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Survey of Pre-inflammation Cytokines Levels in Radiotherapy-induced-mucositis

[1]Farshad Seyyednejad, [2]Amirreza Rezaee, [2]Sarvar Haghi and [3]Mohamad Goldust
[1]Department of Radiotherapy, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Tabriz University of Medical Sciences, Tabriz, Iran
[3]Student Research Committee, Tabriz University of Medical Sciences, Drmogoldust, Iran

Abstract: Mucositis is a toxic side effect of anti-cancer treatments and is a major focus in cancer research. Pro-inflammatory cytokines have previously been implicated in the pathophysiology of chemotherapy-induced mucositis. The aim of this study was to detect a correlation between serum cytokine levels in head and neck (H and N) cancer patients receiving combined chemo-radiation therapy. Thirty patients with H and N epithelial cancer were recruited to this study. All patients received radiotherapy to the H and N region with doses ranging from 50-70 Gray (Gy). Chemotherapy with cisplatin, carboplatin, 5-fluorouracil and taxanes was given to high-risk patients, using standard chemotherapy protocols. Patients were evaluated for mucositis according to WHO common toxicity criteria and blood samples were drawn for inflammatory (IL-1 and TNF-a) and before and during treatment. The mucositis evaluation demonstrated mucositis grade IV in 33.3% of the patients after the 3rd treatment week. At the end of treatment, the number of patients with grade IV mucositis was less. IL-1 and TNF-α did not show any correlation with PEG tube installation. The level of cytokines measured before and during therapy showed decreased TNF-α especially after the third week of therapy. No relationship between IL-1 and TNF-α, level and mucositis grade was shown.

Key words: Mucositis, cytokines, chemoradiotherapy, IL-1, TNF-α

INTRODUCTION

Mucositis is a common complication of cancer therapy, which significantly affects the mucosa. It refers to the erythematous and ulcerative lesions commonly observed in patients undergoing cancer therapy. They are painful and affect nutrition and quality of life of the patient, and contribute to local and systemic infections. (Elad *et al*., 2011; Lin *et al*., 2010). There are a lot of complications if the mucositis affects also the gastrointestinal tract, such as nausea, vomiting, abdominal pain, diarrhoea, blood loss and even sepsis. (Argiris *et al*. 2011; Rodriguez-Caballero *et al*., 2012) Chemoradiotherapy-induced changes in the mucosa have been observed and studied virtually ever since the introduction of radiation as a therapeutic modality. It is often the dose limiting factor, interfering with the intensification of anticancer therapy (Maddocks-Jennings *et al*., 2009; Vayne-Bossert *et al*. 2010). Nuclear factor kappa B (NFκB), cyclooxygenase-2 (COX-2) as well as pro-inflammatory cytokines (in particular interleukin (IL)-1β (IL-6) and tumour necrosis factor (TNF-α) have been suggested to play a key role in the 5 phase mucositis model. Previous research has clearly shown that IL-1 and TNF-α are upregulated in the buccal mucosa, jejunum and colon of rats following administration of chemotherapy. (Goldust *et al*., 2012; You *et al*., 2009). Furthermore, elevated levels of IL-1 and TNF-α have been detected in the buccal mucosa of hamsters who received combined chemotherapy and radiotherapy (Chen *et al*., 2008; Reiter *et al*., 2009). Thus supporting the current view that pro-inflammatory cytokines play a major role in the development of mucositis (Ara *et al*. 2008; Goldust *et al*., 2011). The aim of this study was to evaluate the survey of pre-inflammation cytokines levels in radiotherapy-induced-mucositis.

MATERIALS AND METHODS

In this descriptive analytical study, 30 patients with H and N epithelial cancer in Shahid Madani Hospital, Tabriz from December 2011 to December 2012 were recruited to this study. This study was approved by ethic committee of Tabriz University of medical sciences. Written consent was obtained from all the patients. All patients were treated with radiation therapy (60-72 Gy), or radio-chemotherapy. RTOG/EORTC recommendations for post-operative radio-chemotherapy were adopted in high-

Corresponding Author: Mohamad Goldust, Department of Student Research Committee, Tabriz University of Medical Sciences, Drmogoldust, Iran Tel: 00989111289630 Fax: 00984113368805

risk patients. Chemotherapy was applied as concomitant chemoradiotherapy, adjuvant chemotherapy or neo-adjuvant chemo-radiotherapy. Evaluation prior, during or at the end of treatment. All patients had clinical evaluations one week before treatment, in the third weeks of treatment, and at the end of treatment. WHO common toxicity criteria were used (Grades 1 to 4). Blood samples were drawn three times (one week before treatment, at third week of treatment, and at the end of treatment) for the evaluation of inflammatory cytokines IL-1 and TNF-α. This evaluation was done with DPC's ELISA kits in the quantitative "sandwich" enzyme immunoassay technique. A monoclonal antibody specific for the interleukin molecule evaluated was introduced into the wells. Standard antigens together with the serum samples drawn from patients were also introduced into the wells and the interleukin present was bound by the immobilized antibody. After washing away any unbound proteins, the second enzyme-linked antibody specific for the interleukin was added to the wells to "sandwich" the interleukin immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of the cytokine bound in the initial step. By comparing the optical density of the samples to a standard curve, the concentration of the interleukin in unknown samples is determined. Statistical analyses were conducted using either one-way ANOVA followed by Tukey's *Post Hoc* test, or Kruskal Wallis test followed by Dunn's *Post Hoc* test. Results were deemed significant should $p<0.05$.

RESULTS

Demo graphic data of the study patients is shown in Table 1. Sixteen (53.3%) of the 30 patients required installation of a PEG tube during radio-chemotherapy and two female patient needed installation of a PEG tube after ending therapy. Six patients from the group that received PEG tubes needed a break in therapy because of severe side effects. After a short pause, therapy was renewed. Two patients died shortly after treatment because of multiple metastases. The mucositis evaluation demonstrated mucositis grade IV in 33.3% of the patients after the 3rd treatment week. At the end of treatment, the number of patients with grade IV mucositis was less (in accordance with boost irradiation to a smaller irradiation field). Patients who showed Grade IV mucositis at week 3 needed to have PEG tube installation. The level of cytokines measured before and during therapy showed decreased TNF-α, especially after the third week of therapy. IL-1 did not show any significant changes. IL-1 and TNF-α did not show any correlation with PEG tube installation. In this study no relationship between IL-1, and TNF-α, level and mucositis grade was shown.

Table 1: Patient characteristics

Parameters	No. of patients	%
Site of disease		
Nasopharynx	11	36.6
Oropharynx	1	6.7
Hypopharynx	1	6.7
Tongue	1	6.7
Supraglottic larynx	3	23.3
Tonsil	2	13.3
Cervical lymph nodes of unknown primary	1	6.7
Histology		
Undifferentiated nasopharyngeal carcinoma	5	16.6
Squamous cell carcinoma	25	83.4
Stage		
III	3	10.0
Iva	19	63.3
Ivb	4	13.3
Ivc	2	6.7
Loco-regionally advanced, NOS	2	6.0

DISCUSSION

In the past decade, active research has been conducted to define the pathogenesis of mucositis and the role of proinflammatory cytokines TNF-α and IL-1. Evidence of the upregulation of these proinflammatory cytokines coordinated with the extent of mucosal injury in mucositis proves to be valuable (Patni *et al*., 2005; Xanthinaki *et al*., 2008). This subclass of cytokines is recognized to play an enormous role during inflammation and tissue damage in response to cytotoxic therapy. There remains, however, a huge gap in the knowledge to recognize whether antiinflammatory cytokines such as and IL- 1 and TNF-α are essential tools in down regulating the inflammatory response associated with mucositis (Ryu *et al*., 2007). Lack of this knowledge which ties pro- and anti-inflammatory cytokines together within the complex yet interesting cytokine milieu leaves an incomplete image of immune response associated with mucositis. Furthermore, there is no evidence in literature that interprets the net balance of the subclass of cytokines in accordance with different phases of mucositis development (Cummins *et al*., 2007; Sugimoto *et al*., 2007). Moreover, the underlying mechanisms of action of these anti-inflammatory cytokines in chemotherapy induced mucositis remain under researched (Blijlevens and Sonis, 2007; Guney *et al*., 2007). This study demonstrated decreased TNF-α level in the patients receiving chemoradiotherapy. Patients undergoing chemoradiotherapy showed a significant reduction in TNF-α levels and to a lesser

extent. These observations are in contrast with previous findings where pro-inflammatory cytokine levels in were found to be elevated five days following chemotherapy in patients and 12 days post-chemoradiation treatment. (McAleese *et al*., 2006; Stokman *et al*., 2006). Radiation exposure in the range 1-2 Gy is known to activate the growth stimulatory ERK pathway via EGFR. It has been suggested that this activation is mediated through radiation-induced free radicals. Free radicals are also strongly linked to activation of NFκB and the pro-inflammatory pathway, as well as JNK signalling, indicating a balance between outcomes which is highly dose-dependent and linked to free-radical generation (Gabrilove, 2006; Su *et al*., 2006). This study demonstrated a correlation between inflammatory and anti-inflammatory cytokines and acute mucositis with a need for PEG tube installation. Sixteen of thirty patients who took part in this study needed PEG tube installation due to the severity of radio-chemotherapy side effects. The first phase of mucositis during radio-chemotherapy was characterized by the production of inflammatory cytokines, such as IL-1 and TNF-α that coordinate this process in the mucosa. This study was unable to detect any correlation between IL-1 and TNF-α. In some reports, elevated levels of TNF-α were documented during irradiation of H and N tumors (Hofmeister and Stiff, 2005; Langer *et al*., 2006). This fact leads to suggested treatment with TNF-α cytokines to avoid inflammation. In this study, we observed the opposite effect, a decrease of the TNF-α level. TNF-α has an important interaction with other cytokines and hormones. This cytokine influences cell proliferation, various intra-cellular processes and cytostatic effects that, in the presence of IFN-g, create a cytotoxic effect. This demonstrated no change of IL-1 levels in the patients, in contrast to other studies that showed enhancement of IL-1 in patients with mucositis. Fibroblasts, B lymphocytes, and endothelial cells can produce IL-1, a cytokine-induced cytokine which participates in various biological processes, including acute inflammation.

CONCLUSION

The level of cytokines measured before and during therapy showed decreased TNF-α, especially after the third week of therapy. IL-1 did not show any significant changes. IL-1 and TNF-α did not show any correlation with PEG tube installation. In this study no relationship between IL-1 and TNF-α, level and mucositis grade was shown.

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