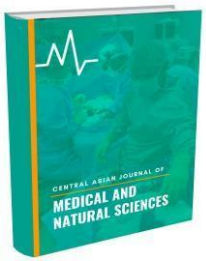

CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
https://cajmns.centralasianstudies.org/index.php/CAJMNS
Volume: 07 Issue: 02 | April 2026 ISSN: 2660-4159

.

Article

Risk Associated with MTHFR-C677T Gene Polymorphism and Immunity in Breast Cancer

Dhuha Abdullah Abbas[*1]

1. Department of Pathological Analyses, College of Science, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

* Correspondence: dhuha.abdallah@qu.edu.iq

Abstract: Background: Breast cancer is a complex malignancy characterized by uncontrolled proliferation of abnormal cells within breast tissue, and representing one of the most prevalent cancers affecting women globally leading to cancer-related mortality. Aim: Gene expression profiling of MTHFR-C677T to estimate association of breast cancer to folate metabolism with evaluation of immune and inflammatory responses in non-treated and treated patients to predict outcome of disease. Materials and methods: A total of 30 adult women diagnosed clinically as newly non-treated (15 women) and treated chemically and/or surgically (15 women) cases in addition to 15 healthy ones (negative control) were subjected to present study. Venous blood was sampled from all study population and used to serological measurement of immune [cluster of differentiation 25 (CD25), CD127, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF)] and inflammatory [C-reactive protein (CRP)] markers by quantitative ELISA; and molecular genotyping of MTHFR-C677T gene by Tetra-ARMS-PCR. Results: Among the population of negative population, differences between the observed and expected data of CC, CT and TT were insignificant. The comparison of allele and genotypes frequency concerning the MTHFR-C677T SNP between the groups of breast cancer patients and healthy negative control detected a significant variation in their values. Concerning alleles, significant elevation was detected in frequency distribution of T allele and the risk in breast cancer patients when compared to negative control population. Regarding genotypes, frequency of TT and the risk were significantly higher in breast cancer patients than those of negative control. Immunologically, the findings of CD25, CD127, IL-6, and TNF-α were shown a significant elevation in non-treated breast cancer patients when compared to those of treated patients and negative control. Subsequently, values of treated breast cancer patients were significantly higher than those of negative control individuals. However, insignificant variation was seen in values of VEGF among the study population; non-treated and treated breast cancer patients as well as the negative control. For inflammatory marker, values of CRP were higher in non-treated breast cancer patients than those of treated breast cancer patients and negative control. Also, the findings of treated breast cancer patients were higher than those of negative control. Conclusion: Significant alteration in alleles and genotypes frequency of MTHFR-C677T gene indicates that folate metabolism contributes to the risk of female breast cancer. Increases in immunologic and inflammatory responses in non-treated and treated breast cancer patients might refer to the relevant of such markers in prognosis of disease.

Keywords: Enzyme-Linked Immunosorbent Assay (ELISA), Folate Metabolism, Methylenetetrahydrofolate Reductase Gene, Polymerase Chain Reaction (PCR), Rs1801133, Single Nucleotide Polymorphism (SNP).

Citation: Abbas D. A. Risk Associated with MTHFR-C677T Gene Polymorphism and Immunity in Breast Cancer. Central Asian Journal of Medical and Natural Science 2026, 7(2), 46-56.

Received: 10th Nov 2025
Revised: 21st Dec 2025
Accepted: 22nd Jan 2026
Published: 15th Feb 2026

1. Introduction

Breast cancer is a group of biologically and molecularly heterogeneous diseases that originating from the breast at different areas such as ducts, lobules, or intervening tissue [1]. The pathogenesis of breast carcinogenesis is understood as a multistep process driven by the accumulation of genetic and environmental events that transform normal cells through stages of hyperplasia, premalignant change, and in situ carcinoma [2], [3]. This progression largely is fueled by genetic mutations that activate oncogenes or deactivate tumor suppressor genes within the mammary cancer stem cells, thereby disrupting the regulatory mechanisms that typically control cellular proliferation and apoptosis [4]. Most DNA mutations related to cancer occur in single breast cells during the life of a women rather than being inherited with acquired mutations of oncogenes and tumor suppressor genes potentially resulting from radiation or carcinogenic chemicals though the specific environmental causes of most acquired mutations remain unknown [5], [6], [7]. Despite uncertainty surrounding many environmental triggers, germline mutations in high penetrance genes in addition to lifestyle variables including physical activity, high body mass index and smoking have been identified as modifiable risk factors that contribute to disease onset [8]. Hormonal influences also play a pivotal role in breast cancer etiology, as reproductive hormones that produced by the ovaries and adrenal glands are involved in the pathogenesis of the disease [9]. Thus, the clinical manifestations of this disease are diverse and driven by intricate pathogenesis possess significant obstacles to effective treatment and prevention [10].

Epidemiological data indicate that the global burden of this malignancy is substantial with over 2.3-2.5 million new cases reported in 2022, accounting for 11.6-12.4% of all cancer diagnosis worldwide [11], [12]. Subsequently, annual rates increased by 1.5% in half of examined countries but the mortality rates were decreased in 29 countries with very high human development index (HDI). By 2050, new cases and deaths might be increased to 38% and 68%, respectively, disproportionality impacting low-HDI countries [13]. In Iraq, Al-Hashimi documented that 72.022 incident breast cancer cases registered during 2000-2019 with an average annual percentage change (AAPC) of +3.192 [14]. The recent national reports and the Iraqi Cancer Registry highlighted the importance of "early detection, strengthen of pathology and imaging services, multimodality treatment, and supporting of related-socioeconomic factors" in improving of life's quality of survivors and reducing mortalities [15]. This high prevalence underscores the critical need for understanding the biological mechanisms of the disease and risk factors to inform public health strategies and clinical interventions. Hence, this study aims to determine the gene expression profiling of *MTHFR-C677T* to estimate association of breast cancer to folate metabolism with evaluation of immune and inflammatory responses in non-treated and treated patients to predict outcome of disease.

2. Materials and Methods

Ethical approval

The Scientific Committee in Department of Pathological Analyses (College of Science, University of Al-Qadisiyah) was licensed the current study.

Samples

A total of 30 adult women 31-49 years old were admitted to some private surgeon clinics in Baghdad province (Iraq) during May (2024)-June (2025), diagnosed with breast cancer and divided as either new non-treated (total number=15 women) or previously treated chemically and / or surgically (total number=15 women) cases. In addition, 15 healthy adult women were selected as a negative control group. Approximately, 10ml of venous blood were collected from each study individuals, and divided into free-

anticoagulant glass-gel tube (8ml) and EDTA-anticoagulant plastic tube (2ml). The free-anticoagulant glass-gel tubes were centrifuged (5000rpm/5min) and the obtained sera were kept in labeled Eppendorf tubes. Tubes of both EDTA-whole blood and sera were saved frozen (-20°C) until be tested.

Genotyping

After preparation at room temperature, the whole blood samples were subjected to extraction of DNAs following the Blood Protocol Procedure of the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan). After estimation the concentration and purity of extracted DNAs, MasterMix tubes were prepared at a final volume of 25µl using Tetra-ARMS-PCR primers (Table 1) and the GoTaqTM G2 Green Master Mix Kit (Promega, USA). Then, the MasterMix tubes were subjected to conditions of Thermal Cycler system as 1 cycle for initial denaturation (95°C/8min); 30 cycles for denaturation (94°C/1min), annealing (63°C/1min) and extension (72°C/1min); and 1 cycle final extension (72°C/5min). Electrophoresis of Agarose-gel (3%) was done to amplification of each SNP, C allele: 198bp, and T allele: 175bp (Sadiq et al., 2019).

Table 1. Sequence of Tetra-ARMS-PCR primer for MTHFR-C677T genotyping.

Primer	5'→3'	Product size
F(inner)	TGAAGGAGAAGGTGTCTGCGGGA	198bp
R(inner)	AGGACGGTGCGGTGAGAGTG	175bp
F(outer)	GCCCCCAAAGCAGAGGACTCTC	344bp
R(outer)	GAGAGTGGGGTGGAGGGAGCTT	

Immunologic and inflammatory markers

Following the manufacturer instructions of the immune markers including CD25 (Cat.No:SL3336Hu), CD127 (Cat.No:SL4940Hu), IL-6 (Cat.No:SL1001Hu), TNF-α (Cat.No:SL1761Hu), and VEGF (Cat.No:SL1811Hu) as well as inflammatory CRP (Cat.No:SL0535Hu) marker of quantitative ELISAs' Kits (SunLong Biotech, China), the sera and contents of each kit were prepared at room temperature, processed, and the optical density (OD) was measured at 450nm. Then, the concentrations of each marker in serum samples were calculated through utilization of Standard Curve in the Microsoft Office Excel.

Statistical analysis

One-Way ANOVA in the GraphPad Prism Software was applied to detect significant differences between study groups (healthy, treated, and non-treated) at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****). Additionally, Chi-square (x^2), 95% confidence interval (95%CI), odds ratio (OR) and relative risk (RR) were estimated to indicate significance between alleles and genotypes frequency (Gharban et al., 2025).

3. Results

Genotyping of MTHFR-C677T

Among the population of negative population, differences between the observed and expected data of CC, CT and TT were insignificant ($p<0.5024$), (Table 2).

Table 2. Hardy Weinberg equation.

Genotype	Observed	Expected	x^2	p-value
Homozygote reference (CC)	12	11	1.3768	0.5024
Heterozygote (CT)	1	3		
Homozygote variant (TT)	2	1		

The comparison of allele and genotypes frequency concerning the MTHFR-C677T SNP between the groups of breast cancer patients and healthy negative control detected a significant variation in their values (Table 3). Concerning alleles, significant elevation ($p<0.0126$; 95%CI: 25.71 to 59.04) was detected in frequency distribution of T allele (20%) and the risk (1.625, 1.0446) in breast cancer patients when compared to negative control population. Regarding genotypes, frequency of TT (23.33%) and the risk (1.9783, 1.6) were significantly higher ($p<0.0349$; 95%CI: 45.20 to 81.86) in breast cancer patients than those of negative control.

Table 3. Frequency of alleles and genotypes in breast cancer patients (Total No: 30) and negative control (Total No: 15).

MTHFR C677T	Breast cancer	Negative control	p-value	OR	RR	95%CI
Allele frequency						
T	9 (20%)	6 (13.33%)	0.0126	1.625	1.0446	25.71 to 59.04
C	36 (80%)	39 (86.67%)	0.0755	0.6154	0.7059	-40.96 to 125.7
Genotype frequency						
TT	7 (23.33%)	2 (13.33%)	0.0349	1.9783	1.6	45.20 to 81.86
CT	3 (10%)	1 (6.67%)	0.0886	0.5	0.1919	-12.82 to 29.49
CC	20 (66.67%)	12 (80%)	0.0433	1.5556	1.2360	11.35 to 158.0

Immune markers

Significant elevation ($p<0.0001$; 95%CI: 1.760 to 6.240) in values of CD25 were determined in non-treated patients (4.07±0.13µg/L) when compared to treated patients (1.61±0.09µg/L) and negative control (1.04±0.07µg/L). Additionally, the findings of treated patients were significantly higher ($p<0.05$) than the values of negative control group (Figure 1).

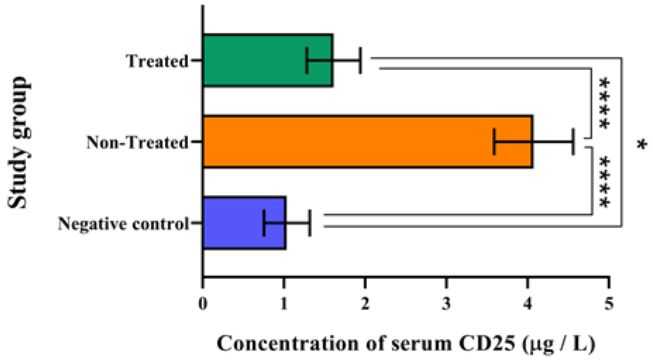

Figure 1. Quantitative measurement of immune CD25 marker among study population.

For CD127 marker, there was a significant elevation (p<0.0001; 95%CI: 551.8 to 1836) in values of non-treated breast cancer patients (1170.8±28.02pg/ml) when compared to treated patients (524.4±40.63pg/ml) as well as the negative control (231.4±10.22pg/ml). Subsequently, the findings of treated breast cancer patients were significantly (p<0.01) higher than those of healthy one (Figure 2).

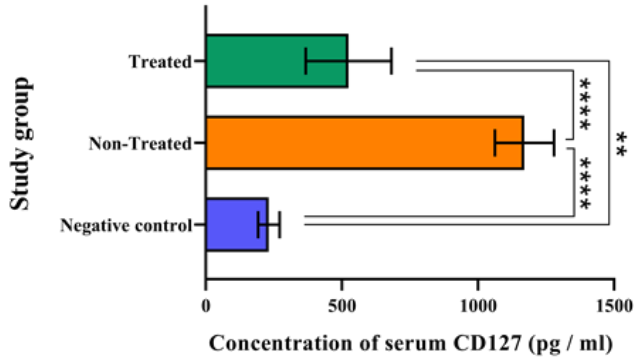

Figure 2. Quantitative measurement of immune CD127 marker among study population.

The findings of IL-6 were shown a significant elevation (p<0.0001; 95%CI: 6.420 to 51.91) in values of non-treated breast cancer patients (36.29±3.02ng/L) than those of treated (16.51±0.4ng/L) and negative control (15.44±1.19ng/L) groups. Also, the findings of treated breast cancer patients were significantly (p<0.05) higher than the values of negative control population (Figure 3).

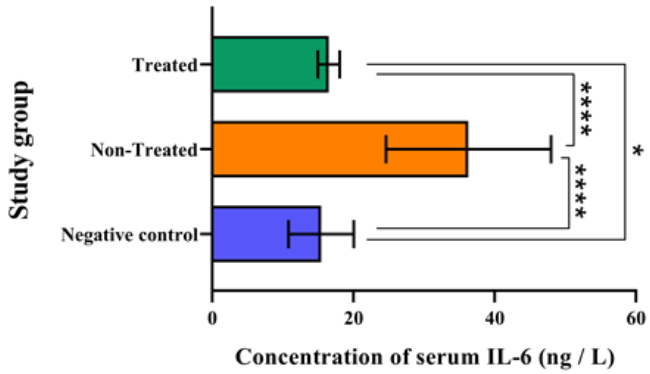

Figure 3. Quantitative measurement of immune IL-6 marker among study population.

Significantly (p<0.0001; 95%CI: 100.6 to 278.7), values of TNF-α were shown an increase in non-treated breast cancer patients (175.4±8.76pg/ml) when compared to those of treated (61.4±5.43pg/ml) and negative control (30.4±1.75pg/ml) groups. Subsequently, the findings of treated breast cancer patients were significantly (p<0.01) higher than those of negative control population (Figure 4).

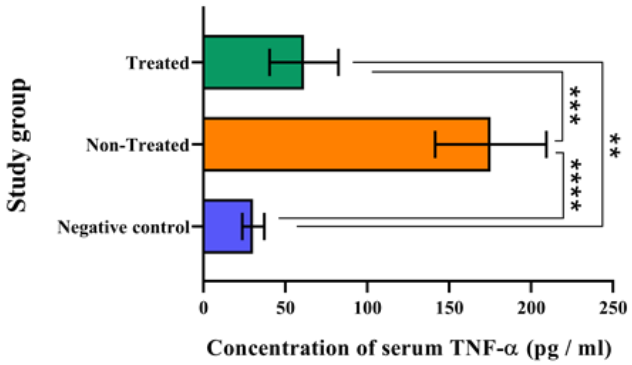

Figure 4. Quantitative measurement of immune TNF-α marker among study population.

The findings of VEGF in non-treated (76.67±4.24pg/ml) and treated (74.33±7.42pg/ml) breast cancer patients were differed insignificantly (p0.0951; 95%CI: 60.70 to 84.85) when compared to those of negative control (72.67±7.07pg/ml), (Figure 5).

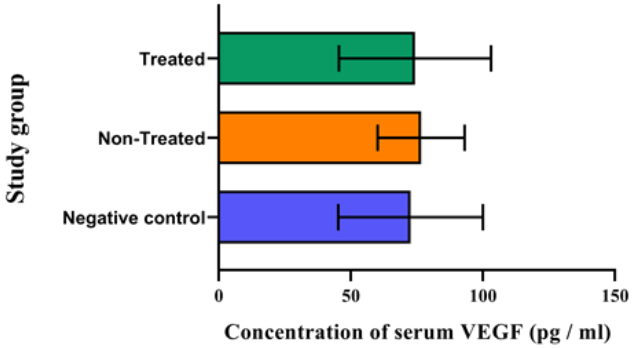

Figure 5. Quantitative measurement of immune VEGF marker among study population.

Inflammatory marker

Values of CRP were shown a significant elevation (p<0.0001; 95%CI: 154.5 to 804.1) in non-treated breast cancer patients (530.93±31.54pg/ml) when compared to treated patients (295±46.89pg/ml) and negative control (148.53±9.23pg/ml). Subsequently, the findings of treated breast cancer patients were significantly higher (p<0.01) than those of negative control individuals (Figure 6).

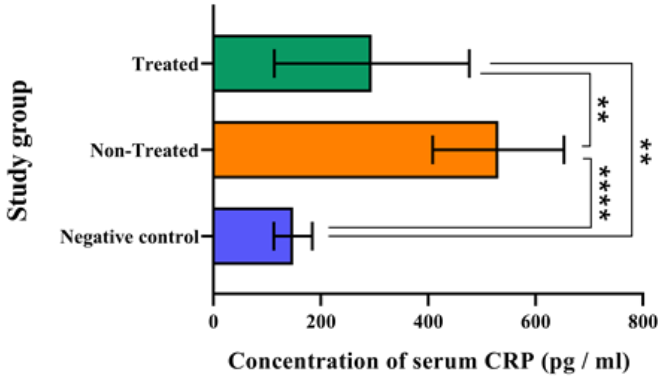

Figure 6. Quantitative measurement of immune CRP marker among study population.

4. Discussion

Breast cancer remains the most prevalent malignancy and the leading cause of cancer-related mortality among women nationally and internationally due to complex etiologies that involving genetic, epigenetic and environmental factors. Among the genetic determinants investigated, *MTHFR* gene, particularly C677T variant (rs1801133), has garnered significant attention due to the enzyme's critical role in folate metabolism and DNA methylation process [16]. In the present study, the findings of *MTHFR-C677T* gene polymorphism were revealed a significant association between folate metabolism and breast cancer. In Iraq, *MTHFR-C677T* gene polymorphism has been investigated extensively to estimate the risk of various diseases such as ischemic stroke [17], colorectal diseases [18], myocardial infarction [19], men [20] and women [21] infertility, rheumatoid arthritis [22], [23], autism [24], toxoplasmosis in pregnant women [25], and type 2 diabetes mellitus [26], nonetheless, few studies have been conducted to evaluate the association of *MTHFR* gene to breast cancer [27], [28], [29].

However, there is inconsistency in distribution of the genetic frequency of the MTHFR polymorphism between patient and control individuals which might be associated with increasing etiological risk of the SNP in breast cancer patients [28]. In addition, this might be attributed to the biological mechanism by which the *MTHFR-C677T* polymorphism reduces enzyme activity, thereby disrupting of folate-mediated DNA synthesis, one-carbon metabolism, and methylation pathways that are essential for maintaining genomic stability and preventing mutagenesis [30], [31]. As identified in present study, the reduction in methyl donor availability could particularly significant under conditions of impaired folate status where the homozygous TT genotype has been associated with elevated plasma total homocysteine levels and an increased risk of neoplastic transformation [32], [33], [34], [35]. Epidemiological studies investigating this association have reported significant variability across different ethnic groups with meta-analysis indicating that the C677T polymorphism is associated with breast cancer risk in Chinese, Iranian, American, European, Mexican, Jordanian, Turkish, Kazakh, Indian, and East Asian populations; whereas, studies involving Caucasians, Canadian, Spanish, German, and Russian cohorts have frequently demonstrated a lack of association [36].

Immunologically, the findings of CD25, CD127, IL-6, and TNF-α were shown a significant elevation in non-treated breast cancer patients when compared to those of treated patients and negative control. Subsequently, values of treated breast cancer patients were significantly higher than those of negative control individuals. However, insignificant variation was seen in values of VEGF among the study population; non-treated and treated breast cancer patients as well as the negative control. Within the tumor microenvironment, several processes have been initiated which characterized by a

consistent expression of pro-inflammatory cytokines and chemokines [37], [38]. Among these, CD25 is highly expressed on tumor-infiltrating regulatory T (Treg) cells in breast cancer, serves as a key surface marker for identification and function, and contributes to an immunosuppressive environment that promotes tumor growth and poor prognosis [39], [40]. Specifically, CD4+CD25+CD127low/- Tregs have been observed to predominate in the peripheral blood mononuclear cells of invasive breast cancer patients compared to healthy individuals and those with benign fibroadenoma [41]. This elevated Treg is population correlates with an increased risk of relapse and shorter relapse-free survival, suggesting that these cells may serve as a critical pathological factor in disease progression [42].

The IL-6 signaling pathway, mediated through the IL-6 receptor complex and downstream JAK/STAT3 activation, represents a promising target for therapeutic intervention in breast cancer [43]. Constitutively, active IL-6/JAK/STAT3 signaling drives cancer cell proliferation and invasiveness while suppressing apoptosis, and STAT3 enhances IL-6 signaling to promote a vicious inflammatory loop [44], [43]. Elevated concentrations of IL-6 are frequently observed in breast cancers and correlate with advanced stages and poor survival outcomes [45]. On other hand, TNF-α is secreted by macrophages and other stromal cells within the tumor microenvironment, where it exerts complex effects on tumor biology through its interaction with TNF-receptor 1 (TNFR1) and TNFR2 [46]. Additionally, Cruceriu et al. reported that TNF-α exhibits a functional duality by promoting signals for activation, differentiation, survival, or cell death depending on the cellular context [47]. Elevated levels of TNF-α, are frequently detected in the serum of breast cancer patients and have been correlated with two distinct receptors on various types with the tumor cells [48].

For inflammatory marker, our findings detected that the values of CRP were higher in non-treated breast cancer patients than those of treated breast cancer patients and negative control. Also, the findings of treated breast cancer patients were higher than those of negative control. Several studies have demonstrated that CRP, a major acute-phase reactant and biomarker of chronic low-grade inflammation, has been implicated in carcinogenesis through mechanisms that include DNA damage, genomic alterations and the promotion of angiogenesis [49], [50], [51]. However, association between CRP levels and breast cancer susceptibility remains controversial as observational studies have reported conflicting results regarding the relationship between systemic inflammation and cancer risk [52], [53]. Lou et al. (2023) mentioned that CRP is a commonly measured as non-specific biomarker of inflammation that has been most frequently studied in the context of breast cancer development [54]. In a number of epidemiological studies, researchers have reported that elevated serum concentrations of CRP are associated with an increased risk of breast cancer development [55], [56], [57].

5. Conclusion

Significant alteration in alleles and genotypes frequency of MTHFR-C677T gene indicates that folate metabolism contributes to the risk of female breast cancer. Increases in immunologic and inflammatory responses in non-treated and treated breast cancer patients might refer to the relevant of such markers in prognosis of disease. Thus, furthermore studies are needed to investigate the role of other folate genes as well as other immune and inflammatory markers in stimulation or inhibition of breast cancer in women.

Acknowledgments

Author thanks the surgeon clinics and laboratories contributed effectively in supporting this work through providing the samples and data.

REFERENCES

[1] M. Roy, J. Biswas, and A. Datta, *Breast cancer: epidemiology, types, diagnosis, and treatment*. Springer Nature Singapore, 2023.

[2] L. F. Mé◎ndez-López, "Revisiting epithelial carcinogenesis," *Int. J. Mol. Sci.*, vol. 23, no. 13, p. 7437, 2022.

[3] D. A. O. A. Pratama, "Molecular Histopathology of Mammary Carcinogenesis as," *Mol. Histopathol. Cytopathol.*, vol. 47, 2023.

[4] X. Xiong *et al.*, "Breast cancer: pathogenesis and treatments," *Signal Transduct. Target. Ther.*, vol. 10, no. 1, p. 49, 2025.

[5] M. Akram, M. Iqbal, M. Daniyal, and A. U. Khan, "Awareness and current knowledge of breast cancer," *Biol. Res.*, vol. 50, no. 1, p. 33, 2017.

[6] A. K. Basu, "DNA damage, mutagenesis and cancer," *Int. J. Mol. Sci.*, vol. 19, no. 4, p. 970, 2018.

[7] A. Karnwal, J. Dutta, A. R. M. S. Al-Tawaha, and N. Nesterova, "Genetic landscape of cancer: mechanisms, key genes, and therapeutic implications," *Clin. Transl. Oncol.*, pp. 1–22, 2025.

[8] S. C. Anton *et al.*, "Current trends in breast cancer genetics, risk factors, and screening strategies," *J. Biol. Methods*, vol. 12, no. 2, p. e99010054, 2025.

[9] M. D. Yussuf *et al.*, "The role of hormones in the onset and progression of endocrine and gynaecological cancers," *Int. J. Adv. Biol. Biomed. Res.*, vol. 13, no. 5, pp. 573–603, 2025.

[10] E. I. Obeagu and G. U. Obeagu, "Breast cancer: A review of risk factors and diagnosis," *Medicine (Baltimore).*, vol. 103, no. 3, p. e36905, 2024.

[11] Y. Liu and Z. Zheng, "Understanding the global cancer statistics 2022: growing cancer burden," *Sci. China Life Sci.*, vol. 67, no. 10, pp. 2274–2276, 2024.

[12] G. Kavgacı and S. Aksoy, "Breast Cancer: General Overview," *Manag. Side Eff. Breast Cancer Treat.*, pp. 3–22, 2025.

[13] J. Kim *et al.*, "Global patterns and trends in breast cancer incidence and mortality across 185 countries," *Nat. Med.*, pp. 1–9, 2025.

[14] M. M. Al-Hashimi, "Trends in breast cancer incidence in Iraq during the period 2000-2019," *Asian Pacific J. Cancer Prev. APJCP*, vol. 22, no. 12, p. 3889, 2021.

[15] A. N. M. Ali and S. N. Muslim, "Breast Cancer in Iraq: A Comprehensive Review of Disease Burden, Risk Factors, and Healthcare Response," *South Asian Res. J. Biol. Appl. Biosci.*, vol. 7, no. 6, pp. 349–360, 2025.

[16] A. F. Araszkiewicz *et al.*, "MTHFR Gene Polymorphisms: A Single Gene with Wide-Ranging Clinical Implications—A Review," *Genes (Basel).*, vol. 16, no. 4, p. 441, 2025.

[17] N. A. Al-Allawi, A. S. Avo, and J. M. Jubrael, "Methylenetetrahydrofolate reductase C677T polymorphism in Iraqi patients with ischemic stroke," *Neurol. India*, vol. 57, no. 5, pp. 631–635, 2009.

[18] E. D. Salman, A. G. Mohamed, and S. J. Al-Awadi, "Molecular Study of the Methylenetetrahydrofolate Reductase Polymorphisms in Iraqi Colorectal Patients," *Curr. Res. Microbiol. Biotechnol.*, vol. 3, no. 3, pp. 637–642, 2015.

[19] A. A. Eissa, C. S. Rasool, and M. H. Mustafa, "Methylenetetrahydrofolate reductase gene (C677T and A1298C) polymorphisms and hyperhomocysteinemia as risk factors for Myocardial Infarction," *Indian J. Biochem. Biophys.*, vol. 56, no. 4, pp. 321–324, 2019.

[20] H. K. I. Al-Sammarraie, M. I. Nader, A. H. B. A. A. Alani, and A. Abdullat, "Effect of C677T Mutation of Methylene Tetrahydrofolate Reductase Gene as a Genetic Risk Factor in Iraqi Infertile Men," *Iraqi J. Biotechnol.*, vol. 1, no. 20, 2021.

[21] R. Z. Al-Yasiry, M. A. Jawad, and M. S. Abbood, "Association of methylenetetrahydrofolate reductase gene polymorphism C677T with oocyte number and embryo quality in Iraqi infertile women undergoing intracytoplasmic sperm injection," *Al-Rafidain J. Med. Sci.*, vol. 6, no. 1, pp. 39–45, 2024.

[22] S. S. Younis, N. K. Issa, and D. M. Sulaiman, "MTHFR gene polymorphisms in Iraqi Kurdish rheumatoid arthritis patients: Relation to methotrexate response and toxicity," *Iraqi J. Sci.*, pp. 5186–5196, 2022.

[23] Q. M. Mutlak and A. A. Kasim, "Impact of MTHFR gene polymorphism on the outcome of methotrexate treatment in a sample of Iraqi rheumatoid arthritis patients," *Sci. Rep.*, vol. 14, no. 1, p. 15119, 2024.

[24] R. M. Nashee, L. M. Al-Janabi, and M. A. Altahan, "Association of MTHFR C677T, MTHFR A1298C polymorphism in relation to patients with autism in Thi-Qar Governorate," *Rom. J. Neurol.*, vol. 23, no. 2, p. 166, 2024.

[25] B. A. Hraija and G. M. A. Abdair, "Serological and Molecular Phylogeny of Toxoplasma gondii in Asymptomatic

Pregnant Women, and Association to MTHFR-C677T gene Polymorphism in Wasit Province, Iraq," *Cent. Asian J. Med. Nat. Sci.*, vol. 6, no. 4, pp. 2131–2144, 2025.

[26] Z. A. Shareef and D. M. Sulaiman, "MTHFR C677T polymorphism and T2DM risk in Iraqi Kurds: a cross-sectional study," *Ann. Med. Surg.*, vol. 87, no. 12, pp. 8121–8129, 2025.

[27] E. D. Salman, E. A. Al Bayyar, and R. K. Ibrahim, "Association between Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphisms and breast cancer in sample of Iraqi women," *Iraqi J. Sci.*, pp. 447–453, 2017.

[28] H. K. I. Al-Sammarraie, "The MTHFR A1298C polymorphism and Breast Cancer Susceptibility: MTHFR A1298C and Breast Cancer Risk," *Iraqi J. Cancer Med. Genet.*, vol. 18, no. 1, pp. 26–29, 2025.

[29] W. H. Hoidy, M. O. Orabiy, S. M. Essa, and L. S. Jasim, "Novel Genetic Susceptibility Markers for Breast Cancer in Iraqi Women: First Evidence of CYP3A4* 1B Protective Effects and GSTP1/MTHFR Risk Associations," *Clin. Breast Cancer*, 2025.

[30] A. Agodi, A. Quattrocchi, A. Maugeri, and M. Barchitta, "The link between MTHFR C677T polymorphism, folate metabolism and global DNA methylation: A literature review," in *Methylenetetrahydrofolate Reductase (MTHFR) in Health and Disease*, R. Evans, Ed., 2015, pp. 71–82.

[31] Y. Menezo, K. Elder, A. Clement, and P. Clement, "Folic acid, folinic acid, 5 methyl tetrahydrofolate supplementation for mutations that affect epigenesis through the folate and one-carbon cycles," *Biomolecules*, vol. 12, no. 2, p. 197, 2022.

[32] M. Hiraoka and Y. Kagawa, "Genetic polymorphisms and folate status," *Congenit. Anom. (Kyoto).*, vol. 57, no. 5, pp. 142–149, 2017.

[33] A. M. Mahmoud and M. M. Ali, "Methyl donor micronutrients that modify DNA methylation and cancer outcome," *Nutrients*, vol. 11, no. 3, p. 608, 2019.

[34] D. Pan *et al.*, "MTHFR C677T genetic polymorphism in combination with serum vitamin B2, B12 and aberrant DNA methylation of P16 and P53 genes in esophageal squamous cell carcinoma and esophageal precancerous lesions: A case–control study," *Cancer Cell Int.*, vol. 19, no. 1, p. 288, 2019.

[35] B. K. Bartak *et al.*, "Examination of Genetic and Epigenetic Characteristics of Patients with Hyperhomocysteinemia Following High-Dose Folic Acid Consumption," *Nutrients*, vol. 17, no. 13, p. 2133, 2025.

[36] X. Chen, H. Ahamada, T. Zhang, Z. Bai, and C. Wang, "Association of intake folate and related gene polymorphisms with breast cancer," *J. Nutr. Sci. Vitaminol. (Tokyo).*, vol. 65, no. 6, pp. 459–469, 2019.

[37] H. D. Saleem, M. A. Razooqi, and H. A. J. Gharban, "Cumulative effect of subclinical mastitis on immunological and biochemical parameters in cow milk," *Arch. Razi Inst.*, vol. 76, no. 6, p. 1629, 2021.

[38] S. Tahmasebi, M. Alimohammadi, S. Khorasani, and N. Rezaei, *Pro-tumorigenic and anti-tumorigenic roles of pro-inflammatory cytokines in cancer*. Springer Nature Switzerland, 2025.

[39] B. K. Banin-Hirata *et al.*, "The prognostic value of regulatory T cells infiltration in HER2-enriched breast cancer microenvironment," *Int. Rev. Immunol.*, vol. 37, no. 3, pp. 144–150, 2018.

[40] M. A. Nelson, W. Ngamcherdtrakul, S. W. Luoh, and W. Yantasee, "Prognostic and therapeutic role of tumor-infiltrating lymphocyte subtypes in breast cancer," *Cancer Metastasis Rev.*, vol. 40, no. 2, pp. 519–536, 2021.

[41] A. Glajcar, J. Szpor, D. Hodorowicz-Zaniewska, K. E. Tyrak, and K. Okoń, "The composition of T cell infiltrates varies in primary invasive breast cancer of different molecular subtypes as well as according to tumor size and nodal status," *Virchows Arch.*, vol. 475, no. 1, pp. 13–23, 2019.

[42] L. Wang *et al.*, "Connecting blood and intratumoral Treg cell activity in predicting future relapse in breast cancer," *Nat. Immunol.*, vol. 20, no. 9, pp. 1220–1230, 2019.

[43] S. G. Manore, D. L. Doheny, G. L. Wong, and H. W. Lo, "IL-6/JAK/STAT3 signaling in breast cancer metastasis: biology and treatment," *Front. Oncol.*, vol. 12, p. 866014, 2022.

[44] D. E. Johnson, R. A. O'Keefe, and J. R. Grandis, "Targeting the IL-6/JAK/STAT3 signalling axis in cancer," *Nat. Rev. Clin. Oncol.*, vol. 15, no. 4, pp. 234–248, 2018.

[45] N. Ahmad *et al.*, "IL-6 and IL-10 are associated with good prognosis in early stage invasive breast cancer patients," *Cancer Immunol. Immunother.*, vol. 67, no. 4, pp. 537–549, 2018.

[46] D. Laha, R. Grant, P. Mishra, and N. Nilubol, "The role of tumor necrosis factor in manipulating the immunological response of tumor microenvironment," *Front. Immunol.*, vol. 12, p. 656908, 2021.

[47] D. Cruceriu, O. Baldasici, O. Balacescu, and I. Berindan-Neagoe, "The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: molecular insights and therapeutic approaches," *Cell. Oncol.*, vol. 43, no. 1, pp. 1–18, 2020.

[48] D. D. Ghafoor, "Correlation between oxidative stress markers and cytokines in different stages of breast cancer,"

Cytokine, vol. 161, p. 156082, 2023.
[49] M. Cifuentes *et al.*, "Low-grade chronic inflammation: a shared mechanism for chronic diseases," *Physiology*, 2024.
[50] G. K. Bhatti *et al.*, "Inflammation Beyond the Prostate: The Role of Systemic Inflammatory Disorders in Prostate Carcinogenesis," *Cureus*, vol. 17, no. 12, 2025.
[51] K. A. Triantaphyllopoulos, N. D. Ragia, M. C. E. Panagiotopoulou, and T. G. Sourlingas, "Integrating inflammatory and epigenetic signatures in IBD-associated colorectal carcinogenesis: models, mechanisms, and clinical implications," *Int. J. Mol. Sci.*, vol. 26, no. 19, p. 9498, 2025.
[52] M. K. Mikkelsen, N. A. F. Lindblom, A. Dyhl-Polk, C. B. Juhl, J. S. Johansen, and D. Nielsen, "Systematic review and meta-analysis of C-reactive protein as a biomarker in breast cancer," *Crit. Rev. Clin. Lab. Sci.*, vol. 59, no. 7, pp. 480–500, 2022.
[53] Y. Cui *et al.*, "CRP, IL-1α, IL-1β, and IL-6 levels and the risk of breast cancer: a two-sample Mendelian randomization study," *Sci. Rep.*, vol. 14, no. 1, p. 1982, 2024.
[54] M. W. Lou *et al.*, "Linking physical activity to breast cancer via inflammation, part 2: the effect of inflammation on breast cancer risk," *Cancer Epidemiol. Biomarkers \& Prev.*, vol. 32, no. 5, pp. 597–605, 2023.
[55] M. Cairat *et al.*, "Circulating inflammatory biomarkers, adipokines and breast cancer risk—a case-control study nested within the EPIC cohort," *BMC Med.*, vol. 20, no. 1, p. 118, 2022.
[56] M. M. Bakheet, H. M. Ali, and T. J. Talab, "Evaluation of some proinflammatory cytokines and biochemical parameters in pre and postmenopausal breast cancer women," *Cytokine*, vol. 179, p. 156632, 2024.
[57] J. B. Holm *et al.*, "Circulating C-reactive protein levels as a prognostic biomarker in breast cancer across body mass index groups," *Sci. Rep.*, vol. 14, no. 1, p. 14486, 2024.