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Study the impact of serum SHMT2 and 5-MTHF levels in patients with breast cancer

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Abstract

Background: Breast cancer is the most common type of cancer in females. Although the majority of one-fourth of breast cancers are benign and curable with surgery, whereas the other quarter are latent and insidious, developing slowly but rapidly metastasizing. Current treatments significantly decrease tumor growth, but recurrence is unavoidable, resulting in high mortality rates. SHMT2 is a one-carbon unit metabolic enzyme that catalyzes the conversion of serine and tetrahydrofolate (THF) to glycine and 5,10 methylenetetrahydrofolates. 5-MTHF provides a methyl group for transmethylation of homocysteine to methionine catalyzed by methionine synthase (MS). 5-MTHF donates a carbon group to the biological methylation pathway which also yields THF. This study aimed to elucidate the levels of markers SHMT2 and5-MTHF in serine synthesis pathway in breast cancer patients.

Methods: The study was conducted between December 2022 and March 2023. The total number of samples taken was 135 from women, with 65 samples collected from cancer patient ranging in age from 28 to 53 years, with a mean age of (43.237.028) years. The study also involved collect 70 sample from healthy women as a control

group, ranging in age from 27 to 45 years, with a mean age of (40.867.948) years. Blood sample (5 ml) were collected from all study groups. Serum was collected from the residual (3 mL) blood clot for biochemical measurement of blood urea nitrogen (BUN) and serum creatinine using a colorimetric technique. SHMT2 and 5-MTHF were measured using an enzyme-linked immunosorbent assay (ELISA).

Results: The study's findings revealed a notable The mean blood urea was not significantly changed between patients and the control ($P > 0.05$), Additionally, there was discernible variation in the mean serum creatinine levels amongst the control subject and individuals with breast tumours, ($P < 0.05$), The result show a significant decrease in SHMT2 levels in patients with breast cancer ($P \leq 0.01$) compared to control, and Serum 5-methyl tetrahydrofolate (5-MTHF) level were significantly increased in the patient group with breast cancer compare to the control group ($P \leq 0.01$).

Conclusion: The results suggest that 5-MTHF and SHMT2 might have a new function independent on their metabolic role and proposed as non-invasive predictive biomarkers for breast cancer.

Keywords: Breast cancer, SHMT2, 5-MTHF, Serine synthesis pathway

1. Introduction

Breast cancer develops when cells in the breast begin to proliferate uncontrollably. These cell frequently combine to create a tumor, which can be seen on an x-ray or felt as a bump. If the cell in the tumor may developing into (invade) surrounding tissues or spread (metastasize) to other regions of the body, the tumor is malignant (cancerous) [1]. Breast cancer is the most frequent cancer in women

and is a serious worldwide health issue. Almost 70% of breast cancer patients are over the age of 50, with just 5% being under the age of 40. Every year, roughly 700,000 instances are recorded globally [2].

Breast cancer develop from cell in the breast that have grown abnormally and proliferated to create a lump or tumor. The earliest stage of breast cancer is non-invasive illness (Stage 0), which has not progressed to healthy breast tissue or distant organs (Stages I IV). Breast cancer is the most prevalent cause of cancer-related deaths in women, and it most commonly affects postmenopausal women over the age of 50 [3]. Whenever cancerous cells penetrate the circulatory or lymphatic systems, they can spread to other parts of the body. Lymph is a transparent fluid that flows via lymph veins to transfer waste and immune system cells from tissues, Lymph vessels convey lymph fluid away from the breast through the lymphatic system. These lymph veins can be invaded by cancer cells in the event of breast malignancy, leading to the development of cancerous lymph nodes. It is believed that axillary (located in the armpits) and supraclavicular (located on top of or behind the collarbone) as well as internal mammary lymph nodes (located in the chest) areal lymph nodes from which lymph fluid drains from the breast [4].

SHMT2 (serine hydroxymethyltransferase) is an enzyme that catalyzes the conversion of serine to glycine. It is essential for one-carbon metabolism. Recently, SHMT has been linked to a variety of disorders. As a result, SHMT has gained interest as a biomarker and therapeutic target [5]. As cofactors, tetrahydrofolate (THF) and N-5, N-10-methylenetetrahydrofolate (CH2-THF) are used in the synthesis. SHMT expression has been demonstrated to connect with tumor development and prognosis in recent years [6]. SHMT2, the mitochondrial version of serine hydroxymethyltransferase, is an important enzyme at the intersection of the

amino acid and folic acid metabolic pathway. SHMT2 begins a series of events in mitochondria that result in one-carbon unit covalently bonded to folate species: it transfers one-carbon unit from serine to tetrahydrofolate (THF), producing glycine and 5,10-methylene-THF [7]. In humans, SHMT is divided into two isoforms: SHMT1, which is found in the cytosol (cSHMT), and SHMT2, which is found in the mitochondria (mSHMT). These two isoforms' amino acid sequences are identical. [6].

In the human body, folic acid is inactive and must be transformed by the liver into the active molecule 5-methyltetrahydrofolate (5-MTHF). Many metabolic activities use 5-MTHF as a methyl donor, include the change of homocysteine to methionine, the manufacture of glycine from serine, and the creation of DNA precursor components [8]. The folate cycle is then connected to the methionine cycle via methyl-THF (mTHF), which adds a carbon to homocysteine, methylate's it, and converts it to methionine. One-carbon metabolism produces a variety of components needed for the synthesis of all macromolecules involved in cellular growth and proliferation, such as proteins, lipids, and nucleic acids [9].

2. Materials and methods

Subject

The total number of samples taken was 135 from women, of whom 65 were from cancer patients whose ages varied from 28 to 53 years, with a mean age of (43.237.028) years, and the samples were collected at an oncology hospital. The study also includes collection 70 sample from healthy women as a control group, their age ranging from 27 to 45 years, their mean age was (40.867.948) years, and the sampling period was from December 2022 to March 2023, and general data from the patient were recorded, including age, gender, family history, and some tests that

were performed, such as the Urea test and creatine test. The Oncology Hospital Laboratory and the Clinical Biochemistry Laboratory conducted all laboratory testing at the College of Medicine, University of Al-Qadisiyah Iraq.

Methods

All study groups provided blood samples (5 mL). EDTA Vacationer tubes were filled with 2 mL of blood. The CBC hematological analyzer was used to perform the neutrophils/lymphocytes and hemoglobin ratio study. Serum was collected for 30 minutes from the residual (3 mL) blood clot by centrifugation at (4000 rpm) for 15-20 minute at room temperature (4°C). The separate serum was stored at (-80 C) in Eppendorf tubes (1.5 ml) for biochemical analysis. blood urea nitrogen (BUN), and serum creatinine by colorimetric method. Enzyme-linked immunosorbent assay (ELISA) was used for, PHGDH, SHMT2, measurements

Statistical analysis

SPSS Statistics 23 was use to analyze data. The data were expressed as means ± the standard deviation (SD) The Andersen-Darling test was use to check for normality. for normal distribution data, a student t-test was used to explore significant differences between the control and patients group. A one-way ANOVA were conducted to determine whether or not there were any statistically significant differences between the 3 groups. For one-way ANOVA, A P value of < 0.05 is considered significant throughout.

3. Results

Assessment of mean age between patient with breast cancer and control group

The mean age of individuals with breast cancer and healthy group was involved in (Table 3.1). The mean ages of the patients and the control subjects were 43.23±7.028 years and 40.86±7.948 years, respectively (p = 0.0951). Family history breast type and location were summarized in table (3.1).

Table 3.1: The Clincpathological data and the mean age in individuals with Breast cancer and control subject

Characteristic	breast cancer *n* = 65	Control subject *n* = 70	*P*
Old (years)			
Mean ±SD	43.23±7.028	40.86±7.948	0.451 I NS
Range	28 -53	27 -54	
Family history Yes No	22(33.8%) 43(66.15%)		
Breast type Ductal Lobular	55 (84.61%) 10 (15.38%)		
Location Lateral, right Lateral , left	32 (49.23%) 33 (50.76%)		

* ***n***: numeral of subjects; NS: significant

The clinical and biochemical assessment.

The blood urea and serum creatinine levels between individuals with breast cancer and the control subject were measured. The mean blood urea was not significantly different between patients and the control ($P > 0.05$), Additionally, there was discernible variation in the mean serum creatinine levels amongst the control subject and individuals with breast tumours, ($P < 0.05$).

Table 3.2: Comparison of the biochemical parameters in study groups.

Characteristic	breast cancer $n = 65$	Control group $n = 70$	*P*
Blood urea (mg/dl)			
Mean ±SD	25.03 ± 9.22	25.59 ± 7.64	0.186 I NS
Range	14 - 52	17 -45	
S. creatinine (mg/dl)			
Mean ±SD	0.67±0.105	0.59 ± 0.18	< 0.05*

** $P < 0.05$;* NS: not significant*

Serum SHMT2 levels in patient with breast cancer and control groups

ELISA was applied to measure SHMT2 levels. The result shows a significant decrease in SHMT2 levels in patients with breast cancer ($P \leq 0.01$, Figure 3.1) compared to control.

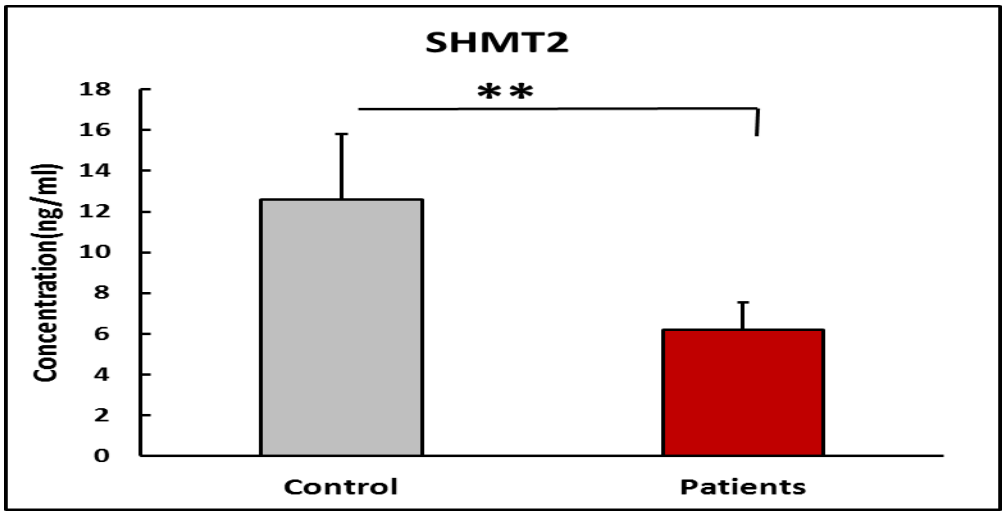

Figure 3.1 Serum SHMT2 levels in patient with breast cancer and control. The data are presented as mean ± SD. ** indicated a significant change among patient and control ($P \leq 0.01$).

In comparison between patient groups, a decreased in SHMT2 is clearly decreased in breast cancer patients who treated with chemotherapy compared to control, but not significantly compared to hormone therapy ($P \geq 0.01$, Figure 3.2)

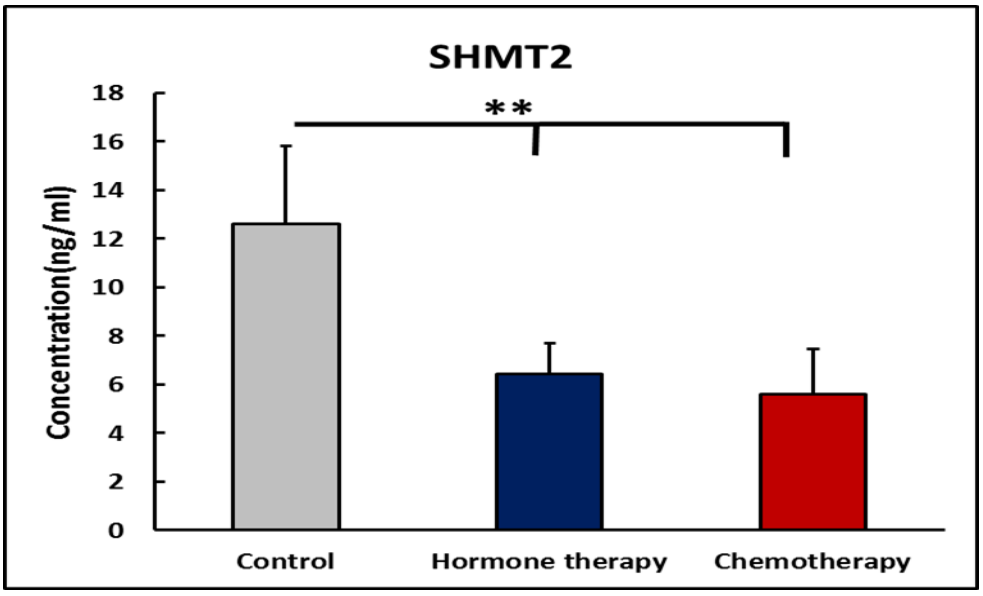

Figure 3.2 Serum SHMT2 level in patient with breast cancer who treated with hormone therapy, chemotherapy and control. The data are present as mean ± SD. ** indicated a significant change among patient and control ($P \leq 0.01$).

Serum 5-methyl tetrahydrofolate (5MTHF) levels in patient with breast cancer and control group

The present study showed that Serum 5-methyl tetrahydrofolate (5-MTHF) level were significantly increased in the patients group with breast cancer compare to the control group ($P \leq 0.01$, Figure 3.3).

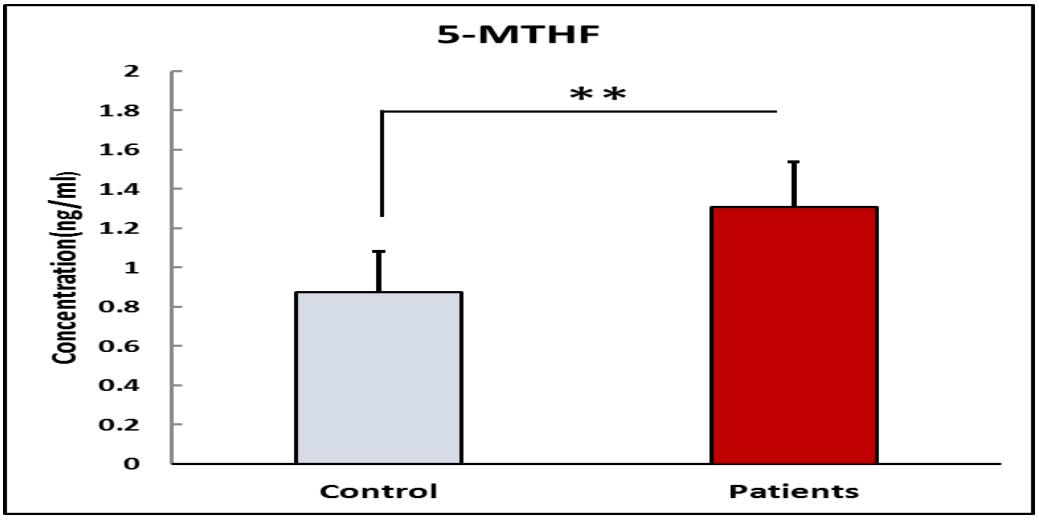

Figure 3.3 Serum 5-MTHF levels in levels in patients with breast cancer and control. The data are present as mean ± SD. ** indicated a significant change among patient and control ($P \leq 0.01$).

A significantly higher level of 5-MTHF was found increased in both hormone and chemotherapy groups compared to control, with Non- significant changes were indicate between the patients with who received hormone and chemotherapy ($P \geq 0.01$) (Figure 3.4).

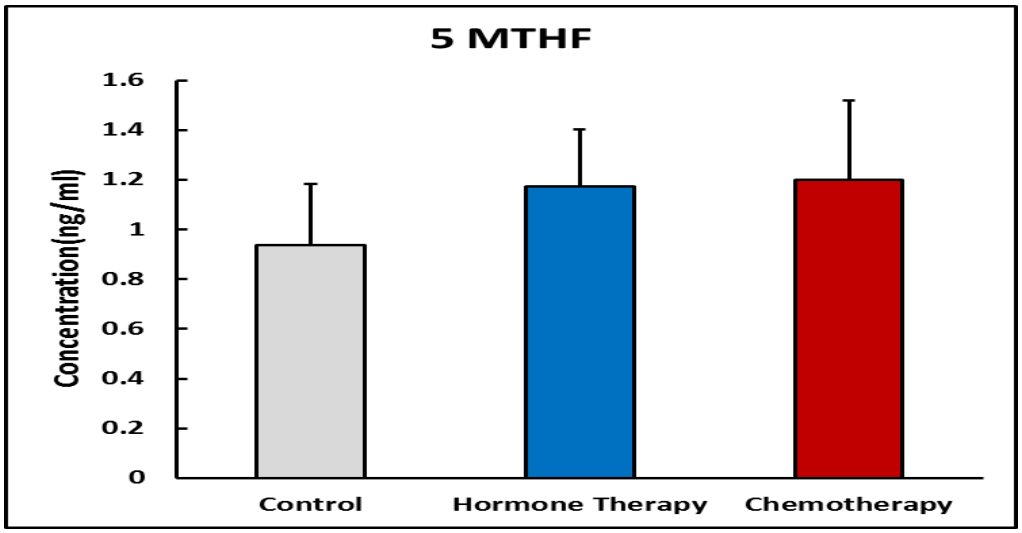

Figure 3.4 Serum 5-MTHF levels in patient with breast cancer who treated with hormone therapy, chemotherapy and control. The data are present as mean ± SD. ** indicated a significant change among patient and control ($P \leq 0.01$).

4. Discussion

Assessment of mean age between patient with breast cancer and control group

The current study found no significant differences in the mean age of breast cancer patients compared to controls. More than 40% of afflicted patients are presently over the age of 65, and this group accounts for over 60% of all breast cancer fatalities [10], [11]. Interestingly, the predicted probability of acquiring breast cancer before the age of 49 is 1/53; however, this doubles to 1/43 for those aged 50-59, and again to 1/23 for those aged 60-69. Significantly, females over the age of 70 had the highest risk, with a 1/15 chance of having breast cancer.

Renal function tests

Urea is the primary nitrogenous waste product of metabolic processes and is produced as a result of protein breakdown. It is the most important nitrogenous byproduct of protein and amino acid degradation. Proteins are first reduced to

component amino acids, resulting in the generation of hazardous ammonia (NH3). In a sequence of five enzymatically regulated reactions known as the "urea cycle," poisonous ammonia produced by protein degradation is converted to non-toxic urea [12].

The present study showed The mean blood urea was not significantly different between patients and the control ($P > 0.05$). is in agreement with this study FU Malya, H Kadioglu [13]. and by T. Kebede, T. Melak [14]. Serum urea levels was non significantly increase in malignant breast cancer patient than in control. This finding is similar with the study conducted in India which showed that the levels of urea were significantly increased in breast cancer cases compare with controls. The study also included an examination of Serum creatinine is one of the most commonly measured product in clinical chemistry laboratories worldwide. Serum creatinine is a commonly use kidney function estimation marker. Given that creatinine is mainly released from muscle tissue, serum creatinine levels is mainly influenced by skeletal muscle mass the serum level reflects not only renal excretion but also the generation, intake, and metabolism of creatinine. Where the results of the study appeared, there was discernible variation in the mean serum creatinine levels amongst the control subject and individuals with breast tumours, ($P < 0.05$). is in agreement with this study B Islam, GI Yousra, D Samir [15]. Our results obtained concerning biochemical parameters revealed a significant increase in serum creatinine levels in Women with breast cancer compared to controls.

Serum SHMT2 in breast cancer patients

SHMT2 is an enzyme that catalyzes the change of serine and tetrahydrofolate (THF) to glycine and 5,10 methylenetetrahydrofolates in one-carbon unit

metabolism. SHMT2 can be activated, promoting the production of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione to maintain redox equilibrium. SHMT2 promotes cells proliferation and tumor formation and is strongly linked to a bad prognosis. and SHMT is composed of two isoforms in humans: SHMT1, which is found in the cytosol (cSHMT), and SHMT2, which is found in the mitochondria (mSHMT). [16].

The current study showed that The result a significant decrease in SHMT2 levels in patients with breast cancer ($P \leq 0.01$, Figure 3.1) compared to control. According to the literature, the current study showed no find references to measure SHMT2 levels in breast cancer patients. SHMT2 was discover to be over-expression in various tumor and correlate with tumor progression and poor prognosis. Regarding breast tumor, SHMT2 was also found to be over-expression in breast tumor cell, and the expression levels of SHMT2 was positively correlated with breast cancer grade [17].

Serum 5-MTHF in breast cancer patients

5-MTHF is the only form of folate that is able to participate in the regeneration of Adenosylmethionine (SAM), a universal methyl donor for most biological methylation reactions including DNA methylation, histone methylation, and protein methylation. 5-MTHF provide a methyl group for transmethylation of homocysteine to methionine catalyzed by methionine synthase (MS). 5-MTHF donates a carbon group to the biological methylation pathway which also yields THF. THF is an important substrate for pyrimidylate and purine biosynthesis, which are the building blocks of DNA and RNA [18].

Folic acid, often known as vitamin B9, is required for DNA and RNA synthesis enzymes. It is required for hematopoiesis and red blood cell creation in addition to purine and pyrimidine production and carbon transfer processes of amino acid metabolism [19].

Current findings showed, 5-methyl tetrahydrofolate (5-MTHF) levels was significantly increased in the patients group with breast cancer compare to the control group ($P \leq 0.01$, Figure 3.3). According to the literature, the current study showed no find references to measure 5-MTHF levels in breast cancer patients. Evidence suggests that changes in folate metabolism, notably 5-MTHF levels, may be linked to an increased risk of various cancers, including breast cancer, 5-MTHF levels may be affected by the chemotherapy medicines used to treat breast cancer [20]. Since the increase of 5-MTHF in breast cancer patients is also considered to be an increase in folate This conclusion is consistent with the findings of Young-In Kim [21] In this study, women who reported supplementary folic acid consumption of 400 g/d had a 20% higher chance of getting breast cancer than those who reported no supplemental intake. Furthermore, whereas dietary folate consumption was unrelated to breast cancer risk, total folate intake, primarily from folic acid supplementation, raised breast cancer risk by 32%.

Conclusion

Decreased SHMT2 and increased 5-MTHF levels in patients with breast cancer associated with rapidly growing cancer cells which may contribute to an increase in folate levels. suggesting that these intermediate might have a novel function independent of their role in metabolism and proposed as non-invasive predictive biomarkers for breast cancer.

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