

## Serum Level of Cytokines and their Expression in Brain Tumors (Meningioma and Glioma) of Iraqi Patients

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### Abstract:

**Objective:** the study aimed to assess serum level and tissue expression of Cytokines in brain tumour (meningioma and glioma) of Iraqi patients.

**Methodology:** In this study, 64 Iraqi brain tumor patients (30 meningioma and 34 glioma) were investigated for serum level of IL-2, IL-8, IL-10 and IFN- $\gamma$ , and tumor-immunohistochemical expression of IL-2, IL-10, IFN- $\gamma$ , TNF- $\alpha$  and TGF- $\beta$ . Two control samples were also enrolled in the study. The first included 30 blood donors, while the second included 15 cadavers for immunohistochemical evaluation.

**Results:** IL-10 ( $15.51 \pm 1.01$  and  $16.57 \pm 1.01$  vs.  $11.98 \pm 1.48$  pg/ml, respectively) and IFN- $\gamma$  ( $0.94 \pm 0.15$  and  $0.99 \pm 0.16$  vs.  $0.18 \pm 0.01$  IU/ml, respectively) levels were significantly increased in meningioma and glioma patients as compared to controls, while IL-2 and IL-8 revealed no significant variation. Assessment of cytokine expressions revealed that TGF- $\beta$  was positive in all cases of meningioma and glioma, while IL-10 was positive in 20.6% of glioma tissues. In contrast, all brain tissues of patients were negative for IL-2, IFN- $\gamma$  and TNF- $\alpha$  expression. The 15 brain tissues of cadavers were negative for the expression of all investigated cytokines.

**Conclusions:** This study shows that meningioma and glioma patients had increased circulating serum levels of IL-10 and IFN- $\gamma$ ; demonstrating that brain tumors might have a systemic effect on the immune system. The data also suggest a possible role of TGF- $\beta$  in pathogenesis of brain tumor.

**Recommendation:** The serum level of IFN- $\gamma$  and IL-10 has to be revisited, with other types of brain tumours, especially if the grading system of tumour is considered.

**Keywords:** Brain tumor, Meningioma, Glioma, Cytokines.

### I. Introduction

Brain tumor is a mass or growth of abnormal cells in brain, which can either be originated from the brain itself or migrated from another part of the body to the brain and form a metastatic brain tumor [1]. Human malignant brain tumor (MBT) is a highly lethal tumor that is paradigmatic for the ability to suppress effective anti-tumor immune response [2]. Different pathways of MBT-associated immune suppression have attracted the attention, and the release of different cytokines and the expression of various cell surface receptors are of an important concern [3].

Cytokines are low molecular-weight soluble protein messengers that are involved in all aspects of immunity, and may be regarded as hormones of the immune system [4]. These molecules can be secreted by various cells and act as signals between cells to regulate the immune responses. They have a variety of functions that facilitate immune effector mechanisms in cancer immunity, and their mechanisms may be either growth stimulation or inhibition of pre-malignant or malignant cells by acquired and/or innate immunity [5]. It has also been reported that they are important regulatory proteins, which control growth and differentiation of normal and malignant glial cells [6]. In addition, it has been further demonstrated that brain tumor cells showed abnormalities in the expression of tumor necrosis factor (TNF)- $\alpha$ , transforming growth factor (TGF)- $\beta$ , interferon (IFN)- $\gamma$ , interleukin (IL)-2 and IL-10 [7].

The cytokines are produced by different types of immune cells, and recently [8] reviewed that subclasses of T helper (Th) lymphocytes can be identified based on their repertoire of cytokines (Th1, Th2, Th17 and Treg). Th1 cells produce IL-2, IFN- $\gamma$  and TNF- $\alpha$ ; Th2 cells produce IL-4, IL-5, IL-9 and IL-13; Th17 cells produce IL-6 and IL-17; while Treg cells produce IL-10 and TGF- $\beta$ . However, Th1/Th2 model is a well-established way of understanding the various cytokines that are secreted by the different CD4+ T helper lymphocyte subsets. The CD4+ Th1 cytokines are collaborated to stimulate a cell-mediated response and they have been shown to exert a potent anti-tumor effect. In contrast, CD4+ Th2 cytokines stimulate the humoral immune response [9]. The Th1 and Th2 cytokines act in an antagonistic fashion; with a balance point that varies

between the physiological and pathological state, and there is evidence that glial tumors may induce a Th1 to Th2 type cytokine shift, and it is likely that such switching is related to the origination of gliomas and the evasion of glioma cells from immune surveillance [10].

The present study aimed to determine the serum level of IL-2, IL-8, IL-10 and IFN- $\gamma$ , in addition to the tissue expression of IL-2, IL-10, INF- $\gamma$ , TNF- $\alpha$  and TGF- $\beta$  in brain tumors (meningiomas and gliomas) of Iraqi Arab patients.

## II. Subjects and Methods

### Part 1: Subjects

The study was approved by the Medical Ethics Committee of the Ministry of Health in Iraq, in which 64 brain tumor patients were enrolled. They were admitted to the Specialized Surgeries Hospital and Neurological Disorders Hospital in Baghdad for a surgical operation to resect brain tumor. Based on a clinical evaluation (the consultant medical staff at the two hospitals) and a histopathological examination of tumor, the patients were distributed into two clinical groups; meningioma (30 cases) and glioma (34 cases). Two further subjects (control samples) were also investigated. The first sample included 30 apparently healthy blood donor volunteers (Control I), while the second included 15 cadavers (less than 20 hours postmortem) from the Forensic Medicine Institution (Baghdad). The first control was employed for the comparisons of cytokine serum levels, while the second control (Control II) subjects were used to obtain brain tissues for immunohistochemical evaluations. Age and gender distribution of patients and controls are given in Table 1.

### Part 2: Specimen Collection

From each participant, 3 ml of venous blood were collected pre-surgical operation, and dispensed in a plain tube for the collection of serum. The serum was distributed into aliquots (0.25 ml) and kept in the freezer (-20°C) until assessment of cytokines. Brain tumors (patients) or normal tissues (cadavers) were also obtained and embedded in paraffin for immunohistochemical examination.

**Table 1:** Age and gender distribution of brain tumor patients and controls.

Groups		Gender	No	Age Mean $\pm$ S.E. (Years)
Patients (No.= 64)	Meningioma	Males	9	57.1 $\pm$ 2.3
		Females	21	44.1 $\pm$ 2.9
	Glioma	Males	19	40.6 $\pm$ 3.8
		Females	15	35.3 $\pm$ 5.1
Control I (No.= 30)		Males	14	38.3 $\pm$ 3.9
		Females	16	40.4 $\pm$ 4.1
Control II (No.= 15)		Males	15	29.1 $\pm$ 3.9

No = Number ; S.E.= Stander Error

### Part 3: Serum level of cytokines

Level of IL-2, IL-8, IL-10 and IFN- $\gamma$  was assessed in the sera by means of ELISA method by using ready-used kits and the instructions of manufacturers (Biosource, Belgium and Cell Company, France) were followed.

### Part 4: Cytokine Immunohistochemical expression

The immunohistochemical expression of IL-2, IL-10, INF- $\gamma$ , TNF- $\alpha$  and TGF- $\beta$  was determined by the envision method, in which the primary antibody (anti-IL-2, -IL-10, -INF- $\gamma$ , -TNF- $\alpha$  or -TGF- $\beta$ ) reacts with its corresponding antigen (IL-2, IL-10, INF- $\gamma$ , TNF- $\alpha$  or TGF- $\beta$ ) in the tissue, and then a secondary antibody system (a polymer backbone to which multiple antibodies and enzyme molecules are conjugated) binds to the primary antibody. When the conjugate is added, the polymer secondary antibody system will form a complex with the peroxidase-conjugated streptavidin, and by adding a substrate, which contains diaminobenzidine (DAB) in a chromogen solution, a brown-coloured precipitate will form at the antigen site [11]. The materials of this detection were obtained from Dako (Denmark). Allred's scoring system [12] was adopted to score the reaction patterned. It is based on two score evaluations: proportion and intensity scores. A proportion score (PS) is assigned to represent proportion of tumor cells with positive nuclear staining, and has a range of 0-5, while an intensity score (IS) is assigned to representing the average staining intensity of all positive tumor cells, and has a range of 0-3. A total score (TS) is the sum of PS plus IS, and has a range of 0–8. Accordingly, TS score of 0-2, 3-4, 5-6 and 7-8 corresponds to negative, weak positive, intermediate positive and strong positive reactions, respectively.

**Part 5: Statistical analysis**

Serum level of cytokines was statistically analyzed using SPSS (Statistical Package for Social Sciences) version 13. Their data were given as mean ± standard error (S.E.), and differences between means were assessed by analysis of variance (ANOVA) followed by Duncan's test. The difference was considered significant when the probability (P) value was ≤ 0.05.

**III. Results**

**Part 1: Serum level of cytokines**

Serum level of IL-2 (20.94 ± 1.63, 21.31 ± 1.41 and 18.82 ± 0.64 IU/ml, respectively) and IL-8 (103.84 ± 4.76, 111.62 ± 5.53 and 103.48 ± 7.49 pg/ml, respectively) showed no significant variation between meningioma and glioma patients and controls. In contrast, the variation was significant in IL-10 (P = 0.018) and IFN-γ (P = 1.4 × 10<sup>-5</sup>). IL-10 demonstrated a significant increased level in meningioma and glioma patients (15.51 ± 1.01 and 16.57 ± 1.01 pg/ml), as compared to controls (11.98 ± 1.48 pg/ml). A similar increased level was also observed in IFN-γ (0.94 ± 0.15 and 0.99 ± 0.16 vs. 0.18 ± 0.01 IU/ml, respectively) (Table 2).

**Table 2:** Serum level of IL-2, IL-8, IL-10 and IFN-γ in brain tumor (meningioma and glioma) patients and controls.

Groups	Number	Cytokine Serum Level (Mean ± S.E.)*			
		IL-2 (IU/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	IFN-γ (IU/ml)
Meningioma	30	20.94 ± 1.63 <sup>A</sup>	103.84 ± 4.76 <sup>A</sup>	15.51 ± 1.01 <sup>A</sup>	0.94 ± 0.15 <sup>A</sup>
Glioma	34	21.31 ± 1.41 <sup>A</sup>	111.62 ± 5.53 <sup>A</sup>	16.57 ± 1.01 <sup>A</sup>	0.99 ± 0.16 <sup>A</sup>
Controls	30	18.82 ± 0.64 <sup>A</sup>	103.48 ± 7.49 <sup>A</sup>	11.98 ± 1.48 <sup>B</sup>	0.18 ± 0.01 <sup>B</sup>
ANOVA Probability		Not significant	Not significant	0.018	1.4 × 10 <sup>-5</sup>

\*Different superscript letters: Significant difference (P ≤ 0.05) between means of columns (Duncan test).

**Part 2: Cytokine Immunohistochemical expression**

Immunohistochemical evaluation revealed that all cadaver brain tissues were negative for the expression of IL-2, IL-10, INF-γ, TNF-α and TGF-β, and in addition, all brain tumor tissues were negative for the expression of IL-2, INF-γ and TNF-α, but IL-10 and TGF-β were an exception. IL-10 showed a positive expression in 20.6% of glioma patients, which were distributed as 5.9% weak and 14.7% intermediate (Table 3). For TGF-β, all brain tumor tissues (meningioma and glioma) showed a positive reaction, although different scores were observed. Meningioma positive cases were distributed as 20.0% weak, 36.7% intermediate and 43.3% strong, while glioma cases showed intermediate (35.3%) and strong (64.7%) positive reactions (Table 4).

**Table 3:** Brain tumour (meningioma and glioma) patients and controls distributed by the expression of IL-10.

Groups	Number	Reaction Score							
		Negative		Positive					
		No.	%	Weak		Intermediate		Strong	
		No.	%	No.	%	No.	%	No.	%
Meningioma	30	30	100.0	0	0.0	0	0.0	0	0.0
Glioma	34	27	79.4	2	5.9	5	14.7	0	0.0
Controls	15	15	100.0	0	0.0	0	0.0	0	0.0

**Table 4:** Brain tumour (meningioma and glioma) patients and controls distributed by the expression of TGF-β.

Groups	Number	Reaction Score							
		Negative		Positive					
		No.	%	Weak		Intermediate		Strong	
		No.	%	No.	%	No.	%	No.	%
Meningioma	30	0	0.0	6	20.0	11	36.7	13	43.3
Glioma	34	0	0.0	0	0.0	12	35.3	22	64.7
Controls	15	15	100.0	0	0.0	0	0.0	0	0.0

**IV. Discussion**

Tumor initiation and progression is a complex process involving genomic mutations, micro environmental factors, and immunological mediators. Within the tumor environment these mediators are responsible for cell proliferation, tumor invasion, angiogenesis and suppression of certain immune functions [3].

The present study highlighted the importance of IL-10 and IFN-γ in pathogenesis of brain tumor of both types (meningioma and glioma), as both cytokines showed a significant increased serum level in the

patients. IL-10 is principally produced by Treg cells, as well as some cancer cell types, B cells, dendritic cells, and monocytes/macrophages [13]. It was originally coined as cytokine synthesis inhibitory factor because of its general suppressive role of certain cytokines; more specifically, its ability to block the synthesis of IL-1 $\alpha$ , IL-1 $\beta$  and IL-12. In addition to cytokine inhibition, IL-10 has also been shown to reduce the antigen presentation capacity of monocytes via down-modulating MHC class II expression [14].

In agreement with the present results, [15] found that patients with benign brain tumors showed a significant increased serum level of IL-10. Such findings came to confirm an earlier demonstration made by [16], who also showed an increased serum level of IL-10 in both anaplastic astrocytoma and glioblastoma patients, but their results in meningioma showed no difference between patients and controls.

For IFN- $\gamma$ , the present increased serum level in brain tumor patients might be expected, because it is an important cytokine in tumor immunity and studies have always augmented the view that IFN- $\gamma$  plays a critical role in the rejection of transplanted tumors by mechanisms such as inhibition of angiogenesis, enhancement of cytotoxic responses against tumors, and by its direct action on tumor cells [17]. The latter effect involves up-regulation of MHC class I expression and thereby increasing tumor cell recognition and killing by cytotoxic T lymphocytes, and in some cases promoting the anti-proliferative and pro-apoptotic effects on tumor cells [18]. Following such theme, [19] studied serum level of IFN- $\gamma$  in brain tumor and brain traumas patients, and the results confirmed that IFN- $\gamma$  was increased in the majority of patient's groups (78%), but the increase was more pronounced in brain tumor patients than brain trauma patients. A further demonstration was made by [15], who showed increased release of pro-inflammatory factors and some cytokines, such as IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$ , which were implicated in both regulation of inflammation and the development of cancer as suggested by the authors.

With respect to tumor expression of cytokines, TGF- $\beta$  was expressed by all tissues of brain tumor patients, while none of the controls showed such expression. In agreement with such findings, it has been demonstrated that the expression of TGF- $\beta$  was positive in all primary typical meningioma, and it was detected in more than 50% of primary brain neoplasms [20] and [21]. Functionally, TGF- $\beta$  inhibits proliferation of meningeal and benign meningioma cells. Thus, it seems likely that TGF- $\beta$  exerts an inhibitory effect on benign meningiomas and that a loss of TGF- $\beta$  signaling and/or resistance to the growth inhibitory effects of TGF- $\beta$  results in progression to malignancy [21]. [22] have been able to correlate that molecularly, and they identified molecular signatures that characterize the different grades of meningiomas. Fourteen genes that participate in TGF- $\beta$  signalling were differentially expressed between grades 1 and 3 meningiomas. The majority of these components had reduced expression levels in grade 3 meningiomas, and they suggested that loss of TGF- $\beta$  signalling as a mechanism contributing to the development of higher-grade meningiomas and signaling to distinguish between benign low-proliferative tumors from malignant high-proliferative tumors.

By using intracranial rat C6 glioma model, it has been demonstrated that gene modification of glioma cells to block the expression of the immuno-suppressive cytokine TGF- $\beta$  was in favor of anti-tumor immune responses and thereby prolonging survival of tumor-bearing animals was observed [23]. Furthermore, [24] demonstrated that high TGF- $\beta$  expression was present in aggressive, highly proliferative gliomas and conferred poor prognosis in patients with glioma. It is also a key player of glioma carcinogenesis and its isoform TGF- $\beta$ 2 plays a pivotal role as an autocrine stimulus of growth and de-differentiation. Besides autocrine effects, various other mainly paracrine functions emphasize the role of TGF- $\beta$  as a highly potent suppressor of immune reactions, inductor of angiogenesis, and promoter of cell motility and malignant invasive capacity [25]. In this regard, TGF- $\beta$ 2 has been implicated in glioma cell motility and migration via several mechanisms that involve cell adhesion factors (e.g. integrins) and extracellular matrix proteins such as versican [26]. Some human gliomas have also been demonstrated to express high levels of TGF- $\beta$ 2 and neuroprogenitor cell markers, and TGF- $\beta$  was regarded to play an essential role in the regulation of glioma-initiating cells in human glioblastoma [27].

TGF- $\beta$  is a multifunctional cytokine which not only interferes with multiple steps of afferent and efferent immune responses, but also stimulates migration, invasion and angiogenesis. Several *in vitro* paradigms and rodent glioma models have been used to demonstrate that the antagonism of TGF- $\beta$  holds promise for treatment of glioblastoma, employing antisense strategies, inhibition of pro-TGF- $\beta$  processing, scavenging TGF- $\beta$ , or blocking TGF- $\beta$  activity by specific TGF- $\beta$  receptor (TGF- $\beta$ R) I kinase antagonists. So that TGF- $\beta$ -antagonistic treatment strategies are among the most promising of the current innovative approaches for glioblastoma, particularly in conjunction with novel approaches of cellular immunotherapy and vaccination [28]. Antagonizing TGF- $\beta$  activity has been shown to inhibit tumor invasion *in vitro*, but a systemically inhibition or lack of TGF- $\beta$  signaling results in acute inflammation and disruption of immune system homeostasis [29].

The low percentage of IL-10 expression by brain tumor cells may be in favor of tumor progression. In agreement with the present results, several cultures of human glioma cells expressed very low levels of IL-10, and accordingly, it is considered as an important factor produced by malignant brain tumors involved in the

local immunosuppression [30]. However, in primary brain neoplasm tumors, transcription of genes encoding for the inhibitory cytokine IL-10 was detected in more than 50% of samples [31]. Such cytokine has been reported to be secreted by glioma cells [32], and functionally it impairs T cell activity and responsible for the development of immunotolerizing Treg cells [33].

The other three cytokines (IL-2, IFN- $\gamma$  and TNF- $\alpha$ ) showed a negative expression in the tissue of brain tumor patients. The same finding was also observed in meningioma patients by [34]. Also, no expression was detected for IL-1 $\alpha$ , IL-2, IL-4, IL-5, IL-7, TNF- $\alpha$ , or IFN- $\gamma$  in any of the meningioma cultures. In glioma patients, similar results have been demonstrated by [31], in which no expression for the cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IFN- $\gamma$  and TNF- $\alpha$  was observed in freshly excised brain tumor samples. Therefore, the expression of these cytokines may not be favored by tumor progression. With respect to IFN- $\gamma$ , [35] also studied IFN- $\gamma$  and IL-17 expression in tissue samples from kidney cancer and brain tumors, and found that IFN- $\gamma$  and IL-17 expression was negatively expressed in brain tumors including gliomas and meningiomas, while they were abundantly present in kidney cancer.

The negative tissues expression of IL-2, IFN- $\gamma$  and TNF- $\alpha$  was also observed in meningioma patients by [34]. Also, no expression was detected for IL-1 $\alpha$ , IL-2, IL-4, IL-5, IL-7, TNF- $\alpha$ , or IFN- $\gamma$  in any of the meningioma cultures. In glioma patients, similar results have been demonstrated by [31], in which no expression for the cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IFN- $\gamma$  and TNF- $\alpha$  was observed in freshly excised brain tumor samples.

It appears from the present results and the literature that IL-10, IFN- $\gamma$  and TGF- $\beta$  are important molecules in brain tumor biology and they should be a subject of future investigations, especially at the molecular level.

## V. Conclusions

This study shows that meningioma and glioma patients had increased circulating serum levels of IL-10 and IFN- $\gamma$ ; demonstrating that brain tumors might have a systemic effect on the immune system. The data also suggest a possible role of TGF- $\beta$  in pathogenesis of brain tumor.

## Recommendation

The serum level of IFN- $\gamma$  and IL-10 has to be revisited, with other types of brain tumours, especially if the grading system of tumour is considered.

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