

Research Article

Irradiation and Immunological and Oxidative Stress Parameters (TNF- α and MDA) in Breast Cancer Patients in Najaf Governorate

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Abstract

Background: Breast cancer treatment can alter systemic inflammatory and oxidative-stress parameters. This study evaluated serum tumor necrosis factor-alpha (TNF- α) and malondialdehyde (MDA) across treatment stages, with particular attention to the reported radiation dose of 2.5 Gy per fraction. Radiation therapy was delivered strictly using a linear accelerator with high-energy radiation. **Methods:** Thirty-six female participants aged 35–60 years were divided into four groups (n=9 each): healthy controls, patients assessed after chemotherapy and before radiation-dose administration, patients after the first radiation-dose session, and patients after the fifteenth session. The reported dose was 2.5 Gy per fraction delivered via linear accelerator X-ray beams. If 15 fractions were each delivered at this dose, the nominal cumulative dose by fraction 15 would be 37.5 Gy. Serum TNF- α and MDA were measured by sandwich ELISA; group comparisons used ANOVA and post-hoc tests. **Results:** Serum TNF- α levels were significantly elevated in all patient groups compared to healthy controls (G1: 97.09 ± 18.03 pg/mL vs. G2: 418.89 ± 98.70 pg/mL, $p=0.0001$). Chemotherapy (G2) triggered systemic pro-inflammatory cytokine surges, whereas the first radiation-dose session (G3: 298.31 ± 59.87 pg/mL) and the fifteenth radiation-dose session (G4: 299.15 ± 69.89 pg/mL) resulted in significantly lower TNF- α levels compared to G2 ($p=0.004$ and $p=0.003$, respectively). Conversely, serum MDA reached a peak post-chemotherapy (G2: 536.54 ± 60.67 pg/mL) and demonstrated a significant progressive reduction during radiation-dose administration (G3: 412.66 ± 62.21 pg/mL; G4: 342.51 ± 37.21 pg/mL, $p=0.001$ compared to G2; $p=0.05$ between G3 and G4). **Conclusions:** Chemotherapy significantly elevates serum TNF- α and malondialdehyde (MDA) levels ($p \leq 0.001$) in breast cancer patients compared to healthy controls, driven by systemic inflammation and oxidative stress. Conversely, localized radiotherapy exhibits lower TNF- α levels and a significant reduction in MDA ($p = 0.01$) compared to chemotherapy alone. Continuous daily radiation sessions further decrease MDA levels ($p = 0.05$), indicating a positive redox balance, subsiding treatment toxicity, and progressive tissue recovery.

1. Introduction

Breast cancer (BC) is the most common type of cancer around the world and the second leading cause of women's death around the world after lung cancer [1]. It occurs both in men and women, but with more incidence in women than men with a ratio of 1:100 [2]. In 2018,

globally, the incidence of BC was about 2.1 million (24.2%) new cases with 627,000 (15%) deaths [3]. More than half of the BC incidence and 60% of deaths occur in low- and middle-income countries (LMICs) [4]. In 2020, about 2.3 million (11.7%) new cases were reported, with 685,000 deaths [5]. According to WHO in 2022, there were 2.3 million women diagnosed with breast cancer and 670,000 deaths globally [6]. It is the most common type of cancer in 157 countries out of 185 around the world [7]. According to the Global Cancer Observatory estimates, BC incidence will increase to be about 46% by 2046 [8].

Among the Iraqi population, BC ranked as the top cancer in women and the second leading cause of mortality after cardiovascular diseases [9]. Between 2000 and 2009, BC rates were found to be constant, but new statistics were reported by the Iraqi Cancer Registry, which detected that since 2009 there has been an increase in BC rates, especially in women older than 50s [10]. According to the Annual Report of the Iraqi Cancer Registry in 2020, 2021, and 2022, BC was reported as the top cancer among females in Iraq (34.35%, 30.99/100,000 FP), (35.02%, 34.87/100,000 FP), and (35.9%, 39.2/100,000 FP), respectively [11]. About AlNajaf governorate a dramatic increase in BC incidence over these years (33.25%, 33.52/100,000 FP) in 2020, (46.88%, 36.96/100,000 FP) in 2021, while in 2022 (33%, 45.9/100,000 FP) [12]. The incidence of breast cancer in Najaf has shown a dramatic increase, moving from 33.52 per 100,000 in 2021 to 45.9 per 100,000 in 2022 [13]. The administration of the Oncology Hospital in Najaf revealed that 2,312 cases were recorded during 2024, an increase of more than 100 cases compared to the previous year [14].

Radioactive pollution is the unwanted presence of radioactive substances (radionuclides) on surfaces or inside a person or environment, which pose a health risk because they emit harmful ionizing radiation (alpha, beta, gamma) [15]. Ionizing radiation has sufficient energy to remove electrons from atoms, causing ionization and potentially damaging cells, DNA, and tissues; the biological effect depends on factors including absorbed dose and exposure conditions [16]. Ionizing radiation damages living cells by knocking electrons out of atoms, creating reactive molecules that destroy DNA and intervene with mobile replica, main to mobile death, mutations, or most cancers [17].

Radiation therapy for breast cancer effectively kills cancer cells to save you recurrence but causes short-term facet results like fatigue and skin inflammation and ability long-time period effects like breast modifications [18]. Tumor necrosis factor-alpha (TNF- α) is a major pro-inflammatory cytokine produced by phagocytic and immune cells to regulate the immune response, inflammation, and programmed cell death [19]. Ionizing radiation at therapeutic doses may influence the expression and blood levels of tumor necrosis factor-alpha (TNF), a pro-inflammatory cytokine [20]. Malondialdehyde (MDA) in blood is a number one biomarker of lipid peroxidation and oxidative pressure, formed while free radicals attack polyunsaturated fatty acids in cellular membranes [21]. Radiation exposure may influence malondialdehyde (MDA) levels in the blood of breast cancer patients through oxidative stress and lipid peroxidation [22]. Uranium and depleted uranium contamination exerts severe biochemical, endocrine, and metabolic toxicities, influencing Vitamin D biosynthetic pathways, Cytochrome P450 enzymes, and nuclear receptor signaling [23].

Previous studies have examined biological responses associated with breast-cancer treatment [24]. Mahmoud et al. investigated DNA-damage responses in breast-cancer cell lines after radiation exposure [24]. Rouia et al. assessed oxidative stress and biochemical and hematological parameters in women with breast cancer during treatment [25], while Arjmandi et al. evaluated serum oxidant–antioxidant status before and after treatment [26]. Meng et al. reported changes in adipose-tissue autotaxin after treatment [27], and Geinitz et al. examined fatigue, circulating cytokines, and blood-cell counts during treatment [28]. Jain et al. studied a yoga intervention in patients receiving chemotherapy and/or other cancer treatment [29]. Khoshbin et al. investigated treatment-associated changes in red-cell osmotic fragility and plasma malondialdehyde [30], whereas Saifullah et al. measured malondialdehyde in sera, red blood cells, and tissue homogenates from Iraqi women with breast tumors [22]. Together, these studies provide context for evaluating TNF- α and MDA across treatment stages [22, 24–30]. The present study compares these parameters in healthy controls and breast-cancer patients after chemotherapy, before the first radiation dose, after the first dose, and after the fifteenth dose.

2. Materials and Methods

2.1. Patient Groups and Healthy Controls

A total of 36 blood samples were collected between July 2024 and January 2025 across specialized oncology centers in Najaf Governorate (National Hospital for Oncology & Hematology, Al-Amal Hospital, and Al-Sadr Teaching Hospital). Female participants aged 35–60 years were stratified into four cohorts (n=9 per group):

- Group 1 (G1 - Control): Healthy female control subjects without history of malignancy or systemic inflammatory disease.
- Group 2 (G2 - After Chemotherapy): Breast cancer patients who completed chemotherapy protocols and were evaluated immediately before the first radiation dose.
- Group 3 (G3 - First Radiation Dose): Breast cancer patients assessed at the first fractionated radiation-dose session.
- Group 4 (G4 - Fifteenth Radiation Dose): Breast cancer patients assessed at the fifteenth fractionated radiation-dose session.

2.2. Radiation-Dose Protocol & Irradiation Technique

Radiation therapy was administered using a Medical Linear Accelerator (LINAC) generating high-energy 6 MV X-ray photon beams. The target treatment area received a daily fractionated dose of 2.5 Gy per fraction over approximately 15 minutes per session. For patients completing 15 fractions at 2.5 Gy per fraction, the nominal cumulative delivered dose was 37.5 Gy (2.5 Gy $\times$ 15 fractions). Target volume definition and radiation delivery were aligned with standard clinical radiation therapy protocols for breast cancer treatment.

2.3. Blood Sampling and Laboratory Instrumentation

Venous blood samples (3.0 mL) were drawn via sterile venipuncture, incubated in sodium citrate tubes at 37°C for 30–40 minutes, and centrifuged at 3,000 rpm for 10 minutes to separate serum. Quantitative determination of serum human TNF- α and human MDA was conducted using Sandwich ELISA techniques on a Bioelisa Reader ELX800 at 450 nm wavelength. The study was approved by the relevant ethics committee under approval No. 3029, dated July 16, 2025.

2.4. Statistical Analysis

All values are expressed as Mean $\pm$ Standard Deviation (SD). Statistical comparisons across the four study groups were conducted using SPSS software. One-way Analysis of Variance (ANOVA) followed by post-hoc Tukey tests and Student's t-tests were applied. Statistical significance thresholds were established at $p < 0.05$ and $p < 0.001$.

3. Results and Discussion

Quantitative Serum TNF- α Analysis

Biochemical quantitative assay of serum TNF- α demonstrated highly significant variation across the study groups ($p = 0.001$) [20]. Healthy control subjects (G1) exhibited a baseline mean TNF- α concentration of 97.09 ± 18.03 pg/mL [28]. Following chemotherapy (G2), serum TNF- α surged dramatically to 418.89 ± 98.70 pg/mL, representing a statistically significant elevation compared to healthy controls ($p = 0.0001$) [29]. The first radiation-dose session (G3) was associated with a significant drop in serum TNF- α to 298.31 ± 59.87 pg/mL ($p = 0.004$ compared to G2) [25]. At the fifteenth fractionated radiation-dose session (G4), this lower level was maintained at 299.15 ± 69.89 pg/mL ($p = 0.003$ compared to G2) [28]. Comparison between G3 and G4 revealed no statistically significant difference ($p = 0.98$), indicating stable systemic cytokine levels between the two sampled radiation-dose stages [27] as shown in Table 1.

Table 1: Serum levels of TNF- α (pg/mL) and MDA (pg/mL) expressed as Mean $\pm$ SD across study cohorts.

Parameter	G1 (Control)	G2 (Chemo Only)	G3 (Rad 1st Session)	G4 (Rad 15th Session)	Overall P-value
TNF- α (pg/mL)	97.09 ± 18.03	418.89 ± 98.70	298.31 ± 59.87	299.15 ± 69.89	0.001
MDA (pg/mL)	418.17 ± 86.00	536.54 ± 60.67	412.66 ± 62.21	342.51 ± 37.21	0.001

Quantitative Serum MDA (Malondialdehyde) Analysis

Serum MDA analysis provided crucial insights into treatment-induced lipid peroxidation [30]. Healthy control baseline MDA stood at 418.17 ± 86.00 pg/mL [22]. Administration of systemic chemotherapy (G2) caused a sharp, significant rise in serum MDA to 536.54 ± 60.67 pg/mL ($p = 0.001$ vs. G1) [26]. Across the radiation-dose stages, serum MDA levels exhibited a progressive decrease [30]. After the first radiation-dose session (G3), mean serum MDA dropped to 412.66 ± 62.21 pg/mL ($p = 0.001$ vs. G2; $p = 0.87$ vs. G1) [25]. By the fifteenth fractionated radiation-dose session (G4), serum MDA fell further to 342.51 ± 37.21 pg/mL ($p = 0.001$ vs. G2; $p = 0.05$ vs. G3; $p = 0.04$ vs. G1) [26] as shown in Table 2.

Table 2: Post-hoc Tukey statistical comparisons (p-values) for TNF- α and MDA among study groups.

Group Comparison	TNF- α P-value	MDA P-value	Statistical Significance
G1 (Control) vs. G2 (Chemo)	0.0001	0.001	Highly Significant
G1 (Control) vs. G3 (Rad 1st)	0.0001	0.87	TNF- α Sig / MDA Non-Sig
G1 (Control) vs. G4 (Rad 15th)	0.0001	0.04	Significant
G2 (Chemo) vs. G3 (Rad 1st)	0.004	0.001	Highly Significant
G2 (Chemo) vs. G4 (Rad 15th)	0.003	0.001	Highly Significant
G3 (Rad 1st) vs. G4 (Rad 15th)	0.98	0.05	MDA Sig / TNF- α Non-Sig

Comprehensive Radiobiological Discussion

Radiation-dose context and interpretation. The reported dose of 2.5 Gy per fraction is important for interpreting the biomarker findings. For 15 completed fractions of 2.5 Gy, the arithmetic cumulative dose is 37.5 Gy. High TNF- α levels during chemotherapy in G2 are primarily driven by systemic inflammation [29]. Conversely, low TNF- α levels during radiation therapy G3 and G4 may reflect a more localized treatment effect [28]. In G2, MDA levels were very high due to chemotherapy [30]. In G3 and G4, MDA levels decreased, suggesting an improvement in the body's redox balance and tissue recovery [22].

Comparative interpretation of TNF- α and MDA

TNF- α increased from 97.09 ± 18.03 pg/mL in healthy controls to 418.89 ± 98.70 pg/mL after chemotherapy. At the first and fifteenth radiation-dose sessions, the means were 298.31 ± 59.87 and 299.15 ± 69.89 pg/mL, respectively.

Magnitude and pattern of oxidative-stress response

MDA followed a different pattern, increasing from 418.17 ± 86.00 pg/mL in controls to 536.54 ± 60.67 pg/mL after chemotherapy, and decreasing to 412.66 ± 62.21 pg/mL after the first session and 342.51 ± 37.21 pg/mL at the fifteenth session.

Relationship between inflammatory and oxidative parameters

Simultaneous elevation of TNF- α and MDA in G2 is consistent with inflammatory response and increased lipid peroxidation after chemotherapy. Subsequent decreases suggest differences across treatment stages.

Comparison with previous research

The findings are considered alongside previous work on oxidative stress and cytokine responses in breast-cancer treatment [22, 24–30].

Strengths and limitations affecting interpretation

A strength of this study is the comparison of a healthy-control group with three clinically distinct treatment-stage groups, using the same reported ELISA approach. Limitations include sample size and cross-sectional group design.

Overall implication of the findings

The observed data indicate that the post-chemotherapy group had the highest mean TNF- α and MDA concentrations, with lower mean values observed following the radiation therapy stages.

4. Conclusions

Chemotherapy induces strong systemic inflammation and oxidative stress, causing significantly higher serum TNF- α ($p = 0.0001$) and MDA ($p = 0.001$) levels due to drug metabolism and membrane lipid peroxidation. Radiotherapy exerts a localized effect, maintaining lower TNF- α levels without significant variation between initial and daily sessions ($p = 0.98$). Serum MDA levels decrease significantly in post-chemotherapy radiotherapy patients ($p = 0.01$), indicating a shift toward oxidative balance. Daily radiation sessions further reduce MDA ($p = 0.05$) from start to finish, reflecting treatment success and reduced systemic damage. The drop in MDA highlights the body's adaptation and recovery from acute chemotherapy side effects over time. Overall, MDA serves as an effective marker for monitoring tissue recovery and tumor cell destruction during therapy transition.

Article Information

Ethical Approval: The study was approved by the relevant ethics committee under approval No. 3029, dated July 16, 2025.

Informed Consent: Written informed consent was obtained from all participants before their inclusion in the study.

Consent for Publication: Written informed consent was obtained from the participant(s) for publication of the relevant information.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Clinical Trial Registration: Not applicable.

Conflict of Interest: The authors declare that they have no conflict of interest.

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