JBAAR

Journal of Bioscience and Applied Research

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Investigation of TYMS (rs 2853542) polymorphism and Cytomegalovirus in patients with Acute Lymphoblastic Leukemia

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DOI:10.21608/jbaar.2025.419350

Abstract

Background: Acute Lymphoblastic Leukemia (ALL) is a type of cancer of blood and bone marrow. It is a fast-growing cancer that predominantly affects the lymphoid lineage of white blood cells, leading to the overproduction of abnormal lymphoblasts. It is the most frequently diagnosed form of leukemia in children, though it can also occur in adults. **Objective:** This study aimed to determine the association between TYMS (rs 2853542) gene polymorphism and the percentage of CMV in patients with ALL. **Patients and methods**: A case-control study was carried out on one hundred twenty blood specimens, including sixty patients referred to the national center of hematology/al Mustansiriyah University and diagnosed with Acute lymphoid leukemia and sixty specimens collected from persons as a healthy control group, from October 2023 to June 2024. Sequencing was used to identify **TYMS (rs 2853542)** gene polymorphism and the genome of Cytomegalovirus by using conventional PCR. **Results:** Out of 120 individuals, 60 patients diagnosed as ALL with a mean age (15.9±11.4) years and 60 healthy control group with a mean age (17.8±12.3) year. The difference in the frequency of the **TYMS (rs 2853542)** genotype distribution between patients and control groups was statistically significant. PCR testing for CMV revealed positive results in 8.3% (5 out of 60 cases) of ALL patients. While 3.3% (2 out of 60 cases) have positive DNA-CMV genome in AHC group, so there is non-significant value between these groups, High significance value was found between TYMS (rs 2853542) SNP and CMV- DNA in patients with ALL.

Keywords: **TYMS (rs 2853542)** gene, Acute Lymphoid Leukemia, PCR, sequencing.

Introduction

Acute lymphoblastic leukemia (ALL) primarily affects children, with the B-cell phenotype accounting for around two-thirds of cases (B-cell ALL) [1,2]. Age is a prognostic factor for B-cell ALL, with older age being linked to a worse prognosis [3]. Within the category of acute lymphoblastic leukemia (ALL), 40% of individuals who are affected receive a diagnosis after reaching the age of 20 [4]. Several retrospective analyses have demonstrated that pediatric treatment regimens are more effective than adult regimens in older adults and young adolescents (AYAs) [5]. The pediatric strategy involves administering

Received: December 5, 2024. Accepted: February 12, 2025. Published: March 25, 2025

greater doses of asparaginase, vincristine, steroids, and cumulative intrathecal therapy compared to the adult technique. This is followed by an extended maintenance therapy period lasting 2 to 3 years. Several recent trials have shown that using pediatric-inspired regimens for adolescents and young adults (AYAs) with acute lymphoblastic leukemia (ALL) is feasible, with controllable adverse effects and maybe better results [6]. The thymidylate synthase enzyme, which is encoded by the TYMS gene, is in charge of the de novo production of deoxythymidine monophosphate. One protein that is essential to cell division is thymidylate synthase (TYMS) because it acts as the limiting factor enzyme in the production of pyrimidines through "de novo" synthesis, essential for DNA synthesis [1]. Nevertheless, the coding domain of the TYMS gene exhibits remarkable stability, resulting in only a limited number of variants that have any functional impact, even in tumor tissues. As a result, the focus has shifted towards investigating the variants found in the noncoding areas of the gene [7]. Literature identifies three polymorphisms that regulate the expression of literature. The original identification of a variable tandem repeat sequence, commonly referred to as VNTR (rs45445694), occurred in the 5′-untranslated region (UTR). The number of repetitions varies between 2 and 9, with the alleles 2R and 3R being the most regularly occurring. To resolve the inconsistency, a second genetic variation was discovered, specifically a G>C alteration in the second inversion of the 3R allele (rs2853542). Although it is quite rare, this modification is also present in the first repetition of 2R alleles [6]. That concludes it should remove the stimulatory upstream transcription factor 1 (USF-1), which has an additional binding site [8]. Some research has found a connection between a positive clinical response and genetic variations in the regulatory regions of the TYMS gene, specifically the 5′UTR and 3′UTR sections. However, other studies have been unable to reproduce these findings [9]. Cytomegalovirus, a member of the herpesviridae family, is a double-stranded DNA virus [10, 11]. It can be a primary factor in the development of viral-associated congenital illnesses [12] or can be acquired [13]. Approximately 50 to 80 percent of the global population tests positive for CMV antibodies [14]. CMV viremia and infections are being more frequently identified in juvenile cases of acute lymphoblastic leukemia (ALL) [15], Adult cases treated with GMALL regimens rarely suffer this [16]. CMV infection is a major contributor to illness and death in adult hemato-oncologists who have undergone a transplant [17].

Materials and Methods:

This research was intended to be a case-control study.

Study groups:

1. Group of blood samples the Acute Lymphoblastic Leukemia (ALL) blood studied was obtained from patients aged from 2 to 20 years. Blood from each study group of Patients with ALL should be enrolled, which is classified into: -
from Patients with ALL.
2. Blood from apparently healthy people (AHP) as a control group.

Samples Collection:

Blood samples were collected from patients with ALL and AHP as control groups from general hospitals in Baghdad province, Iraq.

Genotyping of TYMS (rs 2853542):

DNA was isolated from blood samples using the kit provided by the manufacturer (Intron / Korea). The total DNA extraction that was obtained was stored at -20 °C until it was used. The detection of TYMS (rs 2853542) gene polymorphism was used the specific primer

TYMS-F: GACCAGACGGTTCCCAAAGG
TYMS-R: ATGTGTTGGATCTGCCCCAG
Product: 4536bp.

The PCR condition of **TYMS (rs 2853542)** SNP detection were as follows:

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
TYMS (rs 2853542)	95C^0 /5 min	95C^0 / 1 min	59.2 C^0 /1min	72 C^0 / 2min	72 C^0 /5min	40

PCR analysis for CMV:

The viral genome was extracted from whole blood samples using a blood and tissue kit following the instructions provided by the manufacturer (Intron / Korea). The DNA/RNA that was obtained was preserved at a temperature of -20°C until it was ready for utilization. The primer of CMV was used in these studies:

F.5'-GAAGGTGCAGGTGCCCTG-3,

R.5'- GTGTCGACGAACGACGTACG-3'

The PCR conditions for CMV detection were as follows:

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
CMV	95C^0 /5 min	95C^0 / 30 Sec	57 C^0 /45 Sec	72 C^0 / 2 min	72 C^0 /5min	35

Ethics approval:

The research protocol was granted approval by the Ethics Committee of the National Center of Hematology, Mustansiriyah University, Baghdad, Iraq.

Statistical Analysis:

This study used the Chi-square test to ascertain the statistical and analytic significance among the variables under investigation. The statistical analyses were conducted using the Version-25 SPSS program, and a significance level of $p < 0.05$ was considered significant.

Results

Age distribution of study groups:

The age range of patients with ALL was from 2 to 20 years with mean = 15.9 + 11.4 years); while the healthy control group had a mean of 17.8 + 12.3 years. However, no significant variations were detected when comparing studied groups ($P > 0.05$) (Table 1).

Sex distribution of the patients with ALL and AHC:

Incidence of ALL in males was greater (58.3%: 35) than in females (41.7%: 25). In the control group consisting of apparently healthy individuals, the proportion of males was greater (63.3%: 38) than that of females (36.7%: 22). The statistical study revealed a statistically significant difference ($P<0.05$) between the sex groups in the considered groupings (Table 2).

Genotyping of TYMS (rs 2853542) Polymorphism

The results showed that DNA polymorphism distribution was according to GT; GG and TT were 30%; 56.7% and 13.3%, respectively in patients

with ALL and 16.7%; 75% and 8.3%, respectively in AHC group. The difference in the frequency of genotype distribution of the polymorphism between patients and control groups was statistically significant Table (3).

We have a new TYMS (rs 2853542) recording in the GENE BANK. We have a new TYMS (rs 2853542) recording in GENE BANK and NCBI. LC771099; LC771100; LC771101; LC771102

Detection of CMV Genome by Conventional PCR:

The percentage of CMV in patients with ALL was 8.3% (5 out of 60 cases). While 3.3% (2 out of 60 cases) have positive DNA-CMV genome in AHC specimens (figure 1 and table4). The statistical analysis of the differences between these two groups was non-significant (p = 0.05).

Spearman's Rho statistical testing of age, sex, CMV-DNA-PCR, and TYMS (rs 2853542) SNP to evaluate the studied markers in patients with ALL:

Non-relationship and also non-correlation with high significance was found between TYMS (rs 2853542) SNP and CMV- DNA in patients with ALL (r= **0.867; p=0.07**). In addition, a non-significant correlation was found between SNP of TYMS (rs 2853542) according to the age of the patients who have ALL (p=0.841, r= 0.09).

However, there were significant correlations among sex and SNP of TYMS (rs 2853542) in the current study (r=0.390; p=0.048). Furthermore, there is no significant correlation between CMV and age in the current study (r=0.684; p=0.6). Lastly, significant correlations between CMV-DNA and sex of patients with ALL of current study (r=0.492; p=0.047 Shown in Table 5.

Table (1): Distribution of studied patients with ALL according to the mean and range of their age

Studied groups (Age / Year)	N	Mean	S.D	S.E	Range		(P-value)
					Minimum	Maximum	
ALL	60	15.9	11.4	1.95	2	20	P=0.53
AHC*	60	17.8	12.3	2.25	8	23	
Total	120						

AHC* means apparently healthy control

Table (2): Distribution of the studied patients with ALL according to their sex.

Sex			Studied Groups		(P-value)
			AHC	ALL	
	Male	N	35	38	P=0.04
		%	58.3%	63.3%	
	Female	N	25	22	Sign. (P<0.05)
		%	41.7%	36.7%	
Total		N	60	60	

Table3. Genotyping of TYMS (rs 2853542) in patients with ALL and AHC groups.

Zygosity status	ALL No. (%)	Control No. (%)	Position in PCR fragment	OR (95%)	SNP type	Sig.
GT	18 30%	10 16.7%		1.67 (0.48- 3.44)	Missense Variant	0.04
GG	34 56.7%	45 75%	475	1.84 (0.32-2.92)		0.03
TT	8 13.3%	5 8.3%				0.5
Totals	60	60				
Allele						
G	60	55		1.32		0.048
T	40	45		(0.43-3.381)		

Table 4: Results of CMV- DNA in ALL specimens among study groups.

CMV-GENOME	ALL N/ %	AHC* N/ %	P-Value
Positive	**5 (8.3%)**	**2 (3.3%)**	**0.5 Non-sig**
Negative	**55 (91.7%)**	**58(96.7%)**	
Total	**60 (100%)**	**60 (100%)**	

Table 5. Spearman's Rho statistical testing of age, sex, CMV-DNA-PCR and TYMS (rs 2853542) SNP to evaluate the studied markers in patients with ALL.

Spearman's rho		Age (years)	TYMS (rs 2853542)	CMV-DNA	Sex
Age	**r**			**0.841**	
	P			**0.09**	
TYMS (rs 2853542)	**r**	**0.736**			0.390
	P	**0.07**			0.048
CMV-DNA	**r**		**0.867**		
	P		**0.07**		
Sex				**0.492**	
				0.047	

Discussion

The inconsistent literature about the involvement of TYMS polymorphisms in the modulation of TYMS expression prompted us to reassess this matter. None of the studied polymorphisms showed a statistically significant association with expression. The investigation has determined that the highest allelic imbalance is 1.5, which contradicts the previously reported 2-4-fold change in expression of TYMS resulting from the VNTR polymorphism. The present work represents the initial attempt to measure Investigation of TYMS expression using fluorescence fragment analysis, Sanger sequencing, and allele-specific qPCR in an in vivo setting. It is acknowledged that the statistical analysis is constrained by the small number of samples. However, the purpose of this investigation is not to formulate a hypothesis, but really to analyze and juxtapose a prior one. It is worth mentioning that the expression of the TYMS protein and activity in human cancer cell lines are associated with the rate at which the cells multiply [18]. The TYMS expression level in There are enough peripheral blood mononuclear cells (PBMCs) for a quantitative polymerase chain reaction (qPCR) assay to be accurate. Moreover, the transcription factors that interact with the repetitive regions of the polymorphic 5′-UTR are present in all mammary cells [19]. When comparing cancer cells to normal cells, the expression of this transcription factor varies and can either rise or fall. Consequently, the overall TYMS expression is altered, but the fold change between the two alleles is unaffected, and the relative expression remains constant.Even so, many resistance mechanisms[20].In contrast, QPCR allele-specific variables consist mainly of the tested polymorphisms and those that demonstrate linkage discord. The difficulties related to allele-specific quantitative polymerase chain reaction (qPCR) analysis are addressed by Using a source gene.Using multiplex qPCR reduces variations in PCR efficiency at both the complementary DNA (cDNA) level and during qPCR, as well as inconsistencies regarding mRNA stability and quality, Preservation of sample processing integrity, hence decreasing both intra-sample and inter-sample variability. Furthermore, although Sanger sequencing is not commonly used for precisely quantifying gene expression, Other research has verified its correctness [21,22]. Controlling the allelic imbalance of TYMS to a 1.5-fold shift prompts the inquiry of whether this disparity is adequate for efficient therapy control. The results we have obtained are consistent with a review article that established the pharmacogenetic range for the allelic imbalance of TYMS to be 1.5-2.5%. The validity of a dependable indicator is contradicted by this result, as it demonstrates that the range for pharmacogenetic expression is 5 to 10 times, and for genetic mutations, it climbs to 10 to 30 times [23]. The 2R2R genotype (rs34743033) did not show a significant association with the development of MTX-induced oral mucositis compared to the 2R3R/3R3R genotypes. This conclusion was supported by a meta-analysis, which reported an odds ratio (OR) of 1.17 (0.62-2.19). The low-expression TYMS genotype in patients (2R2R, 2R3RC, 3RC3RC) was associated with a higher likelihood of developing oral mucositis produced by MTX [OR: 2.42 (0.86-6.80)], which did not reach statistical significance. A 6-base pair deletion [rs151264360, odds ratio: 0.79 (0.20-3.19)] did not correlate with the occurrence of oral mucositis caused by MTX [24].The absence of a correlation between these genetic variations and the response to 5-FU "in vitro" tests confirms these findings [25]. An investigation of colorectal cancer using meta-analysis shows a moderate assessment of the relative risk related to both the effectiveness and side effects of the 2R allele and 6 base pair insertion, However, there may be a potential bias in the publication and variations within the available literature when analysing 5'-UTR polymorphisms [26]. Combining the analysis of the three

polymorphisms did not lead to a higher probability of toxicity potential [27].

The results of these analyses validate our hypothesis and raise doubts about the reliability of these genetic variations in determining the most effective dosage of 5-FU, particularly when additional genetic variables are not considered. A recently published work has found a connection between a genetic variation contained within the Enolase superfamily member 1 and an antisense RNA targeting TYMS. This study also refutes the suggestion that the previously described genetic variations in TYMS are involved [28]. There is evidence suggesting that congenital cytomegalovirus (CMV) infection may be a modifiable risk factor for childhood acute lymphoblastic leukemia (ALL). In response to the two preliminary investigations conducted by Wiemels et al., the researchers conducted an extensive case-control study on cCMV infection and pediatric ALL, including a substantial sample size. In our comprehensive investigation of 1189 instances of acute lymphoblastic leukaemia (ALL) and 4756 control subjects, we found no evidence of a connection between leukaemia and exposure to congenital cytomegalovirus (cCMV) infection [2]. Jain et al., (2016) found that CMV disease was detected in 10% of the children with ALL; there is an increasing amount of data suggesting that pediatric ALL is caused by an infectious agent. There exist three primary hypotheses explaining the progression of this disease: Greave's proposal of delayed infection, Kinlen's hypothesis of population mixing, and Smith's concept of direct transformation by an infectious agent are three independent theories within the field of immunology [11].

Each of these models failed to forecast the incidence of a disease, for instance, CMV, that affects the immune system's reaction to subsequent infections. Out of the current hypotheses, CMV is the most suitable according to Smith's criteria. These criteria include: (1) the ability to cause changes in the genetic material; (2) Effects particular to B lymphocytes (3) Greater prevalence of infections in areas characterized by lower socioeconomic development (4) limited ability to cause cancer in general; (5) Minimum symptoms experienced during the early infection (6) The capacity to transfer from the mother to the fetus through the placenta without resulting in significant defects [29]. CMV can directly cause breaks in chromosomes during prenatal infection, which is likely linked to its ability to cause birth defects. The gene encodes several proteins that regulate cell cycle regulation and the host's response to DNA damage. Evidence of a correlation between congenital cytomegalovirus (cCMV) and B lymphocytes is observed in CD34+ cells, which are the basic haematopoietic precursor cells located in the bone marrow. Moreover, one must consider the particular cell type in which CMV forms latency, the influence of congenital cytomegalovirus (cCMV) on the density of hematopoietic progenitor cells present in human cord blood. The findings indicated that neonates infected with cCMV had CD34+ cell counts some 2.6 times greater than those in the control group. These findings indicate that cCMV enhances the likelihood of developing ALL by promoting the growth of cells that are susceptible to undergoing nuclear transformation. All of these findings constitute evidence supporting the probability of CMV contributing to the formation of ALL. In addition to this possibility, two separate investigations have indicated a correlation between cCMV infection and ALL cancer [30,31].

Conflicting Interest

No known conflicting financial interests or personal ties that may have seemed to have influenced the work described in this publication are disclosed by the authors.

ACKNOWLEDGMENTS

No university or institution provided support for this research paper, and all efforts were made by the authors.

Funding: None

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