Cytokine Gene Polymorphisms in Egyptian Cases with Brain Tumors

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Cytokines are proposed to play important roles in brain tumor biology as well as neurodegeneration or impaired neuronal function. To evaluate the association of polymorphisms of cytokine genes with brain tumors in Egyptian patients. This study included 45 cases affected by brain tumors. Their median age was 45, diagnosed as 24 benign cases and 21 malignant cases, and their sex included 20 males and 25 females. They were taken from the cases presenting to the Neurosurgery Department of Mansoura University Hospital, Egypt. Cases genotypes were compared to 98 healthy unrelated controls from the same locality. DNA was amplified using PCR utilizing sequence specific primers (SSP) for detection of polymorphisms related to TNF-α M308 (G/A), IL-10 M1082 (G/A), IL-6 M174 (G/C) and IL-1Ra (VNTR). Cases affected with benign brain tumors, showed a significant higher frequency of IL-10 M1082 A/A genotype (OR=8.04, \(p < 0.001\)), IL-6 M174 C/C genotype (OR=6.3, \(p < 0.001\)) and TNF-α M308 A/A (OR=4.7, \(p < 0.05\)) with a significant lower frequency of IL-10 M1082 G/A genotype (OR=0.1, \(p < 0.001\)), IL-6 M174 G/C (OR=0.2, \(p < 0.001\)) and TNF-α M308 G/A was found significantly low among the same groups (OR=0.2, \(p < 0.001\)) compared to controls. On the other hand these cases have shown no significant difference regarding the distribution of IL-1Ra VNTR genotype and allele polymorphism compared to controls. Although the levels of different studied cytokines were not determined simultaneously in the serum, these cases are expected to have lower levels of IL-10 and high levels of TNF-α being homozygous for IL-10 M1082 allele A (low production allele) and TNF-α M308 A (high production allele). The frequency of cytokines genotype and allele in malignant brain cases and controls. Malignant cases, on the other hand, showed significant higher frequency of IL-10 M1082 A/A genotype (OR=9.4, \(p < 0.001\)) and both TNF-α M308 A/A (OR=4.9, \(p < 0.001\)) and G/G (OR=4.7, \(p < 0.05\)) genotypes as compared to controls. In the mean time these cases have shown a significant lower frequency of genotypes of IL-6 M174 C/C (OR=4.8, \(p < 0.05\)) and both TNF-α M308 A/A (OR=4.9, \(p < 0.001\)) and G/G (OR=4.7, \(p < 0.05\)) genotypes as compared to controls. Comparing studied genotype frequencies among benign and malignant brain tumor cases no significant difference was found in the frequencies of all studied genotypes and alleles with a non significant trend for the benign cases to have higher frequency of IL10 M1082 AA genotype. In conclusion, cytokine gene polymorphisms have a certain pattern among brain tumor cases and can be considered a genetic marker of potential value in counseling and management.

Brain tumors occur particularly in the young so that middle aged incidence of brain tumors is on the increase. Actually, there are two age peaks of incidence, during the first decade and the second during the 5th and 6th decade of life. (Wrench et al., 2002) Both incidence and mortality of brain tumors have been rising particularly in elderly population. Most of adult primary brain tumors are gliomas of which astrocytomas are the most common. These tumors are progressive and tend to recur following treatment with a fatal outcome. (Hoffman et al., 2006, Boyle et al., 1990)

Multiple genetic changes must occur for a tumor to form. Among these factors, cytokines are proposed to play important roles in brain tumor biology. (Iyin et al., 2004) Moreover, there is now evidence that IL-1, TNF-α, several chemokines and interferon-γ may contribute directly to neurodegeneration or impaired neuronal function. (Moynagh, 2005).

Within the central nervous system, IL-6 can be expressed by astrocytes, microglial cells and folliculostellate cells of the pituitary...
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(Mohamed-Ali et al., 1997). Human cerebrospinal fluid also contains IL-6 as well. (Jellinger et al., 2001). The aggressiveness of human gliomas appears to be correlated with the up regulation of (IL-6) gene. It was found that the amplification of IL-6 gene may be a common feature in glioblastomas and may contribute to the IL-6 over-expression in these cases (Tchirkov et al., 2001).

Tumor necrosis factor-alpha (TNF-alpha) is a highly multifunctional cytokine involved in immune and inflammatory responses and affecting angiogenesis and tumor growth. (Szlosarek & Balkwill, 2003). In multiple sclerosis patients, TNF-α is overproduced in the serum and cerebrospinal fluid (Sharief & Hentges, 1991). Tumor necrosis factor (TNF) was initially described as the haemorrhagic necrosis factor produced by lipopolysaccharide (LPS) stimulated tumors. (Liz Grana et al., 2001). Macrophages alter brain tumor development through a TNF-dependent process that culminates in the formation of microcysts. (Villeneuve et al., 2005). In addition, synergistic IL1 beta with TNF-alpha interactions can be involved in the growth of pilocytic astrocytoma. (Powrie et al., 1997).

On the other hand, IL-10 is able to inhibit the production of a variety of cytokines, such as IL-1, TNF-α and IL-12, which have been reported as activating cytokines for T and NK cells (Moore et al., 2001; Sargen et al., 2000). Taking into consideration that cytokine gene polymorphisms are population specific, we were interested to test for the association of these polymorphisms with brain tumor. In a case control study, we attempted testing the association of diagnosis and prognosis of disease in subjects with polymorphisms of 2 pro-inflammatory cytokines (TNFα at position -308 and IL-6 at position -174) and 2 anti-inflammatory cytokines (IL-10 genes at position -1082 and of IL-1Ra VNTR).

**Subjects and Methods**

This work has included cases affected by brain tumors. Their median age was 45 years and their sex included 20 males and 25 females. These cases were taken randomly from those who were presenting to the Neurosurgery Department of Mansoura University Hospital, the main referral hospital of the Nile Delta region of Egypt. They were diagnosed as brain tumors cases by computerized imaging (C.T. to brain or MRI).

Cases genotypes results from PCR amplification were compared to 98 healthy unrelated adult volunteers with negative family history of the disease from the same locality. These included 52 males and 46 females and their mean age was 44.9 ± 6.7 years.

**DNA Extraction and Purification**

After obtaining an informed consent from all cases and controls, venous blood samples (3 ml) were collected in tube containing EDTA (ethylene diamine tetra acetate). DNA was prepared promptly using a DNA extraction and purification kit (Gentra Systems. Include DNA Purification Solution and a DNA Elution Solution, USA) according to manufacturer's instructions and then stored at -20 C until use.

**PCR Amplification**

Four single nucleotide polymorphisms (SNPs) were analyzed including promoter sites TNF-α -308 (G/A), IL-10 -1082 (G/A) and IL-6 -174 (G/C) as well as IL-1Ra VNTR as previously described (Cavet et al., 1999; Cavet et al., 2001; Wilkinson et al., 1999; Krieger, 1997). For TNF-α, IL-6 and IL-10 SNPs identification, PCR with sequence-specific primers (PCR-SSP) in two reactions employing a common forward and 2 reverse primers was used, and for IL-1Ra VNTR polymorphism, a single PCR reaction employing a forward and a reverse primers was used. All primers, Taq polymerase, dNTP, and MgCl2 were purchased from QiaGene. (USA). PCR reaction were performed using a Techne-Genius thermal-cycler (Techne Ltd, Cambridge, UK). Briefly, 100-500 ng of genomic DNA was added to 25 μl of reaction mixture containing 1 μM of each common/specific primer, 200 μM of each dNTP, and 1 U of Taq DNA polymerase. We were have master mixes for multiple cases and also for different polymorphisms at the same sitting with confirmation of the negative amplification to obtain accurate subject genotyping.

**Detection of Amplified Products**

The entire reaction volume (48 μl) plus 5 μl of bromophenol blue track dye were loaded into 2% agarose gel (Boehringer Mannheim) containing ethidium bromide (5mg/ml). Gels were electrophoresed for 20 minutes at 200 V, photographed under UV light.
(320 nm) and then scored for the presence or absence of an allele specific band. Figure (1) shows the amplified PCR products of IL-6\textsuperscript{F174} (G/C), IL-10\textsuperscript{F1082} (G/A) and TNF\textsuperscript{F308} (G/A) compared to size marker whereas figure (2) shows amplified alleles of IL-1Ra VNTR region in intron 2 of the gene.

**Statistical Analysis**

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 10.0). The frequency of studied allelic polymorphisms among cases and comparing to controls describing number and percent of each and tested for positive association using Fisher’s exact test and Odds ratio with a minimum level of significance of $<$0.05.

**Results**

The overall distribution of cytokines genotype and allele studied in benign and malignant brain tumor cases and controls are shown in Table 1. IL-10\textsuperscript{F1082} A/A genotype, IL-6\textsuperscript{F174} C/C genotype and TNF-\textalpha\textsuperscript{F308} A/A were more likely to be detected in nonmalignant and malignant cases than in controls (Table 1). While the frequency of IL-10\textsuperscript{F1082} G/A genotype, IL-6 \textsuperscript{F174} G/C and TNF-\textalpha\textsuperscript{F308} G/A was found significantly lower among both groups than in controls (Table 1). On the other hand, patient groups were not significantly different
regarding the distribution of IL-1Ra VNTR genotype and allele polymorphism when compared to controls. Malignant cases, on the other hand, showed significant lower frequency of IL-6-174 C/C genotype (OR=4.8, \(P<0.05\)) and higher TNF-\(\alpha\)-308 A/A (OR=4.9, \(P<0.001\)) and lower frequency G/G (OR=4.7, \(P<0.05\)) genotypes as compared to controls.

Table 1. Frequency of genotypes and alleles related to IL-10-1082 (G/A), IL6-174 (G/C), TNF\(\alpha\)-308(G/A), IL-Ra (VNTR) gene polymorphisms among benign and malignant brain tumor cases.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Malignant</th>
<th>Benign</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 21 (%)</td>
<td>OR(95% CI)</td>
<td>n = 24 (%)</td>
</tr>
<tr>
<td><strong>IL10-1082</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>1 (4.8%)</td>
<td>0.9 (0.1-8.4)</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>G/A</td>
<td>16 (76.2%)</td>
<td>0.5 (0.1-1.6)</td>
<td>12 (50.0%)**</td>
</tr>
<tr>
<td>A/A</td>
<td>4 (19.0%)</td>
<td>2.6 (0.7-9.8)</td>
<td>10 (41.7%)**</td>
</tr>
<tr>
<td>alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>18 (8%)</td>
<td>0.8 (0.4-1.6)</td>
<td>16 (33.3%)</td>
</tr>
<tr>
<td>A</td>
<td>24 (12%)</td>
<td>1.2 (0.6-2.4)</td>
<td>32 (66.7%)</td>
</tr>
<tr>
<td><strong>IL6-174</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>3 (14.3%)</td>
<td>3.1 (0.7-14.1)</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td>G/C</td>
<td>13 (61.9%)**</td>
<td>0.2 (0.1-0.6)</td>
<td>14 (58.3%)**</td>
</tr>
<tr>
<td>C/C</td>
<td>5 (23.8%)*</td>
<td>4.8 (1.3-17.59)</td>
<td>7 (29.2%)**</td>
</tr>
<tr>
<td>alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>19 (45.2%)</td>
<td>0.8 (0.4-1.6)</td>
<td>20 (41.7%)</td>
</tr>
<tr>
<td>C</td>
<td>23 (54.8%)</td>
<td>1.2 (0.6-2.3)</td>
<td>28 (58.3%)</td>
</tr>
<tr>
<td><strong>TNF(\alpha)-308</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>5 (23.8%)*</td>
<td>4.7 (1.3-17.6)</td>
<td>4 (16.7%)</td>
</tr>
<tr>
<td>G/A</td>
<td>8 (38.1%)**</td>
<td>0.1 (0.04-0.3)</td>
<td>11 (45.8%)*</td>
</tr>
<tr>
<td>A/A</td>
<td>8 (38.1%)**</td>
<td>4.9 (1.6-14.3)</td>
<td>9 (37.5%)*</td>
</tr>
<tr>
<td>alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>18 (42.9%)</td>
<td>0.8 (0.4-1.6)</td>
<td>19 (39.6%)</td>
</tr>
<tr>
<td>A</td>
<td>24 (57.1%)</td>
<td>1.2 (0.6-2.3)</td>
<td>29 (60.4%)</td>
</tr>
<tr>
<td><strong>IL-1Ra</strong></td>
<td>n =15 (%)</td>
<td>n = 21 (%)</td>
<td>n = 97 (%)</td>
</tr>
<tr>
<td>genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1/A1</td>
<td>11 (73.3%)</td>
<td>1.9 (0.6-6.5)</td>
<td>13 (61.9%)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>4 (26.7%)</td>
<td>0.5 (0.1-1.7)</td>
<td>8 (38.1%)</td>
</tr>
<tr>
<td>alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>26 (86.7%)</td>
<td>1.7 (0.5-5.1)</td>
<td>34 (81.0%)</td>
</tr>
<tr>
<td>A2</td>
<td>4 (13.3%)</td>
<td>0.6 (0.2-1.8)</td>
<td>8 (19.0%)</td>
</tr>
</tbody>
</table>

\(P< 0.05\) is significant. *\(P = \leq 0.05\) ** \(P = \leq 0.001\).
In the meantime these cases have shown a significant lower frequency of genotypes of IL-6$^{174}$ G/C (OR=0.2, $P<0.05$) and higher frequency of genotypes of TNF-$\alpha^{308}$ G/A (OR=0.1, $P<0.001$) compared to controls.

On the other hand, these cases have not shown any statistical significant difference of polymorphic genotypes or alleles related to IL-10$^{1082}$ (G/A) and IL-Ra VNTR genes compared to controls.

Comparing the frequency of the studied genotype among benign and malignant brain tumor cases, no significant difference was found in the frequencies of all studied genotypes and alleles with a non significant trend for the benign cases to have higher frequency of IL10$^{1082}$ AA genotype.

Discussion

When the causes of the inflammatory reaction are of a high intensity, the production of cytokines is increased and released in the circulation provoking the "acute phase response". Increased release of key pro-inflammatory factors and some cytokines, such as IL-1$\beta$, TNF-$\alpha$ and interferon (IFN)-$\gamma$, implicated in both the regulation of inflammation and the development of cancer (Kilpinen et al., 2001).

Interleukin-10 (IL-10) is a regulatory cytokine, whose main role in vivo is to limit inflammatory response (Hohaus et al., 2007). IL-10 is an immuno-regulatory cytokine that plays a crucial role in inflammatory and immune reactions. It has potent anti-inflammatory and immuno-suppressive activity on myeloid cell functions which forms a solid basis for its use in acute and chronic inflammatory diseases (Federico et al., 2005).

IL-10 and its polymorphisms had reported to play a role for both the susceptibility and prognosis of various diseases (Eskdale et al., 1998; Helminen et al., 2001). On the other hand, "inhibitory" cytokines such as IL-10 damp down the activation of some effectors functions of T lymphocytes and mononuclear phagocytes, by inhibiting the release of pro-inflammatory cytokines and therefore turning off the inflammatory processes (Caruso et al., 2000; Hussain et al., 2003). Some authors have reported increased IL-10 production in vitro associated with the presence of the -1082 G allele (Eskdale et al., 1998; Kurreeman et al., 2004). Other investigators reported that the -1082G allele is associated with decreased IL-10 production (Eskdale et al., 1998, Gibson et al., 2001). Cancer susceptibility and severity may also be associated with functional polymorphisms of cytokine genes involved in regulation of inflammation (Caruso et al., 2000; Hussain et al., 2003).

In fact, inflammatory cells and cytokines found in tumors can contribute to tumor growth, progression, and immuno-suppression as well as mounting an effective host anti-tumor response. However, in most cases inflammation plays a role in the development of solid cancer (Federico et al., 2005). Cytokine polymorphisms, especially those involving IL-6 and IL-10 genes, may influence susceptibility to, and in some cases prognosis in neoplastic diseases. It is intriguing that these two cytokines are involved in longevity (Caruso et al., 2000; Parkin et al., 2000). Wang et al., 2001 attributed that to pleiotropic action of cytokines. In addition to the IL-10 genotype, the IL-6 polymorphic site at position -174 had a prognostic role. Hohaus et al., 2007

In the current study, analysis of IL-10$^{1082}$ (G/A) polymorphism between benign cases and controls showed a significant increased frequency of the homozygous form, AA, with a simultaneous high significant decrease of the heterozygous form, GA. On the other hand, analysis of IL6 G/C polymorphism between benign cases and controls showed that homozygous form, CC genotype had a
significant higher frequency whereas IL6 GC heterozygous form had a significant lower frequency.

Macrophages and some of their secreted products, especially tumor necrosis factor (TNF), act as tumor promoter, inhibition to these inflammatory components are currently regarded as potential therapeutic tools to block tumor progression (Villeneuve et al., 2005). Infiltrating macrophages represented a significant population of non-neoplastic cells within malignant gliomas, in which they were the exclusive producers of TNF. Macrophages alter brain tumor development through a TNF-dependent process that culminates in the formation of microcysts (Villeneuve et al., 2005). Macrophages had been long recognized as a critical component of innate immunity against tumors. When appropriately stimulated, they can attack neoplastic cells through contact-dependent interactions as well as the secretion of cytotoxic and cytostatic humoral factors (Burke & Lewis 2002). Nonetheless, malignant cells can escape immune surveillance through mechanisms that are not fully understood, leading to tumor growth and metastasis. The fact that tumorigenesis occurs despite the presence of a large number of tumor-associated macrophages has led to the speculation that macrophages contribute to tumor development, presumably by secreting growth signals, angiogenic factors, matrix-degrading proteinases, and immuno-suppressors (Balkwill & Mantovani 2001; Badie & Schartner 2001; Coussens & Werb 2002; Graeber et al., 2002; Bingle et al., 2002) This possibility has gained substantial support from the observation that colony stimulating factor 1, a key determinant of the monocytic lineage, promotes tumor progression by controlling the recruitment and function of tumor-associated macrophages (Nowicki et al., 1996; Lin et al., 2001; Aharinejad et al., 2004; Pollard, 2004) Yet, the precise mechanisms by which macrophages can facilitate tumor progression remain to become shown clearly. The pro-inflammatory cytokine tumor necrosis factor (TNF) may be one of the ways through which macrophages exert dual functions in tumor biology. TNF promotes tumor invasion and growth in many tissues. Thus far, the evidence suggests that anti-TNF therapy might be useful for reducing the growth of glioblastomas, the most frequent and malignant tumors affecting the central nervous system. Glial cells tumors are characterized by their rapid growth and ability to infiltrate diffusely in the parenchyma.

A similarity between glioblastomas and other types of solid tumors can be found in the abundance of tumor-associated macrophages, which represent up to one third of cells found in glioma biopsies (Morimura et al., 1990; Roggendorf et al., 1996). TNFα is produced in gliomas by different cell types, including macrophages Roessler et al., 1995). Functional studies showed that TNF stimulates the expression of proangiogenic factors by glioma cells (Ryuto et al., 1996; Nabors et al., 2003).

Cases affected with benign brain tumors in the current study, showed a significant higher frequency of IL-10-1082 A/A genotype, IL-6-174 C/C genotype and TNF-α-308 A/A with a significant lower frequency of IL-10-1082 G/A genotype, IL-6-174 G/C. In addition, TNF-α-308 G/A was found significantly low among the same groups as compared to controls. On the other hand, these cases have shown no significant difference regarding the distribution of IL-1Ra VNTR genotype and allele polymorphism compared to controls. On the other hand, cases affected with malignant brain tumors in the current study, showed a significant higher frequency of IL-6-174 C/C genotype, TNF-α-308 A/A and G/G genotypes when compared with the control cases. In the mean time these cases have shown a significant lower frequency of genotypes of IL-6-174 G/C and TNF-α-308 G/A as compared to controls. On the other hand,
these cases have not shown any statistical significant difference of polymorphic genotypes or alleles related to IL-10 \(^{-1082}\) (G/A) and IL-Ra VNTR genes as compared to controls.

Benign and malignant brain tumor cases have shown an interesting pattern in term of their cytokine polymorphic genotypes among the studied Egyptian cases. These genotypes may be considered risk genotypes and can be utilized as important genetic markers for family counseling and for therapeutic trials as well.

It can be concluded from this study that, benign and malignant brain tumor cases have shown an interesting pattern among studied Egyptian cases in term of their cytokine polymorphic genotypes. These genotypes may be considered risk genotypes and can be utilized as important genetic markers of potential value for family counseling and for therapeutic trials as well.

Reference


