Abstract: Cytokines are extensively studied small molecules, but their role in childhood CNS neoplasias is still to be elucidated. CNS tumors are of major interest because even if the outcome is favorable, the side-effects stemming from the nature of the disease are devastating. Even after successful treatment, side-effects may influence the quality of life of the patient. This makes the discovery of new prognostic and therapeutic factors more imperative. In the current report, ten cytokines have been chosen to be further studied, which were: EGF, Eotaxin, FGF2, FLT3 ligand, Fractalkine, IL-6, IL-8, MCP-1, TNF-alpha and VEGF.

Key-Words: - Childhood CNS tumors, cytokines, chemokines, prognosis

1 Introduction

Cytokines are signaling molecules that participate in all processes of the organism. They are produced from endothelial cells, fibroblasts, lymphocytes and macrophages. Cytokine and chemokine (a sub-family of the cytokine family) excretion is related to cell proliferation, cell survival and cell death [1, 2]. Chemokines are low molecular weight molecules that participate in similar functions. The sub-family of chemokines is the CXC family or (alpha-family), which contain cysteine molecules at the N-terminus like interleukin 8 (IL-8). These molecules participate in angiogenic and anti-neoplastic actions [3]. A second chemokine sub-family is the CC family (or beta-family). In humans CXC chemokines are coded in chromosome 4 while the CC family are coded in chromosome 17. Recently, two new chemokine molecules have been reported, namely lymphotactin and neurotactin (fraktalkine) [4-6]. In particular, fraktalkine has been reported to participate in attracting immune cells in a tissue-specific manner. Attraction of immune cells takes place in cases of inflammation, a mechanism that constitutes a defence mechanism against intruders or tissue malfunction [5, 6]. Cytokines function through their respective receptors which in turn belong to the family of serpentine proteins, and functionally consist of G-protein-coupled receptors.

2 Problem Formulation

Childhood Central Nervous System (CNS) tumors are the second most frequent type of tumor in childhood and the leading group of disease in children between 1-14 years old. To the best of our knowledge there not many studies have been conducted on the role of cytokines in childhood CNS tumors and therefore it is urgent that they are investigated for their role in disease pathogenesis and prognosis. We have studied 10 chemokines in both Cerebrospinal Fluid (CSF) and serum. These included: EGF, Eotaxin, FGF2, FLT3 ligand, Fractalkine, IL-6, IL-8, MCP-1, TNF-alpha and VEGF.

2.1 Samples and Materials

56 patients with diagnosed CNS tumor have been chosen for the present study and 32 children with hydrocephalus have been included as controls. CSF and serum has been obtained at the time of diagnosis from all patients. Samples used in the present study are summarized in Table 1.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patient Nr.</th>
</tr>
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<tbody>
<tr>
<td>Medulloblastoma</td>
<td>9</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>27</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>4</td>
</tr>
<tr>
<td>Optic Chiasm Gioma</td>
<td>6</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>4</td>
</tr>
<tr>
<td>Other Tumors</td>
<td>6</td>
</tr>
</tbody>
</table>
CSF and serum samples have been stored at -80°C immediately after collection and kept there until further processing.

2.2 Methods
Cytokine levels in serum and CSF have been measured with the Luminex xMap system. Luminex is a Microsphere-based suspension array technology using polysterene spheres of 5.6μm diameter.

2.3 Analysis and Statistical Methods
Cytokine levels in serum and CSF have been calculated as the mean of similar tumor types i.e. medulloblastomas, glioblastomas, ependymomas etc. The mean expression values and the log2 ratio of CSF/Serum levels have been calculated. Further classification methods have been used in order to find other patterns of expression, such as hierarchical clustering with Euclidian distance and k-means clustering.

3 Results
In examining blood cell distribution, it appeared that all tumors manifested a reverse pattern as compared to control samples. That is lymphocytes appeared to be in higher numbers in control samples than in tumor samples. This is presented diagrammatically in Fig. 1.

The expression levels of each cytokine have been presented individually. EGF showed higher levels in serum than in CSF as compared to control samples, with the exception of choroid plexus carcinoma (Fig. 2). Similarly, Eotaxin manifested the same profile as EGF, as presented in Fig. 3.
FGF-2, demonstrated a very diverse pattern of expression, as presented in Fig. 4.

Fig. 4. Expression levels of FGF2 in serum and CSF as compared to control samples along with the expression ratio.

The FLT3 ligand, whilst also manifesting a diverse pattern of expression, there were no significant differences between tumor and control samples. The result is presented in Fig. 5. Fraktalkine on the other hand manifested significant difference, as compared to control, in xanthoastrocytoma samples, while glioma tumors showed a reversal in the profile expression of CSF over serum levels. The result is presented in Fig. 6.

Xanthoastrocytoma showed a very interesting pattern for IL6 and IL8, where it appeared that it is highly expressed in CSF as compared both to serum and control samples. The result is presented in Fig. 7 for IL6 and Fig. 8 for IL8 respectively.

However, the ratio of CSF over serum for those cytokines appeared to be very diverse as presented in Fig. 7 and Fig. 8. MCP1, on the other hand, was up-regulated in CSF in all samples and it was significantly higher in glioblastoma and xanthoastrocytoma samples as compared to control samples. The results are presented in Fig. 9. TNFA, a very well-studied factor, manifested higher levels in serum than in CSF as compared to control samples, with a notable difference in glioblastoma, rhabdoid tumors and xanthoastrocytoma. The result is presented in Fig. 10.
Finally, VEGFA did not manifest any significant expression differences between tumor and control samples. Also, the CSF over serum ratio presented with a very diverse profile of expression, indicating that this cytokine does not participate in CNS tumor signaling. The result is presented in Fig. 11.

**Fig. 7.** Expression levels of IL6 in serum and CSF as compared to control samples along with the expression ratio.

**Fig. 8.** Expression levels of IL8 in serum and CSF as compared to control samples along with the expression ratio.

**Fig. 9.** Expression levels of MCP1 in serum and CSF as compared to control samples along with the expression ratio.

**Fig. 10.** Expression levels of TNFA in serum and CSF as compared to control samples along with the expression ratio.
Fig. 11. Expression levels of VEGFA in serum and CSF as compared to control samples along with the expression ratio.

Finally, in an attempt to find expression patterns in cytokine expression we have used hierarchical clustering in order to classify the data. The results are presented in Fig. 12. Red coloring represents cytokine overexpression and blue coloring represents cytokine down-regulation, with respect to control samples.

Hierarchical Clustering with Euclidean Distance of All CNS Tumor Samples

4 Conclusion

EGF is one of the best-studied cytokines and its role in neoplasias has been reported [7]. EGF has been reported to be a therapeutic marker where inhibition of its receptor is a possible anti-neoplastic treatment [8]. It has also been reported to play a role in meningiomas [9], gliomas [10], glioblastomas [11], ependymomas [12] and others.

Eotaxin manifested similar expression patterns in oligodendroglioma as in the case of EGF. While there have been reports for the role of Eotaxin in lymphomas [13] and multiple myeloma, there are no reports on the role of Eotaxin in CNS tumors. To the best of our knowledge it is the first time that the role of Eotaxin is investigated in childhood CNS tumors.

FGF2 has been shown to participate in glioma proliferation and survival [14]. It has also been reported that FGF2 activation participates in neuroblastoma anti-sensitization to therapy, meaning that it acts as an anti-neoplastic factor. On the contrary its silencing leads to glioblastoma sensitization to chemotherapy [15].

For Fraktalkine it has been reported that it is excreted from glioblastoma and cancer stem cells [16]. Its natural function is to support the communication in neural cells, whilst also known to participate in tumor progression and aggression [17, 18]. Those reports agree with our findings where Fraktalkine is up-regulated in certain tumor types.

Finally, MCP1 has been reported to participate in meningiomas through the NFκB factor [19]. MCP1 is also known to be a therapeutic target in synergy with IL12 [20].

It appears that cytokines are important molecules in CNS tumor biology and they should be the subject of future investigations.

References:


