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Prognostic value of interferon-gamma, interleukin-6, and tumor necrosis factor-alpha in the radiation response of patients diagnosed with locally advanced non-small-cell lung cancer and glioblastoma multiforme

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Background/aim: This study aimed to investigate the effect of chemoradiotherapy (CRT) on interferon-gamma (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which are critical markers of the clinical radiation response of patients with locally advanced non-small-cell lung cancer (NSCLC) and glioblastoma multiforme (GBM).

Materials and methods: Thirty patients who were treated with CRT and 20 healthy controls were prospectively evaluated. Circulating levels of cytokines were measured by enzyme-linked immunosorbent assay procedure. Post-CRT and pre-CRT levels were compared.

Results: Post-CRT, TNF-α and IFN-γ levels were significantly lower than pre-CRT levels in the NSCLC and GBM groups, respectively. The statistical analysis did not show any significant difference between the post- and pre-CRT IL-6 levels. However, the pre-CRT IL-6 levels in the GBM group and post-CRT IL-6 levels in the NSCLC group were significantly higher than those of the control group.

Conclusion: CRT affected TNF-α levels in NSCLC and IFN-γ levels in GBM, with the levels of both decreasing significantly. The IL-6 levels of the post-CRT NSCLC group were higher than those of the post-CRT GBM group. Irradiation-induced IL-6 may be responsible for tumor regrowth. Therefore, treatment with IL-6 inhibitors could be a potential therapeutic strategy for sensitizing NSCLC to irradiation in the clinic.

Key words: Interferon-gamma, interleukin-6, tumor necrosis factor-alpha, cancer, radiotherapy

1. Introduction

Lung cancer is a leading cause of mortality. The most common cancer subtype, non-small-cell lung cancer (NSCLC), accounts for 85%–90% all cases of NSCLC, which is mainly caused by environmental and genetic factors (1). Chronic inflammatory conditions have been reported to play an important role in the progression of lung cancer (2–4). Despite recent advances in treatment, the prognosis of patients with NSCLC remains poor, with 5-year overall survival of approximately 15% (5). NSCLC typically presents at an advanced stage, where multimodal therapy, including systemic therapy, radiotherapy (RT), and surgery, is necessary (6).

Glioblastoma multiforme (GBM) is one of the most malignant neoplasms in humans (7). Despite multimodal treatment consisting of debulking surgery, RT, and chemotherapy (CT), the prognosis remains extremely poor, with a median survival of 14.6 months (8). There is an urgent, unmet need for novel, effective therapeutic strategies for this devastating diseases so several immunotherapies are under development (9).

RT is used to treat patients with NSCLC and GBM. Cellular damage caused by ionizing radiation induces specific proteins involved in DNA repair, cell death, inflammation, and other pathophysiological responses (10). The majority of biomarker studies in radiation oncology have focused on predicting tumor responses and survival (10,11).

The plasma levels of a range of cytokines have been investigated in both murine (12) and cell models (13). Proinflammatory cytokines that are expressed as acute phase reactants include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (14–17).

IL-6 is a multifunctional cytokine that plays an important role in a wide range of biological activities in different types of cells, including tumor cells. IL-6 is involved in the host immune defense mechanism, as well as in the modulation of growth and differentiation in various malignancies (18).

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TNF-α is a proinflammatory cytokine inducing a broad range of cellular responses, ranging from inflammatory cytokine production to cell survival, cell proliferation, cell differentiation, and cell death. TNF-α can trigger different forms of programmed cell death that are morphologically distinguished as apoptosis and necroptosis (19).

Interferon gamma (IFN-γ), a cytokine secreted by activated T cells and natural killer cells, exhibits dramatic antiviral, antitumor and immunomodulation effects by enhancing the activity of immune cells, upregulating antigen presentation, and increasing the sensitivity of tumor cells to apoptotic signals (20).

The inflammatory response is a classical feature of radiation exposure (21). RT may result in the onset of local inflammation and an acute phase reaction, which causes radiation-related toxicity. Ionizing radiation is known to damage cells within the irradiated volume by generating oxygen species and cytokine-mediated multicellular interactions (22).

To shed light on the association between ionizing irradiation and the induction of inflammatory response, circulating levels of IFN-γ, IL-6, and TNF-α were measured in a group of patients with NSCLC and GBM who were treated with chemoradiotherapy (CRT).

2. Materials and methods

2.1. Patients

This study was approved by the Meram Medical Faculty Ethics Committee of Necmettin Erbakan University on 01.03.2013, decision no: 2013/365. All the participants provided written informed consent. Blood samples were taken from 20 NSCLC patients, 10 GBM patients, and 20 healthy volunteers. The patients were selected from those admitted to the Department of Radiation Oncology, Meram Medical Faculty of Necmettin Erbakan University. In all cases, the NSCLC was inoperable, and all the GBM patients had postoperative residual tumors. Neither group of patients had previously undergone CT or RT.

Heparinized blood samples were taken before and after 6 weeks of RT and CT (temozolomide in GBM and paclitaxel, etoposide, and carboplatin in NSCLC) from the patients and once from the control group. Chemotherapeutics were used for radiosensitization, not for curative treatment. Healthy volunteers did not receive CRT during the study.

The inclusion criteria were: 1) histologically confirmed GBM and stage III cases of NSCLC; 2) age between 40 and 70 years with a Karnofsky Performance Status score of ≥70; and 3) being from a similar ethnicity. Exclusion criteria included cases of: 1) previous CT or RT experience; 2) a secondary malignancy; 3) history of any drug addiction, chronic smoking habit, or alcohol addiction; 4) any metabolic or endocrine disorders; 5) any infection; and 6) any chronic inflammatory condition.

Plasma was separated by centrifugation at 4000 × *g* for 5 min. The plasma was stored at –80 °C prior to cytokine analysis. Cytokine measurements in the control and patient groups were studied in the Department of Biochemistry, Meram Medical Faculty of Necmettin Erbakan University. IFN-γ, IL-6, and TNF-α were analyzed with enzyme-linked immunosorbent assay kits (Boster Biological Technology, Fremont, CA, USA) with an ELx800 universal microplate reader and ELx50 microplate strip washer (Bio-Tek, Winooski, VT, USA). The cytokine levels before and after RT were compared.

2.2. Statistical analysis

Statistical analysis was performed using SPSS for Windows 21.0 (IBM Corp., Armonk, NY, USA). The statistical significance was calculated using general linear models. The relationship between survival and cytokine levels was evaluated by LR Cox regression analysis. $P < 0.05$ was considered statistically significant. All the results were expressed as mean value and standard deviation (mean ± SD).

3. Results

IL-6 levels were reduced in GBM cases and increased in NSCLC cases with CRT (Figure). There was a significant difference between the pretreatment IL-6 levels of the GBM group and those of the control group (23.93 ± 14.38 and 10.77 ± 10.48, respectively; $P < 0.05$). The post-CRT IL-6 levels were significantly higher in the NSCLC group compared with those of the control group (22.55 ± 14.82 and 10.77 ± 10.48, respectively; $P < 0.05$).

With CRT, TNF-α levels decreased in the patients with NSCLC but increased in those with GBM (Figure). Posttreatment TNF-α levels were significantly lower than pretreatment levels in NSCLC (6.44 ± 1.74 and 12.45 ± 15.6, respectively; $P < 0.05$) (Table).

The levels of IFN-γ decreased in both the GBM and NSCLC groups with CRT, but the decline was statistically significant only in the GBM group. There was a significant difference between the pre-CRT and post-CRT IFN-γ levels of the patients with GBM (8.7 ± 4.21 and 5.13 ± 3.79, respectively; $P < 0.05$) (Table).

The LR Cox regression analysis revealed that the pre-CRT and post-CRT IL-6, TNF-α, and IFN-γ plasma levels were not significantly associated with the survival of patients with NSCLC and GBM ($P > 0.05$). The values of LR Cox regression analysis in the NSCLC and GBM groups are presented in the Table.

4. Discussion

Recent studies have demonstrated that RT induces immune responses (23–25) and that the altered tumor

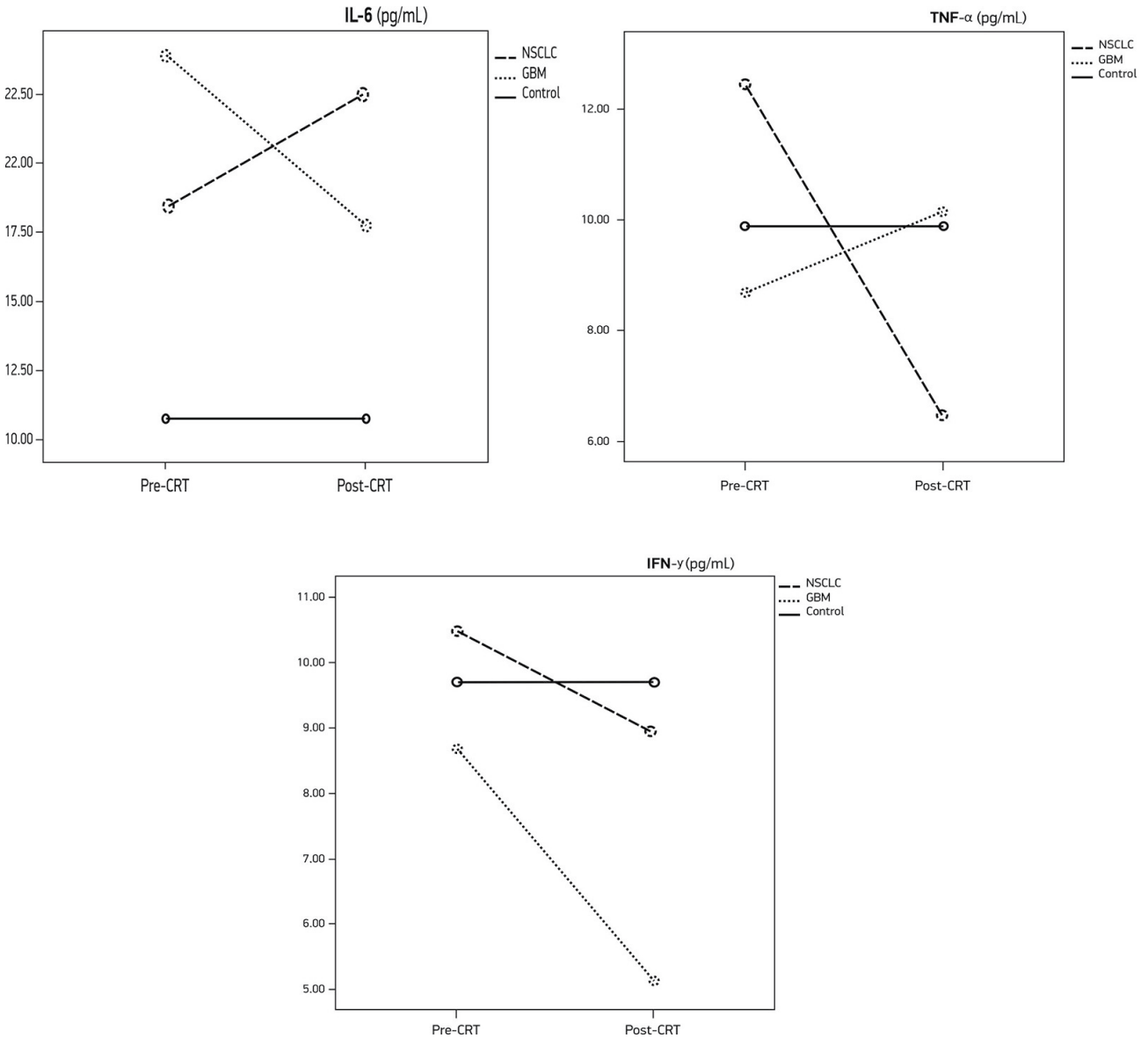

Figure. Cytokine levels from the start of the chemoradiotherapy (CRT) to the completion of CRT.
The tumor necrosis factor-α (TNF-α) levels of the non-small-cell lung cancer (NSCLC) patients and interferon-γ (IFN-γ) levels of the glioblastoma multiforme (GBM) patients decreased significantly during CRT ($P < 0.05$).

microenvironment contributes, in part, to the antitumor response after RT. Although the critical roles of cytokines in carcinogenesis have been highlighted, their roles in the radiation response require further investigation. In the present study, we analyzed radiation-induced changes in IFN-γ, IL-6, and TNF-α levels to investigate the usefulness of cytokines as a marker during RT**,** and we examined the relationship between survival and cytokine levels in NSCLC and GBM patients.

A reduction in IL-6 levels can sensitize tumor cells to irradiation by increasing cell death and DNA damage, and it can mitigate tumor regrowth after irradiation (26). Lopes and Callera (27) demonstrated that IL-6 was sensitive to irradiation in patients with prostate cancer, in contrast to IL-2, IL-4, IL-5, IL-6, TNF-α, macrophage inflammatory protein-1-alpha, and leukemia inhibitory factor. The increased levels of IL-6 following RT, without concurrent elevation of those of the other cytokines involved in the acute phase reaction, are not typical of the classical inflammatory response to radiation exposure. In the present study, the plasma IL-6 level of both cancer groups was higher than that of the control group. During

Table. The pre-chemoradiotherapy (CRT) and post-CRT levels of interferon-γ (IFN-γ) interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in the groups.

	Mean ± SD	P-value	HR	95.0% CI, lower–upper
Pre-CRT IL-6 NSCLC GBM Control	 18.39 ± 8.61 23.93 ± 14.38 [a] 10.77 ± 10.48	 0.404 0.152	 1.032 1.071	 0.958–1.111 0.975–1.177
Pre-CRT TNF-α NSCLC GBM Control	 12.45 ± 15.6 8.69 ± 5.26 9.89 ± 10.12	 0.702 0.430	 1.007 0.946	 0.974–1.041 0.824–1.086
Pre-CRT IFN-γ NSCLC GBM Control	 10.48 ± 12.7 8.7 ± 4.21 9.7 ± 7.27	 0.282 0.406	 0.946 1.156	 0.854–1.047 0.821–1.629
Post-CRT IL-6 NSCLC GBM	 22.55 ± 14.82 [a] 17.75 ± 12.47	 0.603 0.188	 1.012 1.044	 0.968–1.057 0.979–1.113
Post-CRT TNF-α NSCLC GBM	 6.44 ± 1.74 [b] 10.15 ± 6.99	 0.443 0.687	 1.109 1.037	 0.851–1.444 0.868–1.240
Post-CRT IFN-γ NSCLC GBM	 8.91 ± 7.84 5.13 ± 3.79 [b]	 0.121 0.350	 1.054 0.907	 0.986–1.126 0.738–1.113
Survival NSCLC GBM	 287.8 ± 193.8 466.7 ± 377.46	 -	 -	 -

SD, Standard deviation; HR, hazard ratio; CI, confidence interval; NSCLC, non-small-cell lung cancer; GBM, glioblastoma multiforme. The P-value, HR, and 95.0% CI values for survival (days) and cytokine levels in the LR Cox regression analysis are presented. The IFN-γ, IL-6, and TNF-α levels were not associated with patient survival.

[a] Significantly different when compared with the control group ($P < 0.05$).

[b] Significantly different when compared with pre-CRT levels ($P < 0.05$).

CRT, IL-6 decreased in the GBM group and increased in the NSCLC group. One question arising from our results is why we did not observe the same changes in IL-6 levels among patients with GBM and NSCLC. Usually, the total volume of irradiated tissue is assumed to influence the development of tissue injury. In our study, the IL-6 levels of the post-CRT NSCLC group were higher than those of the post-CRT GBM group. This difference in the levels of post-CRT IL-6 levels in each type of cancer may be due to differences in the irradiated tissue volume. Furthermore, in vitro and in vivo experiments have demonstrated that the expression of IL-6 was linked to irradiation and radiation resistance (28,29).

Chen et al. (26) demonstrated that IL-6 was important in determining the radiation response of liver tumor cells. Given the potential role of irradiation-induced IL-6 in tumor regrowth, they proposed that concurrent treatment with IL-6 inhibitors could be a potential therapeutic strategy for increasing the radiation response of tumors. Chen et al. (30) also explored the predictive power of IL-6 in the treatment response of pharyngeal cancer, and they suggested that regulating IL-6 signaling might be a promising therapeutic approach. In our study, the post-CRT level of IL-6 was significantly higher in the NSCLC group compared with that of the control group. An antibody targeting IL-6 might be useful in NSCLC patients undergoing RT.

Several clinical trials have demonstrated the therapeutic effects of human interferons on malignancies and infectious diseases. The effects of IFN alone or combined with other treatment modalities (RT and CT) in NSCLC have been investigated. Arpin et al. reported that IFN-γ did not display cytotoxic activity and that it may actually induce repair mechanisms (31). We found that IFN-γ levels significantly decreased following CRT only in GBM. Due to decreased levels of IFN-γ after CRT, we propose that integrating immunotherapy in the primary standard treatment for GBM might benefit patients. Combining cytotoxic therapy with glioma vaccination has been conducted, and encouraging preliminary efficacy has been reported (32,33). Ardon et al. (34) concluded that including tumor vaccination as part of the standard primary postoperative treatment for patients with newly diagnosed GBM was feasible and that it was well tolerated.

Two distinct apoptotic pathways have been identified: the intrinsic and the extrinsic pathway (35). In the extrinsic pathway, cell surface death receptors are activated by specific ligands such as TNF-α (36). It is well known that both pathways are involved in radiation-induced apoptosis (37). Certain NSCLCs have been shown to be resistant to current therapeutic approaches due to defects in apoptotic mechanisms. Overexpression of inhibitors of apoptosis proteins was shown to be associated with resistance to conventional therapies (e.g., RT) and poor patient outcomes. Inhibitors of apoptosis proteins modulated nuclear factor-kappa B signaling and inhibited TNF-α-mediated cancer cell apoptosis. Recently several Smac mimetics have been introduced to eliminate resistance to ionizing radiation (IR). According to one study, Debio 1143 was shown to be a potent sensitizer of NSCLC cells to effects of IR-induced apoptosis and it remarkably enhanced the radiosensitivity by autocrine TNF-α production. Furthermore, it has been detected that the radiosensitization effects could be potentiated by an increase in TNF-α stimulation (6). In another study, El-Mesery et al. (26) reported that novel second mitochondria-derived activator of caspases mimetic BV6 induces cell death and sensitizes different cell lines to TNF-α-induced apoptosis (38). In the present study, the levels of TNF-α decreased with CRT in the NSCLC patients. Therapies that promote the sensitization to RT when used alone or in combination with existing modalities could be promising.

We also evaluated the relationship between patient survival and cytokine levels to determine the potential role of the latter in the prognosis of patients. Arpin et al. (39) correlated variations in IL-6 and TNF-α levels with the occurrence of radiation pneumonitis during RT in patients with NSCLC to evaluate the predictive value of cytokine levels. They suggested that early variations in circulating IL-6 and IL-10 levels during RT were significantly associated with the risk of radiation pneumonitis. In a clinical study, Shariat et al. (40) found that plasma IL-6 levels were significantly elevated in patients with prostate cancer that had metastasized to the bones, and they observed that preoperative plasma IL-6 levels predicted biochemical progression and lymph node metastases in men following radical prostatectomy (41). However, according to our study, pre-CRT and post-CRT IFN-γ, IL-6, and TNF-α plasma levels were not significantly associated with the survival of patients with NSCLC and GBM. This finding may be due to the small sample size in our study.

In conclusion, the present study was undertaken to examine the effect of CRT on the levels of cytokines in patients with NSCLC and GBM. Our data demonstrated that IFN-γ, IL-6, and TNF-α were sensitive to irradiation. Within the framework of the present study, the significantly decreased levels of TNF-α in NSCLC and IFN-γ in GBM following RT did not suggest a classical inflammatory response to radiation exposure. The levels of IL-6 were high in both NSCLC and GBM cases when compared to the control group, but they decreased in GBM and increased in NSCLC during CRT. Given that the GBM and NSCLC patients in this study received the same doses of RT, it is unusual that the IL-6 levels of the two groups differed in response to the treatment. Further studies with a larger number of cases should be designed to elucidate the roles of IFN-γ, IL-6, and TNF-α as biomarkers of malignancy and to discover new anticancer strategies.

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