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Methylenetetrahydrofolate reductase levels and gene expression in leukemia

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ABSTRACT

Leukemia is a neoplastic disease that affects the proliferation of white blood cells and their progenitors. Folic acid deficiency is well-known as a risk factor for leukemia. Methylenetetrahydrofolate reductase (MTHFR) enzyme functions in the folate metabolism route, DNA synthesis and methylation processes. Numerous research has been conducted to investigate the link between MTHFR and several human illnesses, including cancer, cardiovascular disease, psychiatric disorders and neurological issues. The current study aimed to examine MTHFR levels and gene expression in patients with leukemia. The research was carried out between February and October of 2022. There were 130 subjects in total, 80 with leukemia, 45 with AML (Female = 16, Male = 26) with a mean age of (32.32±2.69), and 35 with ALL (Female = 16, Male = 19) with a mean age of (35.794±0.63). The study included 50 healthy subjects as a healthy control group (Female = 28, Male = 22), with an average age of (41.8±1.57) years. MTHFR and Folic acid were measured using ELISA, while MTHFR mRNA was assessed using qPCR in blood and plasma. Folic acid and MTHFR levels were found to be lower in patients with AML and ALL compared to controls ($p < 0.01$). MTHFR gene expression was found to be downregulated in leukemia patients ($p < 0.01$).

The findings of the study give support for future research into the effect of folic acid level and supplementation on leukemia-associated pathological outcomes. Folic acid deficiency may affect the folate mediate-one carbon metabolism pathway, as evidenced by changes in the expression of the mRNA MTHFR gene. As a result, more research into the effects of folate status and folic acid supplementation on leukemia is needed to investigate potential therapeutic strategies.

INTRODUCTION

Leukemia is a type of blood cancer that affects the bone marrow and white blood cells (WBCs). It is a broad term that refers to a group of cancers that affect the bone marrow and blood cells, and that causes an increase in WBCs in the human body; these WBCs compete for space with the RBCs and platelets needed by the body to function normally, and the extra WBCs are ineffective [1]. Leukemia is the primary or secondary generation of abnormal leukocytes; it can be acute or chronic, myeloid or lymphoid, depending on the originating cell and the rate of growth. Acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) of the myeloid lineage are the most common types of leukemia. Maturated WBCs can also give rise to less common types of leukemia, such as B-cell, T-cell and NK cell lineages [2,3]. Leukemia is responsible for nearly 2.5% of all new cancer cases and 3.1% of cancer-related deaths. Leukemia is distinguished by alterations in hematopoietic progenitors, as well as widespread bone marrow invasion [4].

Folic acid is essential for humans and other animals because it is one of the basic building blocks and catalyst for several critical metabolic processes [5]. Folate metabolism is a fundamental metabolic process that facilitates various biosynthetic activities, such as the synthesis of pyrimidines and thymidine, as well as the remethylation of homocysteine, through the activation and transfer of 1-carbon units. Folate metabolism facilitates a wider array of biochemical conversions referred to as one-carbon (1C) metabolism [6,7].

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Folate-mediated one-carbon metabolism (FOCM) encompasses a network of interconnected folate-dependent metabolic pathways, including the interconversion of serine and glycine, de novo purine synthesis, de novo thymidylate synthesis, and the remethylation of homocysteine to methionine. The aforementioned pathways are spatially distinct, with marked localization within the mitochondria, cytosol and nucleus. Enzymes within the FOCM network engage in competitive interactions to regulate intracellular folate levels. The distribution of folate cofactors across folate-dependent pathways is regulated by feedback mechanisms. However, the impact of cell cycle regulation on folate one-carbon metabolism (FOCM) is not extensively understood [8].

The enzyme MTHFR operates within the cytoplasm and is accountable for the irreversible transformation of 5,10-MTHF (methyleneTHF) into 5-MTHF (methylTHF). The process necessitates the utilization of NADPH as an electron donor and FAD as a cofactor. The human MTHFR protein consists of two identical subunits, known as homodimers. Each subunit is composed of a short linker region that connects the N-terminal catalytic domain, responsible for binding methyleneTHF, NADP and FADH2 to the C-terminal regulatory domain. The catalytic domain is capable of carrying out the entirety of the enzymatic reaction [9]. The homodimeric human MTHFR protein is composed of several subunits, each of which is comprised of two distinct regions. The N-terminal catalytic domain, spanning amino acids 1-356, is responsible for binding methyleneTHF, NADPH and FAD. In contrast, the C-terminal regulatory part, spanning amino acids 363-656, is involved in the regulation of these molecules. Specifically, amino acids 357-362 within the C-terminal region play a role in this regulatory process [9,10]. The MTHFR gene polymorphism is characterized by the presence of two common variations, namely C677T and 1298C. One of the two genetic variations of the MTHFR gene leads to a decreased activity of the MTHFR enzyme, resulting in a reduction in folic acid levels and an elevation in homocysteine levels within the bloodstream. Mutations in the MTHFR gene lead to a significant deficiency in 5,10-methylenetetrahydrofolate reductase (MTHFR), resulting in hyperhomocysteinemia and a spectrum of disease severity, ranging from neonatal fatality, to adult onset [9].

Several relevant studies have investigated the association between MTHFR and a range of human diseases, including psychiatric disorders, cancers, cardiovascular diseases and neurological conditions [11,12]. One of the identified polymorphisms in the MTHFR gene is the 5,10-MTHFR 677COT (C677T, rs1801133). The substitution of valine for alanine at codon 222 results in a reduction in the enzyme's activity. The prevalence of the 5,10-MTHFR 677CO polymorphism exhibits ethnic variability. Several studies have identified a correlation between the 5,10-MTHF 677COT polymorphism and increased susceptibility to ALL [13,14]. The MTHFR gene mutation has the potential to negatively impact the activity of enzymes involved in the folate metabolism pathway, resulting in an elevation of homocysteine levels in the bloodstream and significant impairment of normal physiological processes in the human body. The etiology of ALL is significantly impacted by MTHFR polymorphisms, specifically the MTHFR C677T gene mutations. Hence, it can be postulated that the presence of MTHFR polymorphism might be associated with increased susceptibility to acute lymphoblastic leukemia and alterations in mitochondrial function [15]. The methylene tetrahydrofolate reductase (MTHFR) gene has been associated with a range of cancers, including acute leukemia [16].

The objective of this study was to investigate the levels of MTHFR and gene expression in individuals diagnosed with leukemia. Additionally, the study sought to explore the levels of folic acid and iron so as to identify potential targets for novel leukemia therapies.

MATERIAL AND METHODS

Subjects

These patients were divided into two groups: the patient group and the control group. A total of 130 subjects were included in the study. It consisted of 80 patients diagnosed with leukemia from whom blood samples were obtained, 45 patients with AML (Female = 19, Male = 26) with a mean age of 32.32±2.69 years old, and 35 patients with ALL, (Female = 16, Male = 19), with mean age (35.794±0.63). As a healthy control group, 50 healthy subjects were included in the study (Female = 28, Male = 22). Their average age was (41.8±1.57) years.

This study employed a case-control design and focused on patients diagnosed with hematological cancer (specifically, acute and chronic leukemia) who received medical care at the Medical City Department/Hematology Center in Baghdad, Iraq. The study period spanned from February 2022 to October 2022, and the diagnoses were made by experienced physicians. The clinical diagnosis was established by evaluating the morphological features of bone marrow aspirates stained with Wright-Giemsa, as well as by conducting immune-phenotyping analyses of leukemic cells. Such hematological data obtained from the peripheral blood sample revealed whether a notable increase in the proportion of leukemia blasts had occurred. The patients in this study were diagnosed with leukemia based on classification and diagnostic criteria.

Methods

A total of 6 milliliters (6 ml) of anticoagulated K3-EDTA blood samples were obtained from each research group. These samples were then divided into 1 milliliter (1 ml) portions for Complete Blood Count (CBC) analysis and 500 microliters (500 µl) for RNA extraction. The extracted RNA was used for measuring MTHFR mRNA gene expression through quantitative polymerase chain reaction (qPCR). The plasma component of the blood was isolated through centrifugation at a speed of 4000 revolutions per minute (rpm) for a duration of 15-20 minutes at a temperature of four degrees Celsius. The isolated plasma was subsequently stored at a temperature of negative 20 degrees Celsius. The purpose of this procedure was to assess the levels of MTHFR using the ELISA method, following the guidelines provided by the manufacturer.

Statistical analysis

The mean and standard deviation (SD) are statistical measures utilized to describe data. The Andersen-Darling test was employed to assess the normality of the data, with a significance level of $p < 0.05$. The distinction between control and experimental subjects was assessed using the Student's t-test. The researchers employed a one-way analysis of variance (ANOVA) to examine potential significant distinctions between the control group and the group of patients. Tukey's post hoc analysis was applied in the analysis of variance (ANOVA) to evaluate the statistical significance. The statistical significance of the results was determined by evaluating the P-value, which was considered significant if it was less than 0.05 in all cases.

RESULTS

One hundred and thirty subjects were involved in the present study. Eighty patients with leukemia; 45 patients with AML (Female = 19, Male = 26) with a mean age of 32.32±2.69 years old, and 35 patients with ALL, (Female = 16, Male = 19) aged 35.794±0.63 years old. Fifty healthy subjects were included as a control group (Female = 28, Male = 22), whose average age was 41.8±1.57 years. All clinical and hematological parameters are summarized in Table 1.

The clinical and biochemical variables of patients with leukemia were compared with the control group as shown in Table 2. Iron concentration was increased significantly in AML and ALL patients compared to control ($p < 0.05$, Table 2). The hemoglobin (Hb) levels exhibited a notable decrease in the groups of patients with leukemia, in comparison to the control group ($p < 0.05$, as shown in Table 2).

Table 1. Comparison of the clinical variables of study groups

Variables	Groups			p-value
	Patients with leukemia		Control	
	AML	ALL		
Total Number	45	35	50	
Females, N (%) Males, N (%)	19(33.3%) 26 (44.4%)	16 (45.7%) 19 (54%)a	28 (56%) 22 (44%)	
Age (Years)	32.32 ±2.69[a]	35.794 ±0.63[a]	41.8 ±1.57[a]	p≥0.05
BMI (kg/m^2)	28.80 ±4.14[a]	23.50 ±0.21[a]	24.85 ±0.49[a]	p≥0.05
Family history of leukemia	3(6.6%)[c]	1 (2.85%)[b]	0[a]	p≤0.05

BMI - Body Mass Index, A one-way ANOVA test was used to validate the data. Different letters indicated a statistical significance

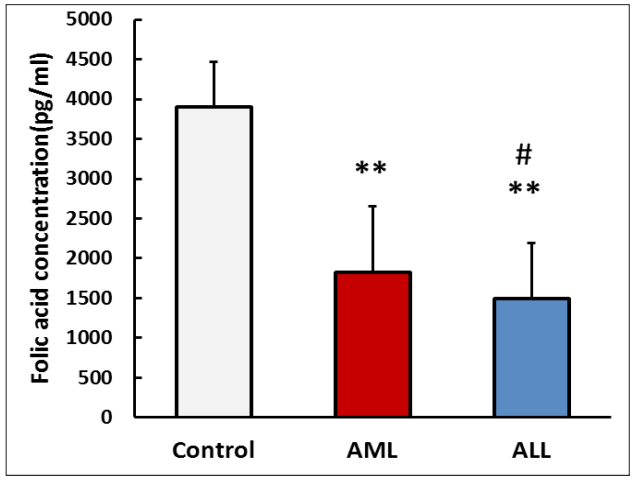
Table 2. Comparison of the biochemical parameters in study groups

Parameters	Groups			p-value
	Patients with leukemia		Control	
	AML	ALL		
Iron	124.77 ±72.19b	287.21 ±179b	81.52 ±24a	p≤0.05
Hb (g/dl)	7.73 ±1.67b	8.2 ±2.31b	13.71 ±1.09a	p≤0.05

Hb - Hemoglobin. A one-way ANOVA test was used to validate the data. Different letters indicated statistical significance

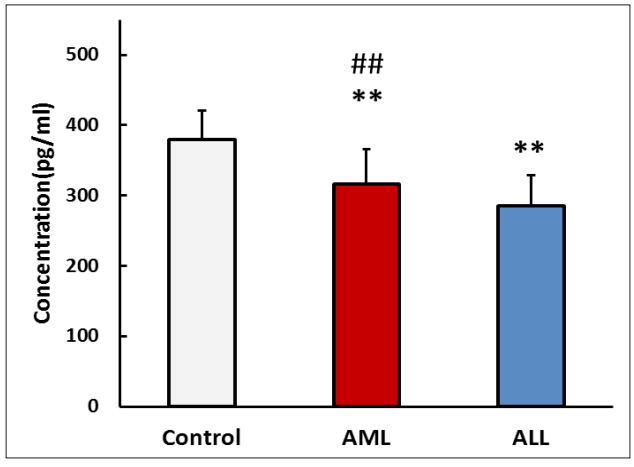
A recent proposition has been made regarding a potential correlation between a deficiency in folate and the development of leukemia. The current investigation observed a notable reduction in serum folic acid levels among leukemia patients when compared to the control group, with statistical significance ($p \leq 0.01$). A significant decrease in folic acid (FA) concentrations was observed in patients diagnosed with ALL when compared to both the control group and the AML group ($p \leq 0.01$), as depicted in Figure 1. Additionally, there has been a suggestion of a potential association between levels of MTHFR and the occurrence of leukemia.

The enzyme MTHFR facilitates the biochemical process of converting 5,10Methylenetetrahydrofolate into 5-Methyltetrahydrofolate, which serves as a co-substrate for the remethylation of homocysteine to methionine. The study revealed that the levels of serum 5,10-MTHFR were significantly lower in both AML and ALL groups, as compared to the control group ($p \leq 0.01$), as depicted in Figure 2. A marked and statistically significant reduction in MTHFR levels was observed in patients with ALL, when compared to those with AML ($p \leq 0.01$).



Data are expressed as mean ± SD. ** indicates a significant change between patient groups *vs* control ($p < 0.01$), # indicates a significant change *vs* ALL ($p < 0.05$)

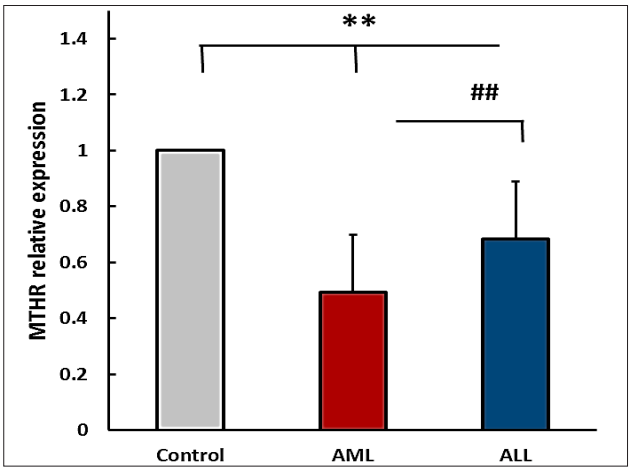
Figure 1. Serum folic acid levels in patients with AML, ALL, and control



Data are expressed as mean ± SD. **indicates a significant change between patient's groups *vs* control ($p < 0.01$), ## indicates a significant *vs* ALL ($p < 0.01$)

Figure 2. Serum MTHFR levels in patients with AML, ALL, and control

The current investigation focused on examining the gene expression of MTHFR. The findings of this study indicate a notable decrease in the expression of MTHFR in both the AML and ALL groups when compared to the control group ($p \leq 0.01$, Figure 3). The results of the study also demonstrated a substantial and statistically significant reduction in MTHR expression in the AML group when compared to ALL and control group ($p < 0.01$).



Data are means ± SD. ** indicates significant differences vs control ($p < 0.01$, ## significant differences vs ALL ($p < 0.01$)

Figure 3. MTHFR expression in patients and control groups. Gene expression is expressed as a fold change in expression *vs* control

DISCUSSION

ALL and AML are commonly occurring malignancies of hematopoietic stem cells, which are characterized by an aberrant accumulation of white blood cells (WBCs) within the bone marrow. This abnormality disrupts the normal process of blood cell production in the body. There is a belief that the metabolic fate of folic acid may have an impact on AL, as this disease arises from rapidly proliferating tissues that have an increased need for DNA synthesis [17]. The findings of the study indicate that there is a higher prevalence of leukemia among males compared to females ($p < 0.05$, Table 1). This observation is consistent with the results reported in a previous study conducted by Bawazir *et al*. [18].

According to the study conducted by Sonja Loges *et al*. [19], the observed gender disparity in hematologic malignancies could potentially be attributed to inherent biological distinctions between males and females, including variations in chromosomes, hormones and immunological reactions. Males afflicted with certain variants of leukemia commonly exhibit alterations in genes located on the X chromosome, potentially elucidating the greater resistance observed in females. In contrast, males possess a pair of chromosomes, specifically an X and a Y chromosome, resulting in a singular instance of protective genes. Consequently, these genes are susceptible to potential mutation-induced alterations. Incorporating considerations of sex within the design of clinical trials is imperative to mitigate the potential risks of over- or under-treatment in either sex.

The concept of age-related clonal hematopoiesis has garnered significant attention in the field of leukemia biology, as it pertains to individuals of diverse age groups who are affected by this disease [20], with AML is 32.32±2.69, and in ALL patients is 35.794±0.63, These findings suggest that individuals of advanced age are more susceptible to developing this particular ailment, and the advancement in age is a notable determinant in the susceptibility to hematopoietic malignancies. These malignancies have been associated with specific somatic mutations, in particular, genes. It is widely accepted that the transformation of normal cells into cancerous cells occurs as a result of the progressive accumulation of genetic or epigenetic modifications [21].

Iron is an essential mineral that plays a critical role in fundamental metabolic processes. Excess iron is stored in tissues in the form of Ferritin. Conversely, increased iron levels result in cellular damage through the mechanism of free oxygen radicals. The lack of an active excretion system in the body leads to the generation of reactive oxygen species (ROS), resulting in cellular damage [22]. In our work, the concentration of iron exhibited a significant increase in patients diagnosed with AML and ALL when compared to the control group. However, no significant alterations in iron levels were observed among patients with AML ($p < 0.05$). The results of the present study are similar to the previously found results [23,24]. Elevated levels of iron, which are closely associated with transfusion procedures, can potentially lead to the accumulation of iron in the heart and liver, resulting in adverse effects.

Iron overload is a significant complication that arises in patients with diseases such as acute leukemias as a result of multiple blood transfusions administered during intensive chemotherapy. Cytotoxic chemotherapy and ineffective erythropoiesis have been identified as contributing factors to the occurrence of iron overload [23]. The hemoglobin levels exhibited a significant decrease in the groups of patients with leukemia when compared to the control group ($p < 0.05$). This finding aligns with the results reported by Ramzi Shawahna and Farah Arshad *et al*. [25,26]. Certain types of leukemia and lymphoma are distinguished by the presence of abnormally reduced levels of hemoglobin and red blood cells. The leukemia group exhibits a reduced concentration of folate, leading to a decrease in hemoglobin levels. Anemia can occur as a consequence of both cancer and cancer treatment.

Anemia in acute leukemia can be attributed to several common causes, including hemorrhage, infiltration of the bone marrow, excessive loss of red blood cells in the peripheral circulation, defective synthesis of red blood cells, and suppression of hematopoiesis due to abnormal iron metabolism [27]. Folic acid, also referred to as vitamin B9, is a crucial coenzyme involved in the synthesis of DNA and RNA [28]. The present study's results indicate a notable reduction in FA levels among individuals diagnosed with AML and ALL, when compared to a control group of healthy individuals ($p < 0.01$). The findings of this study are consistent with the research conducted by Jogamaya *et al*. [27]. Chromosomal instability arises as a consequence of folate deficiency, primarily attributed to the heightened occurrence of DNA strand breakage, uracil misincorporation and impaired repair mechanisms [29].

Various types of cancers, such as leukemia, carcinomas and lymphomas, have the potential to significantly increase the body's requirement for folate, thereby posing a risk of reaching hazardous levels in the absence of appropriate supplementation [30,31]. Several factors can contribute to deficiencies in folic acid, such as inadequate dietary intake and medical conditions that impair the absorption of folate. Furthermore, insufficient availability of folate can also arise from congenital abnormalities in the enzymes responsible for its metabolism [32]. The 5,10-methylenetetrahydrofolate reductase (5,10-MTHFR) enzyme plays a crucial role in regulating intracellular folate metabolism by facilitating the irreversible conversion of 5,10-methyleneTHF to 5-methylTHF [33] and contributes to the progression of cancer through the process of DNA methylation [34]. The one-carbon-methionine pathway is of fundamental importance in regulating various fundamental cellular processes, such as DNA repair, neurotransmitter activation and membrane transport. When this pathway is deficient, it leads to a decrease in 5-MTHF levels and an increase in Hcy levels in the bloodstream [35,36].

In the present study, Serum 5,10-MTHFR levels were lower in both AML and ALL than control ($p < 0.01$). Moreover, a significant decrease in MTHFR levels was observed in ALL patients compared to AML ($p < 0.01$). The low levels of MTHFR in patients may be attributed to a combination of environmental factors, such as inadequate dietary intake, and genetic factors, specifically polymorphism, in MTHFR genes that are involved in homocysteine (Hcy) metabolism. Based on the findings of a published study conducted by M. Karst [37], The available literature suggests that with regard to the T allele of the MTHFR gene, specifically, C677T, polymorphism involves the substitution of a cytosine nucleotide with a thymine nucleotide at position 677. This genetic variant has been proposed as a protective factor against various types of cancer, such as colon cancer and blood cancer, including acute lymphatic leukemia [38]. Furthermore, the study conducted by Rim Frikha [39] suggested that C677T polymorphism has the potential to serve as a reliable biomarker for ALL. It is important to be caution, however, in making such a conclusion, as other factors such as folic acid intake, gene-gene interactions and gene-environment interactions may need to be taken into account. Under circumstances characterized by a deficiency of folate, the occurrence of heightened uracil misincorporation (which, in turn, increases susceptibility to leukemia) can be attributed to mutations in the MTHFR gene. These mutations lead to a decrease in the activity of the MTHFR enzyme, and conversely, reduced enzyme activity is associated with such mutations [40].

The analysis of MTHFR mRNA expression was conducted using quantitative polymerase chain reaction (qPCR) to determine if there was a correlation between the protein level of MTHFR and genetic upregulation in leukemia. The findings of this study indicate a notable decrease in the mRNA expression levels of MTHR in both the AML and ALL groups, when compared to the control group ($p \leq 0.01$). Furthermore, the results of the study indicate that the expression of MTHR was significantly reduced in the AML group when compared to ALL and control group ($p < 0.01$). Laboratorio *et al*. [41] also reported comparable results, demonstrating a significant decrease in the mRNA expression levels of MTHFR among patients with ALL.

This observation aligns with previous research indicating a similar trend in another form of cancer [29]. The potential cause for the decreased levels of MTHFR in patients could stem from a confluence of genetic and environmental elements, including inadequate dietary intake and variations in the MTHFR genes implicated in homocysteine metabolism [40]. According to Zhongjun and Jianheng [42], the expression level of the MTHFR gene exhibits a notable decrease in 17 tumor samples, and significant variations in the gene alterations of MTHFR have been observed among different types of tumors. Of note, the expression of MTHFR exhibits a negative correlation with mitochondrial DNA levels in the majority of cancer types. Given that both colorectal carcinomas and leukemias arise in tissues that undergo rapid proliferation and have a high demand for DNA synthesis, it is expected that the metabolic fate of FA would have a similar influence on both cancer types [17].

CONCLUSIONS

The results of the study indicated that there was a significant decrease in the expression levels of the MTHFR gene in leukemia patients when compared to the control group. This phenomenon played a role in the impact of leukemia on mRNA functionality, metabolic rate and ROS generation. The aberrations in serum protein levels of MTHFR were found to be correlated with the mRNA gene expression levels in patients diagnosed with AML and ALL.

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