal metastatic capacity of BxPC-3 cells.

Moreover, **Che et al., 2016** measured FUT1 gene expression in the peripheral blood mononuclear cells (PBMC) of chronic myeloid leukemia (CML) patients and CML/multidrug resistance (MDR) cell lines, which was one of few studies that measured FUT1 gene expression in leukemic patients or leukemic cell lines. When CML/MDR patients' peripheral blood mononuclear cells were compared to chemosensitive CML groups, FUT1 gene expression was 32.101-fold higher. Additionally, MDR cell lines expressed more FUT1 than drug-sensitive parental cell lines. These findings revealed that FUT1 gene regulation influenced the EGFR/MAPK signaling pathway and P-GP expression, which could account for the unusual attribute of the association with CML cell multidrug resistance (MDR).

Conclusively, the present results show that the FUT1 gene expression and the O blood group have no significant correlation with ALL risk in the present studied cohort (OR=1.513, 95% CI= 0.983-2.330) and (OR=1.465, 95% CI= 0.602-3.567) respectively. Previously, numerous studies

reported the association between ABO blood type and the FUT1 gene and the risk of diseases and cancers. For example, the risk of SARS-CoV-2 infection and severe COVID-19 illness was marginally lower in people with the O blood group **(Ray et al., 2021),** while the risk of death was significantly higher in people with the A blood group **(Muñiz-Diaz et al., 2021).** In addition, ABO blood type was correlated with an increased risk of developing some cancers. For instance, blood types B and AB were significantly more likely to develop esophageal squamous cell carcinoma than those with blood type O. Adenocarcinoma of the gastric noncardiac was strongly related to blood types A and AB **(Chen et al., 2021).** Also, a higher risk of developing stomach cancer was seen in people with blood type A **(Edgren et al., 2010).**

Furthermore, it was recently found that the FUT1 gene is a prognostic antigen gene for kidney renal clear cell carcinoma (KIRC) **(Wang et al., 2022).** In another study of the FUTs family's prognostic value in acute myeloid leukemia, a high or low expression of the FUT1 gene had no correlation on event-free survival (EFS) or overall survival (OS) in the chemotherapy alone and allogeneic hematopoietic stem cell transplantation (allo-HSCT) groups. Nevertheless, high expression levels of other FUTs family genes, FUT3, FUT6, and FUT7, had a bad prognosis on EFS. However, in the allo-HSCT group, only FUT3 expression subgroups demonstrated a significant correlation in EFS between the high and low groups. In contrast, other FUT members did not affect survival **(Dai et al., 2020).**

Conclusion and recommendations:

Overall, the FUT1 gene expression is significantly higher in the O blood group ALL patients than in its corresponding healthy control. Moreover, there was significant overexpression of the FUT 1 gene in O blood group ALL patients than in non-O blood group ALL patients. On the other hand, there was no significant difference in FUT1 gene expression in non-O blood group patients and its control. However, the FUT1 gene and the O blood group are not considered risk factors for acute lymphoblastic leukemia in patients in the Egyptian population. Admittedly, additional research using larger sample size and from different locations could validate these results and fully explain the process.

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