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**Role of the circulatory interleukin-6 in the pathogenesis of gliomas**

Role of circulatory IL-6 in gliomas

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## **Abstract**

Gliomas are most common primary tumor in brain. In spite of extensive research, the overall survival is not enhanced. Interleukin-6 (IL-6) has been found to have significant role in progression and apoptosis resistance of glioma. This article reviewed published case-control studies investigating the role of circulatory IL-6 in the development and progression of glioma and its utility as biomarker. Data were collected using various combination of keywords using electronic databases to find relevant published articles. Examined 5 studies have been included investigating the role of circulating IL-6 in glioma cases and controls appear to be an independent diagnostic and/or prognostic marker. The published results are inconsistent; however, majority of studies found a significantly higher IL-6 Level in glioma cases as compared to controls. Comparative IL-6 Level among the different grade of glioma cancer observed a higher level with low grade gliomas and lower levels with high grade gliomas. In conclusion, IL-6 Level was more than 2-fold in cases therefore, clears the criterion of acceptance and can be considered as potential biomarker for the diagnosis. On the basis of tumors with progressive growth the circulating level also raised and hence can be employing as markers of prognosis and monitoring glioma.

**Key Words:** Gliomas; Interleukin-6; Circulatory markers; Diagnostic marker; Prognostic marker

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**Core Tip:** In spite of extensive research in the field of brain oncology, the overall survival is not much improved. There is emergent need to explore the circulatory markers for the diagnosis and prognosis purpose. This systematic review has focused on role of IL-6 in brain cancer and it showed potential to be explored in the same field.

## INTRODUCTION

Gliomas are the most common primary brain tumors in adults accounting for 80% of malignant brain tumors originated from glial cells (1). Globally, gliomas showed a wide variation in the incidence and it is 0.01 to 12.7 in males and 0.01 to 10.7 in females per 1,00,000 people (2). The minimal incidence is in Africa and highest in Northern Europe (2). Gliomas are the increasing cause of death in children and third in adolescents and adults (2). According to World Health Organization (WHO) classification the most common occurring histologic type gliomas grade can be astrocytic tumors (grades I-III) and oligodendroglial tumors (grades II-III), ependymomas (grades I-III) and glioblastoma tumors (grade IV) (3,4). Glioblastoma is very aggressive in nature and the survival rate is very marginal considering death within 2 years of diagnosis while receiving of maximal surgical removal of tumor and medical therapies including chemotherapy and radiotherapy, therefore, there is an urgent need to find the comprehensive treatment strategies to enhance the survival rate (5).

Adapting the Virchow theory various studies concluded inflammation is one of the major hall marks of the cancer formation (6,7). Within the cancerous microenvironment inflammatory cells and cytokines have pleomorphic role, on one hand these aid in tumor suppression while on the other hand they support malignant cell transformation, tumor growth, inhibition of apoptosis, invasion, angiogenesis, cell migration, tumor cells differentiation and immunosuppression (8,9,10,11). A number of evidences showed varied circulating levels of cytokines in glioma. On the basis of The Cancer Genome Atlas (TCGA) database Interleukin-6 (IL-6) is one of the immune related gene expressed IL-6 protein has a significant role in progression and apoptosis resistance of glioma (12,13,14,15).

### *Interleukin (il-6) physiology*

IL-6 a pleiotropic pro-inflammatory cytokine is a with 21-28 kDa 4-helix bundled glycoprotein involving of 184 amino acids (16,17). In the normal conditions, IL-6 secretion is initiated in response to stimuli such as viruses, ultra violet rays (UV) and secretion of other cytokines and it is released by a variety of cells including

macrophages, monocytes, hematopoietic cells, stromal cells, muscles cells and epithelial cells. IL-6 has a significant role in the process of immunity, inflammation, angiogenesis, neural development, reproduction, metabolism hematopoiesis, and bone remodeling (18,19). In tumor vasculature, IL-6 is released by tumor cells, tumor infiltrating immune cells and fibroblast stromal cells and induced by several factors for example prostaglandin E<sub>2</sub>, IL-1 $\beta$ , hypoxia, nuclear factor kappa B (NF- $\kappa$ B), microRNAs (miRNAs) and lack of STAT3-inhibitors, *etc.* (16,18,20,21,22,23). IL-6 exerts its function by binding to its receptor either by membrane bound receptor (mIL-6R), the classical pathway or by soluble receptor (sIL-6R), the trans-signaling pathway. Binding of IL-6 to its receptor cause the activation of gp130 which subsequently activates cytoplasmic tyrosine kinases (Janus Kinase, JAK) *via* its phosphorylation that is responsible for intracellular signaling by phosphorylation of signal transducer and activator of transcription (STAT) factors (specially STAT3 pathway). Phosphorylated STAT3 dimer translocate to the nucleus which leads to the transcription of targeted genes (Bcl-2, Bcl-xL, Cyclin D1, VEGF, *etc.*) and production of other pro-inflammatory cytokines and exerts acute-phase response (16,18,24). These activated genes may code for the proteins involved in cell survival (cyclin D1, surviving and MYC) (18), anti-apoptotic condition (Bcl-x and MYC) (16,25), angiogenesis (VEGF) (16), invasion (MMP) (16), tumor growth and immunosuppressive factors secretion (TGF- $\beta$ , IL-10 and VEGF) (26,27). A systematic diagram showing the physiology of IL-6 has been elucidated in Figure 1. The STAT3 signaling pathway is down-regulated by different way such as Suppressor of cytokine signaling 3 (SOCS3) inhibits the phosphorylation of JAK proteins and protein inhibitor of activated STAT3 (PIAS3) inhibits dimerization of STAT3 monomers.

Besides these key roles IL-6 also plays key role in inflammation, proliferation and differentiation of B and T lymphocytes and natural killer cells (28). Recently published article shows that IL-6 blocks the MHC class II expression of Th1 cells and halt the secretion of IL-2 and INF- $\gamma$  and hence reduced cytotoxic T-lymphocyte activity (29). The hamper activity of T-lymphocyte helps cancer cells to inhibit the immune response.

Several miRNA has also been found to involve in the production of IL-6 in a paracrine manner (30).

### ***IL-6 pathophysiology in glioma***

In various studies the higher level of IL-6 is found to be associated with tumor progression and poor survival rate in several cancers including glioma. In glioma IL-6 affects tumor formation and progression by triggering JAK-STAT3 signaling pathway which may further lead to continuous cell growth (31), tumor development and cell invasion and migration (32,33), angiogenesis (34) and inhibition of apoptosis (35,36). The mRNA expression of IL-6 gene has been found to correlate with higher grade of glioma (glioblastoma) (37), in addition IL-6 gene amplification in tissues samples was 54% (15 out of 36) on glioblastoma and 0% (0 out of 17) in lower grade of glioma (38). The immunohistochemistry (IHC) reveals that IL-6 receptors were totally absent in normal brain tissue and 100% (6 out of 6) in the tissues of glioblastoma samples (39). STAT3 promote tumor growth by inhibiting apoptosis in glioma and increased level of phosphorylated STAT3 is found in recurrent glioblastoma as compared to primary glioma (40).

In this systematic review we have reviewed all the published case-control studies investigating the role of circulatory IL-6 in the development and progression of glioma and its utility as a diagnostic or prognostic biomarker.

## **MATERIAL AND METHODS**

The “Preferred reporting items for systematic reviews and meta-analysis” (PRISMA) guidelines (41) have been adapted to execute this systematic review article.

### ***Literature Search strategies***

An exhausted literature search on March 01, 2021 was done by two research scientists independently using various combination of keywords “Glioma”, “glioblastoma”, “Interleukin-6”, “IL-6”, “Case-control study”, “ELISA”, “enzyme linked immunosorbent assay” “circulatory levels of IL-6” using electronic databases “PubMed”, “ScienceDirect”, “Medline”, “Embase” and “Google Scholar” databases to

find the relevant published studies. The systematic search was limited for articles published in English language. The full articles of relevant studies were obtained. References cited in the relevant studies were also evaluated by research scientists to retrieve additional studies. The researchers thoroughly evaluated full length original articles based on inclusion and exclusion criteria to scrutiny the eligible articles for the inclusion in this systematic review article.

#### *Inclusion criteria*

All the retrieved studies were screened and filtered on the basis of PICOS strategy as followed (i) Participants: histopathological confirmed cases of glioma (ii) Intervention: conditions including progression and invasion of glioma (iii) Comparison: controls free from any malignancy (iv) Observation: IL-6 expression level by ELISA or multiplex assay (v) case-control study. A flow chart (PRISMA) showing the search strategy has been plotted Figure 2.

#### *Exclusion criteria*

The studies were excluded based on following criteria (i) Studies with insufficient information regarding the level of IL-6 (ii) Published review articles, meta-analysis, editorial letters and duplicity of published articles. (iii) published conference proceedings (iv) articles published not in English language.

#### *Data Extraction and study characteristics*

Gathering of information from the relevant articles was carefully done on the basis of inclusion criteria. From each relevant study, the following information was collected and organized in Table 1: the first author's last name, year of publication, ethnicity of the study population, sample size, sample collected (serum or plasma), method of analysis (ELISA), IL-6 expression and glioma outcome (increased or decreased) in comparison to controls.

## **RESULTS**

A total of 953 studies identified in web search using above mentioned keywords and five studies have been included for the full evaluation in this systematic review on the

basis of inclusion criteria (Figure 2). The critically evaluated studies have been summarized in Table 1.

In this review literature one study, Doroudchi *et al*, 2013, comprising 38 cases and 26 controls found a statistically significant ( $P = 0.01$ ) decreased level of IL-6 in the serum of glioma cases ( $2.34 \pm 4.35 \text{ pg/mL}$ ) as compared to controls ( $4.67 \pm 4.35 \text{ pg/mL}$ ) while some other studies have observed a significantly increased level of IL-6 in cases as compared to controls (8,42,43). A study including 55 cases of glioblastoma and 20 healthy controls has found four-fold up-regulation of IL-6 in the cases of glioblastoma as compared to controls (8). On contradictory, Schwartzbaum *et al*, 2017 with the large number of cases of glioma ( $n = 487$ ) and healthy controls ( $n = 487$ ) did not find any significant ( $OR = 0.77$ ; 95 % CI 0.45-1.33) association of case-control correlation in differentially expressed level of IL-6 (44).

Level of IL-6 in glioma patients aged more than 30 years showed a lower value as compared to young patients however the investigators did not find statistically significant correlation ( $P = 0.6$ ) (42).

Comparative level of IL-6 among the different grade of glioma cancer observed a higher level ( $4.02 \pm 7.80 \text{ pg/mL}$ ) with low grade of cancer and lower levels ( $1.74 \pm 1.55 \text{ pg/mL}$ ) with high grade of cancer (42). On contrary, in few studies the serum levels of IL-6 increased with the progression of glioma grading that is higher level in high grade of glioma (43). Univariate analysis indicated that increased level of IL-6 has been found to decline after the surgical removal of the glioma (43), this result indicates that along with immune cells including inflammatory cells, tumor cells can also release the IL-6. Recently published article Zhenjiang *et al*, 2018 has compared the circulating level of IL-6 along with other cytokines between glioblastoma multiforme (GBM) and non-GBM malignant glioma observed detectable concentration of IL-6 in 45-50% of cases along with IL-4 and IL-5 in GBM patients, while 55-60% cases with non-GBM glioma expressed the IL-6 along with IL-4 and IL-5 (45). The investigators also analyzed the combinational effects of selected cytokines (IL-4/ IL-5/ IL-6) on patient's survival found if all present or all absent associated with better survival rate.



## **DISCUSSION**

Many biomarkers have been found to be differentially expressed in cases vs. controls using tissue samples, however, the current need is based and concentrated on circulatory biomarkers, recently trend as a liquid biopsy, to find out the disease diagnosis and progression using samples like blood or urine or saliva, *etc.* Through the published literature evidences found that there is scanty of studies done on human brain cancer and IL-6 association and published results are contradictory, however, in-vivo studies showed a strong relationship of IL-6 with the disease initiation and progression indicates an urgent need to design study to find out how IL-6 can exploit as diagnostic or prognostic markers as well as, as a tailored therapy based on the individual needs.

Glioma is a fatal disease with the reported survival rate of 5% while delivering surgical resection along with radiation and/or chemotherapy. In spite of extensive research, the overall survival is not much improved (46). Several published experimental studies are available with evidences that IL-6 can be produced by tumor cells itself and glioma is characterized by systemic immunosuppression which hinders the response of delivered immunotherapy and helps in tumor progression. The Immunotherapy, now a day, is being the mostly explored area of cancer biology and has been observed increasing survival rate in malignancies, however, for glioma its efficacy is currently being discovered (47). In glioblastoma, programmed death-ligand1 (PD-L1) is the critical mediator of immunosuppression and myeloid cells (non-cancerous cells) in tumor microenvironment and circulation express an elevated level of PD-L1 (48,49). Experimental studies have shown that glioblastoma-derived IL-6 is mandatory and sufficient for the induction of PD-L1, and thus correlation between IL-6 and immunosuppression was recognized in-vitro and in-vivo (50,51).

In this systematic review the overall result was inconclusive, however, we found mostly studies observed an elevated level of IL-6 in serum of glioma patients as compared to controls which may clear the its immunosuppressive role in the development of tumor

(3,43). IL-6, IL-8 and IL-1 $\beta$  are the pro-inflammatory cytokines and their circulatory expression level were found to be up-regulated along with down-regulated level of anti-inflammatory cytokine IL-4 in glioma and higher secretion of pro-inflammatory cytokines was also related with the progression of glioblastoma and poor survival rate (8,52,53). In addition, immunohistochemistry studies observing the expression of IL-6 in glioma cases has been found significantly differed from controls and among the grading of glioma the intensity of IL-6 staining increases with the advancement of grading that is patients with poorly differentiated tumor have a higher level of IL-6 (43). Therefore, by measuring the circulatory levels of IL-6 before and after surgery can be standardized for the prediction of clinical prognosis of the glioma.

The uptake and role of IL-6 in glioma invasion using glioma cell lines has been proven by several experimental studies by using glioma cell lines including (U251 cells, U87 cells T98G cells and A172 cells) incubated with exogenous IL-6 and in this trans-well invasion assay IL-6 helps in the invasion of glioma cells (43). These studies observed IL-6 in the supernatant of the glioma cell lines (43). STAT3 gene is considered as conserved sequence and mutation is rare, therefore, it is believed that its constitutive expression is regulated by upstream regulators and IL-6 is one of them (54). This relationship has been observed by in-vivo study and concluded that STAT3 expression is depend on IL-6 and it increases with tumor (55) and hence, IL-6 have an important role in the development and progression of glioma. Studies in this review literature, found a significant association with the disease progression (43,45) except one study with lower level of IL-6 in high grade of glioma (42).

The exact regulatory network of IL-6 in the tumor microenvironment is very complex; therefore, targeting the underlying mechanism of IL-6 regulation should be undertaken to understand how it's up-regulation or over-active signaling pathways (especially IL-6-JAK/STAT3 signaling pathway) can help in tumor development, progression or recurrence of tumor (56). Tumor formation is not a consequence of single risk factor it may consider a panel of cytokines including chemokines, angiogenesis factors and growth factors therefore, combinational effects of cytokines with proved role in glioma

can be planned to assess their benefits for future tailored immunotherapy and immune-monitoring procedures. In addition, by targeting and reducing the molecules hindering the activity of specific therapy may lead to re-sensitization of delivered therapy. Few clinical trials are going on with this idea (57).

## **CONCLUSION**

This systematic review literature found 05 published research articles investigating the role of IL-6 as a potential biomarker of glioma in case-controls studies. The overall results are inconsistent, however mostly studies found an elevated level of IL-6 in cases of glioma as compared to controls. The level of IL-6 was more than 2-fold in cases and therefore, clears the criterion of acceptance and can be considered as potential biomarker for the diagnosis. On the basis of tumors with progressive growth (advanced grade) it's circulating level also raised and hence can be employing as markers of prognosis and monitoring the glioma. Furthermore, focus on immunotherapy which can produce durable and tumor-specific immune-response can be planned by disrupting IL-6 signaling and re-sensitizing immune-response to halt or reduce the tumor growth and enhance the survival rate based on REMARK (reporting recommendation for tumor biomarker prognostics studies) guidelines (58,59).

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1	doaj.org Internet	24 words — 1%
2	Noomi Vainer, Christian Dehlendorff, Julia S. Johansen. "Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer", Oncotarget, 2018 Crossref	17 words — 1%
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