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Detection of Interleukins 6 and 18 Circulating Levels in Patients with Brain Tumors

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Abstract

***Introduction*:** The balance between T helper type 1 (Th1) and Th2 cytokines is thought to have an important role in simplified chaos of tumor progression. So we aimed to evaluate both IL-18 and IL-6 (pro-inflammatory cytokines for Th1 and trigger the Th2 cytokine release, respectively) in patients with brain tumors.

***Materials and Methods*:** In present case- control study serum samples were collected from 65 newly diagnosed patients with brain tumor and also 40 cases as control from normal population that were matched about their age and sex. Then the patients were divided into various groups according to pathologic reports of tumor type and median serum level of the cytokines determined by ELISA technique for each tumor types. Two major groups of malignant tumors (N=33) and benign tumors (N=32) was compared by nonparametric test of Kruskal-Wallis.

***Results*:** There was significant increase in serum IL-6 level in malignant group ($p<0.01$), while IL-18 was reduced in this group ($p<0.05$). Acoustic schowanoma patients (N=13) divided into 2 groups, one with involvement of facial nerve (N=8) and another without this complication (N=5). We found IL-6 serum level was higher in complicated patients than the remained patients ($p<0.01$).

***Conclusion*:** We concluded that the patients with more invasive and advanced brain tumors had more circulating levels of IL-6 in association of lower levels of IL-18, that present the immune balance due to humoral immunity in brain tumors is polarized in favor of Th2.

Introduction

Brain tumors have the major systemic effect on the immune system [1]. Therefore the immune therapy is generally thought to be promised beneficial role in most of the cancer treatments [2]. Imbalance of cytokine levels are associated with several cancers such as liver, prostate, brain tumor, and acoustic schwannoma which is common cerebellopontine angle tumor and presented with the hearing loss or an addition facial palsy, depends on involvement of facial nerve or not, respectively [3, 4]. The balance between Th1 and Th2 cytokines is thought to be an important factor in tumor prognosis [1].

IL-6 attributed for the regulation of innate immunity and Th2 production [5] and plays a major role in the response to injury or infection and is involved in the immune response, inflammation, and hematopoietic. Its deregulation impacts numerous disease states, including many types of cancer [3, 5]. High levels of circulating IL-6 are observed in almost every type of studied tumor [3]. IL-6 is a growth and survival factor in human glioblastoma cells and plays an important role in malignant progression [6]. Anti-IL-6/IL-6R therapy using monoclonal antibodies has been included in clinical trial for numerous diseases and cancers and first results indicate that targeting the IL-6 pathway has beneficial effects in the treatment of IL-6-dependent cancers [7] and therapeutic targeting of IL-6 and its receptor in cancer has strong biological association [2, 3, 6, 7]. The IL-18 (a cytokine from IL-1 family with potent IFN-γ-inducing activities) plays an important role in the Th1-mediated immune response in collaboration with IL-12 [8-12], and known as a proinflammatory cytokines [13]. Its receptor system and its signal transduction pathway are analogous to those of the IL-1 receptor.

Mice with IL-18 deficient have demonstrated the critical role in natural killer cell activity and in vivo Th1 response [11]. The elevation of IL-18/IL-4 ratio suggests an imbalance of cytokine profile to mediate the inflammatory response [13]. Dendritic cells (DCs) load with total tumor lysate and IL-18 may represent a method for inducing Th1 immune-responses against the entire repertoire of glioma antigens [14, 15]. The role of IL-18 modulating in tumors demonstrated [8]. Previous study mentioned the protective role of IL-18 in the disease and it induces IFN-γ from NK cells in a caspase-1 dependent fashion and known as a primary IFN-γ inducing cytokine in promoting Th1 responses [10]. One of the pro-inflammatory mediators is IL-6 through the contribution

to inflammation by Th17 cells and demonstrated Th1 cells being required for the recruitment of Th17 cells into the central nervous system in some diseases related to inflamed tissue [16]. On the other hand, IL-18 is required to induce Th1 response [9]. Together, these indicate that the cells co-expressed IL-6 and IL-18 in inflammation diseases. In one study about Marek's disease shown that IL-6, IL-18, IFNA (Interferon A), and IFNG (Interferon G) were nondifferentially expressed, which indicates host inflammatory response was impaired [17].

Further study is required to define the role of IL-6 and IL-18 in brain tumors. Together these data led us to evaluate the relationship of serum IL-6 and IL-18 levels with brain tumor.

Materials and Methods

In this case-control study the patients with brain tumor that conducted to oncology Biobank Center, Shiraz University of Medical Sciences, Shiraz, Iran were requested to enroll this study. At first step 100 patients entered to study. The patients that underwent replacement chemotherapy, radiotherapy, and whom with certain systemic diseases receiving glococorticoid and with active infection were excluded. So 65 patients entered as a case group and divided them into two groups malignant (N=33) and benign (N=32) tumors. Also acoustic schwanoma patients was picked up (N=13) and divided them into 2 groups, one with involvement of facial nerve (N=8) and another without this complication (N=5). Finally the age and gender of control and case studied groups were matched and informed consent form was obtained from all participants and by administration the previous designed check list, the symptoms of disease, kind and the local of tumor, age, sex, smoking habit and kind of therapy were obtained from all participants.

Serum samples were obtained by centrifugation of their participant's blood samples and that stored at -20ºC. The analysis of IL-6 and IL-18 concentration was conducted through Elisa technology (Bender Med Systems).

Statistical analysis was performed by SPSS-18. The nonparametric test, Kruskal-Wallis, was conducted to compare the two major groups of malignant tumor and benign tumor. We considered the significant level *p*-value=0.01 for IL-6 and 0.05 for IL-18 respectively. Also One-way ANOVA test was used to compare the two studied groups with involvement of facial nerve between acoustic schwanoma patients. Informed consent form was obtained from all participants.

Results

The median levels of IL-6 in malignant and benign tumor were 7.36 pg/mL and 3.7 pg/mL, respectively. There was significant difference of IL-6 levels between the malignant and benign groups serum ($p \leq 0.01$). The median level of IL-18 in malignant and benign tumor was 333.58 pg/mL and 423.31 pg/mL, respectively. Serum IL-18 level was conversely, lower in malignant group than benign group ($p \leq 0.05$) (Table 1). Figure 1 presented the median level of IL-6 and IL-18 in control and patients with malignant and benign tumor. In spite of, the median levels of IL-6 and IL-18 in control group were lower and higher than malignant group, respectively also higher and lower than benign group, respectively but no significant difference was seen (Table 1).

In another approach, the mean serum level of IL-6 in patients with involvement of facial nerve and without this complication was 9.55 pg/mL and 1.4 pg/mL, respectively. They were shown in table 1. One-way ANOVA test showed that there was a significant difference between with and without involvement of facial nerve patients (Fig. 2).

Table 1. The median levels of IL-6 and IL-18 in different groups

Groups		IL-6 (pg/mL)	IL-18
Malignant		7.36	333.58
Benign		3.7	423.31
Control		4.23	413.45
Schwannoma	With facial nerve involvement	1.4	-
	Without of facial nerve involvement	9.55	-

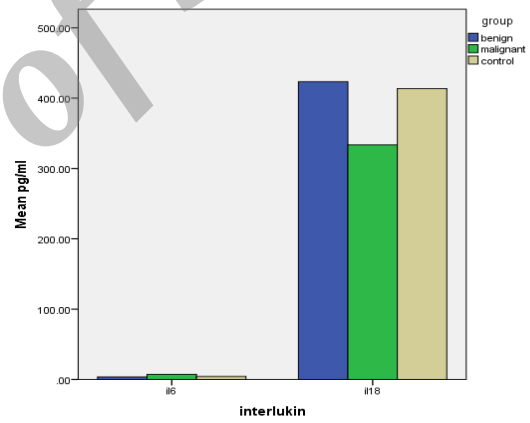
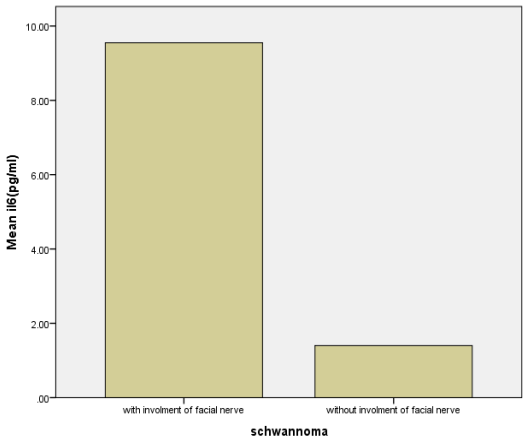


Figure 1. The median level of IL-6 and IL-18 in patients with malignant and benign tumor



Figuer 2. The level of IL-6 in patients with/without involvement of facial nerve

Discussion

In this study, we investigated the relationship between serum cytokines (IL-6, IL-18) level and brain tumors (malignant and benign). We found that serum IL-6 level as a modulation of immune and inflammatory responses was significantly higher in malignant group than benign group. Study by the Liu et al. was proposed that IL-6 as one of the detection mediators, identified the interaction between tumor cells and endothelial cells and several transmitted signals associated with cell invasion and migration in various tumors is related to IL-6 and it promote globlastoma cell invasion and according to this result, it seems that elevation of IL-6 level in the serum should be observed and it similar to the results of this study [6]. Our results confirmed by previous studies [18, 19]. Weisenberger et al. [18] and Chaudhry et al. [19] showed the significant increase in serum IL-6 level in glioma brain tumor and also they reported the correlation between severity of malignancy and serum IL-6 level [18].

Although we demonstrated that the serum IL-18 was reduced in the malignant group. Kato et al. [12] reported that macrophages stimulated with IL-12 and IL-18, then produced IFN-γ and NO, which in turn mediated the antiglioma response and similarly Yamanaka et al. [20] reported that interferon-γ is partly responsible for IL-18 mediated anti tumor immunity and IL-18 is one of the factors which enhanced the induction of the Th1 response as well as antitumor immunity. Lebel-Binay et al. [8], Gillespie et al. [21] and Donatello et al. [22] showed the similar results and focuses on the ability of IL-18 as a induction of interferon gamma production and also modulation in tumours, infections, and autoimmune and inflammatory disease. Similar results were obtained at the study of Donatello et al. [10] which is about of the contribution of IL-18 to active macrophage because of its capacity to induce IFN-γ and it seems that the reduction of IL-18 inhibit the stimulation of Th1 response by decreasion of IFN-γ and even may promote the secretion of IL-10 cytokines (as a pivotal Th2) by decreasion of the blocking production of this cytokine [9]. As Kumar et al. [1] also showed the increase of IL-10 in menangiom, astrocytom and globlastoma brain tumors [1]. Hence IL-18, IL-12 and IFN-γ are likely to play important role in the malignant brain tumor [1], we proposed that decreased of IL-18 cytokines in the brain tumor may improve the malignant process and these results suggest that immunotherapy of patients with the malignant brain tumor is important and IL-18 may represent a method for inducing Th1 immune responses against the malignant brain tumor. Surprisingly, we also detected the high level of IL-6 in serum of the patients with involvement of facial nerve. This finding suggested that Th2 (humoral immunity) response was elicited in the patients with involvement of the malignant tumor and the facial nerve and it is not benefit against tumor cells. It seems, these patients under inhibition of IL-6 may contribute at inhibiting the growth of invasive cells [8] and even if it combined by exhibition of IL-18 cytokine with potent IFN-γ inducing activities, may bring an additional advantage in treatment and can provide a partial to completed protection in the invasive brain tumor while its reduction was observed.

The elevation of IL-6 level in serum of patients with involvement of facial nerve was observed so the immunotherapy of these patients also may promise the new approach due to difficulties, long and side effects of surgical methods. Altogether the immune therapy by performing the IL-18 that induce Th1 (cellular immunity) response may be a good candidate for development of a new treatment protocol, in malignant brain tumor.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis, and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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