Serum Tumor Necrosis Factor Alpha Concentration in Children with Guillain Barré Syndrome: Correlation with Clinical Disability and IVIG Therapy

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ABSTRACT

Background: Guillain-Barré syndrome (GBS) is an acute autoimmune post-infectious demyelinating polyradiculoneuropathy. Activated T lymphocytes and macrophages are the principle source of cytokines, including TNF-α, a primary mediator of inflammation. There is evidence that TNF-α is capable of inducing selective and specific damage to myelin in vitro. Objectives: To assess the role of TNF-α in the pathogenesis of GBS and correlate its serum level with clinical disability and IVIg therapy.

Subjects and Methods: Twenty patients presented by acute GBS with established clinical criteria for diagnosing GBS were recruited. Twenty age and sex matched healthy control subjects were also studied. All patients were submitted to thorough history taking, including demographic data and antecedent events, neurological examination and neurophysiological tests were performed. IVIg was given to all patients within the first week after admission. The patients were followed up for 6 months. The outcome of all patients was determined.

Results: Serum TNF-α concentration in the 20 GBS patients with elevated levels showed a steady decline from (21.43 to 14.18 pg/ml) following treatment with IVIg. At the time of discharge from the hospital, there was a positive correlation between neurological disability and level of TNF-α concentration in these 20 GBS patients.

Conclusion: The results of this study indicate that elevated serum level of TNF-α occur in a proportion of patients with GBS and in these patients elevated serum TNF-α level declines with IVIg therapy.


Key Words: Tumor necrosis factor alpha, Guillain Barré syndrome, Children, IVIg.

INTRODUCTION

Guillain Barré syndrome (GBS) is an acute autoimmune post-infectious demyelinating polyradiculoneuropathy. GBS is the most frequent acquired neuropathy. Detailed immunopathologic features have been described in GBS. Most current investigations are centered on the hypothesis of molecular mimicry in GBS together with the pathogenic role of cell-mediated immunity. Different antibodies have been discovered in GBS which interfere with nerve impulse conduction on neuromuscular transmission. Activated macrophages and T-cells with the participation of T-1 helper cell related cytokines seem to play a fundamental role in demyelination. The nature of antigen presenting cells, T-cell receptors, adhesion molecules and the proinflammatory cytokines need to be explored to design more specific immunotherapies. Asbury et al. demonstrated lymphocytic infiltration in the peripheral nerves, especially during the active phase of the disease. Immunohistochemical methods revealed that many of these lymphocytes are T cells. Autoimmune damage to peripheral nerves, mediated by activated T lymphocytes and macrophages, underlies the pathogenesis of inflammatory demyelination in GBS. Both T lymphocytes and macrophages secrete tumor necrosis factor-alpha (TNF-α), a cytokine that exerts toxic effects on myelin, Schwann cells, and endothelial cells. The level of circulating TNF-α is also elevated in patients with GBS and is implicated in the pathogenesis of the disease. The reported high serum level of this cytokine in patients with GBS may reflect the degree of immune activation rather than a direct pathogenic effect. Activated T lymphocytes and macrophages are the principle source of cytokines, including TNF-α; a primary mediator of inflammation. There is evidence that TNF-α is capable of inducing selective and specific damage to myelin in vitro.
Intravenous immunoglobulin (IVIg) has emerged as a therapeutic agent in the management of several immunologically mediated demyelinating disorders of the peripheral nervous system, including GBS. Though the precise role of IVIg is undetermined, several immune-modulatory mechanisms of action of IVIg have been described. Understanding the mechanism of action of IVIg will not only promote the judicious use of IVIg but also will help in monitoring its dosage in the management of autoimmune demyelinating disorders of the central nervous system.

The objectives of this study were (a) To assess the role of TNF-α in the pathogenesis of GBS and (b) To correlate TNF-α serum level in GBS patients with clinical disability and efficacy of IVIg.

**SUBJECTS AND METHODS**

This study was conducted between January 2008 and January 2009. After obtaining informed written consent, patients were admitted to the ward or to pediatric ICU (PICU) according to their clinical presentation. Age ranged from 3 to 15 years. Diagnostic criteria for GBS were based on the published international research diagnostic criteria of Ashury and Clornblath.

Patients group (Group 1): formed of twenty patients presented by acute GBS. Patients with non confirmed diagnosis were excluded. Patients with other causes of polyneuropathy such as diabetes mellitus, chronic renal failure, toxic or hereditary polyneuropathy were also excluded.

All patients were submitted to thorough history taking, including demographic data, antecedent events and detailed neurological examination.

Nerve conduction studies (NCS) were done using Medtronic machine in the acute phase of the disease within one week of the onset. Motor nerve conduction studies were conducted on median and ulnar nerves in upper limbs, common peroneal and posterior tibial in lower limbs. Sensory nerve conduction studies were conducted on median and ulnar nerves in upper limbs and sural nerve in lower limbs. F-wave response and latency were studied together with tibial H-reflex and latency. All results were compared to age matched normal values. Electromyography (EMG) was also done for all patients. Lumbar puncture for CSF examination was done in the first week to search for cytoalbuminous dissociation. All these tests were done to establish the diagnostic criteria.

IVIg was given to all patients within the first week after admission (0.4 g/kg/day for five consecutive days). Follow up was done for all patients at discharge. The outcome of all patients was determined using the ordinary disability scale.

Control group (Group 2): formed of twenty healthy subjects, age and sex matched with patients group (brothers, sisters or relatives of the patients).

**TNF-α estimation:**

TNF-α estimation in the sera of patients with GBS was carried out using enzyme linked immunosorbant assay (ELISA) kits (Sigma Chemicals, St. Louis, USA).

At the time of admission, venous blood samples (6-8 ml) were extracted from GBS patients and control groups then collected in sterile glass tubes and were allowed to clot spontaneously over one hour. All the sera samples were collected and frozen at -80 °C in aliquots until the time of the assay.

**Statistical Analysis**

Data were collected and analyzed using SPSS software package version 11. Descriptive data were presented as mean±standard deviation. Chi-square test, t-test and analysis of variance [ANOVA] test were used. Statistical significance was defined as P < 0.05.

**RESULTS**

The study included 20 patients with clinically diagnosed GBS according to diagnostic criteria. 12 males and 8 females. Age ranged from 3 to 15 years. Antecedent events either respiratory infection or diarrhea one month before neurological manifestations were reported among 17 (85%) of our patients (Table 1). Most of our patients 95% at presentation were at disability score between 2 and 4 using ordinary disability scale (Table 2). 60% of them had evidence of demyelinating neuropathy in nerve conduction studies, 25% showed axonal neuropathy and 15% were inexcitable (Table 3).

Using ordinary disability scale at discharge, 55% of the patients were able to walk 5 meters without aid and 35% with aids. 5% were able to run and 5% were unable to walk and unable to lift the legs (Table 2). Table 4 showed that the mean TNF-α level was higher among patient group than among control group with statistically significant difference. After IVIg therapy, serum TNF-α was statistically significantly reduced in GBS group compared to their pretreatment levels (Table 5). Comparison was done between serum TNF-α in both demyelinating and axonal groups at presentation and discharge (Tables 6 and 7) and no significant
difference was found. Table 8 showed no significant relationship between serum TNF-α level and the presence of antecedent events. A positive correlation was found between mean serum TNF-α level and clinical disability among GBS patients at presentation and at discharge (Table 9).

Table 1. Antecedent events among patients with GBS (Guillain-Barré syndrome).

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with antecedent events</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>Patients with no antecedent events</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2. Degree of disability in patients with GBS (Guillain-Barré syndrome) at presentation and at discharge using ordinary disability scale.

<table>
<thead>
<tr>
<th>Degree of disability</th>
<th>At presentation</th>
<th>At discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>0 Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Able to run</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 Able to walk 5 m unaided</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 Able to walk with aids</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>4 Unable to walk, able to lift legs</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>5 Unable to walk, not able to lift legs</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>6 Intubated, with artificial ventilation</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

$X^2$ = 3.256
P. value < 0.050*

* Significant at p<0.05

Table 3. Neurophysiological results among GBS (Guillain-Barré syndrome) patients.

<table>
<thead>
<tr>
<th>NCS</th>
<th>Demyelinating</th>
<th>Axonal</th>
<th>Inexcitable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>NCS</td>
<td>12</td>
<td>60</td>
<td>5</td>
</tr>
</tbody>
</table>

$X^2$ = 4.253
P. value = 0.042*

NCS nerve conduction studies
* Significant at p<0.05.

Table 4. Comparison between serum TNF-α in GBS (Guillain-Barré syndrome) group and control group at the time of admission.

<table>
<thead>
<tr>
<th>Serum TNF-α (pg/ml)</th>
<th>Mean±SD</th>
<th>T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS (n = 20)</td>
<td>21.43±4.76</td>
<td>7.89</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>11.01±3.78</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at p<0.05.
Table 5. Comparison between serum TNF-α (Tumor necrosis alpha) before and after treatment with IVIg in patients with Guillain-Barré syndrome

<table>
<thead>
<tr>
<th>Serum TNF-α</th>
<th>Mean±SD</th>
<th>T-Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before IVIg treatment (n = 20)</td>
<td>21.43±4.76</td>
<td>6.798</td>
<td>0.001*</td>
</tr>
<tr>
<td>After IVIg treatment (n = 20)</td>
<td>14.18±3.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IVIg intravenous immunoglobulin, SD standard deviation
* Significant at p<0.05.

Table 6. Comparison of serum TNF-α (Tumor necrosis alpha) among demyelinating and axonal groups of patients with Guillain-Barré syndrome at presentation.

<table>
<thead>
<tr>
<th>Serum TNF-α</th>
<th>Mean±SD</th>
<th>T-Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demyelinating (n = 12)</td>
<td>21.3±5.37</td>
<td>0.217</td>
<td>0.831</td>
</tr>
<tr>
<td>Axonal (n = 5)</td>
<td>21.85±4.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD standard deviation

Table 7. Comparison of serum TNF-α (Tumor necrosis alpha) among demyelinating and axonal groups of patients with Guillain-Barré syndrome at discharge.

<table>
<thead>
<tr>
<th>Serum TNF-α</th>
<th>Mean±SD</th>
<th>T-Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demyelinating (n = 12)</td>
<td>13.78±3.08</td>
<td>0.239</td>
<td>0.814</td>
</tr>
<tr>
<td>Axonal (n = 5)</td>
<td>14.16±3.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD standard deviation

Table 8. Relationship between serum TNF-α (Tumor necrosis alpha) and antecedent events in patients with Guillain-Barré syndrome.

<table>
<thead>
<tr>
<th>Serum TNF-α</th>
<th>Mean±SD</th>
<th>T-Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antecedent events (n = 17)</td>
<td>21.58±4.55</td>
<td>0.345</td>
<td>0.734</td>
</tr>
<tr>
<td>No antecedent events (n = 5)</td>
<td>20.53±6.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD standard deviation

Table 9. Correlation between mean serum TNF-α (Tumor necrosis alpha) level and clinical disability among GBS (Guillain-Barré syndrome) patients at admission and discharge.

<table>
<thead>
<tr>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At admission</td>
<td>0.564</td>
</tr>
<tr>
<td>At discharge</td>
<td>0.856</td>
</tr>
</tbody>
</table>

*Significant at p<0.05.

**DISCUSSION**

GBS is the most common cause of rapidly evolving flaccid weakness in children.14

TNF-α is a cytokine derived from T cells and macrophages together with other products of activated T cells (such as serum interleukin-2), all are raised in GBS.15,16 Ultrastructural studies support this hypothesis, as macrophages are closely opposed to demyelinated axons in necropsy material from patients with GBS. TNF-α is a multifunctional, cytotoxic polypeptide that can induce myelin damage and necrosis of oligodendrocytes in vitro.16 Our study demonstrated that elevated serum level of TNF-α occurs in patients with GBS. These results were in accordance with three previously published
studies. Zhang et al. also reported high serum level of TNF-α in patients with GBS. These studies highlighted that the elevated serum level of TNF-α was correlated with the disease severity and that the elevated level of TNF-α returns to normal parallel with the clinical recovery. So TNF-α may have a prognostic significance in cases with GBS.15

Whether TNF-α is directly involved in the pathogenesis of GBS or it is a result of other basic processes still remains unclear. Possible mechanisms to explain a pathogenic role of TNF-α include a direct myelinotoxic effect of locally produced TNF-α and an indirect effect via disruption of the blood-nerve barrier. Also through release of toxic oxygen free radicals and nitric oxide.15

Apart from the myelinotoxic effect, TNF-α in GBS could play an important role in modulating vascular endothelial function, an effect that would contribute to the development of nerve lesions in GBS. TNF-α can also induce endothelial cell damage.20 Alteration in the endothelial homeostasis increases vascular permeability and this will result in the breakdown of the blood-nerve barrier, and this is a key feature of both GBS and experimental allergic neuritis which may be critical in allowing activated T cells access into peripheral nerve. In experimental allergic neuritis, demyelination occurs when both damage to the blood-nerve barrier and activated T cells or their products are present.21 Studies identified mRNA for TNF-α in necropsy specimens of peripheral nerve from patients with GBS. All these mechanisms play an important role in the pathogenesis of GBS.

The results of this study suggest that the elevated levels of serum TNF-α in patients with GBS decreased following a complete course of IVIg therapy. There was a positive correlation between neurological recovery and a decrease in the serum TNF-α concentration in all the 20 patients with GBS. Reuben et al.3 summarized several modes for therapeutic actions of IVIg in patients with demyelinating diseases these include: a) down regulation of cytokine production;22 b) decrease of TNF-α and interleukin-1β levels;23 c) blockage of Fc receptors of macrophages so inhibits phagocytosis;24 d) inhibits complement mediated immune damage of the capillaries.25

History of previous infection was found in the majority 17 (85%) of patients, the finding which was consistent with Hughes and Rees.26

Patients with GBS following campylobacter jejuni enteritis frequently have IgG antibody to GM1 ganglioside.27

Our study showed significant improvement and recovery of GBS patients with IVIg therapy without any mortality. On the other hand, only three trials have compared IVIg with no treatment or placebo and all concerned children. One trial allocated 18 children alternately to IVIg or supportive treatment alone.28 After 4 weeks, seven of the nine patients in the IVIg group but only two of the nine untreated patients had recovered full strength. The other trial randomized children into three groups: dexamethasone alone in a dose of 5–10 mg daily for 5 or 6 days and then tailed over 7–10 days, or the same dose of dexamethasone and either IVIg or PE.29 This trial included 20 children treated with IVIg and corticosteroids and 16 with corticosteroids alone who could be used to investigate the efficacy of IVIg. The children who received IVIg recovered muscle strength significantly faster than those treated without. Korinthenberg et al.12 compared IVIg in a dose of 1.0 g/kg (half the usual dose) with supportive treatment in 21 mildly affected children who could still walk unaided. The mean improvement in the IVIg group was significantly more than in the untreated participants.

Although plasmapheresis is another therapeutic modality, its therapeutic effect in GBS is through removal of circulating factors. Plasma levels of interleukin and other cytokines are high in GBS, but their circulating half lives is only few hours, so the effect of plasmapheresis on their plasma levels would be short term. In contrast, the half life of IgG is 3 weeks which is much longer than that of the other plasma proteins. It was indicated that IgG is the most important factor in the development of GBS.30

Five trials including 582 participants compared IVIg with plasma exchange.11,15

The weighted mean disability grade improvement was almost identical. There was also no significant difference between the treatments for any of the available outcome measures.

As the therapeutic effect of IVIg is not short term and its mode of action is immunomodulatory we consider that IVIg therapy is less cumbersome than plasmapheresis and also less invasive with satisfactory results, decreased morbidity and mortality in GBS.

Conclusion and Recommendations

In conclusion, serum TNF-α monitoring during IVIg therapy can be used as one of the markers of neurological recovery in GBS patients. IVIg therapy is an immunomodulatory therapy, less invasive and less cumbersome than plasmapheresis and hence we recommend the use of IVIg in the management of GBS in children to decrease morbidity and mortality.

[Disclosure: Authors report no conflict of interest]
REFERENCES


الملخص العربي

تعد متلازمة جيلان باري من أهم أسباب إلتهاب الأعصاب الطرفية الحادة في الأطفال. تهدف هذه الدراسة إلى دراسة التغيرات المرضية في متلازمة جيلان باري ومدى علاقة ذلك بالأعراض الإكستيرميكية في متلازمة جيلان باري.

تمت هذه الدراسة على 20 مريض مصابون بمتلازمة جيلان باري و20 شخصاً من نفس الفئة العمرية. وقد خضع هؤلاء المرضى للآتي:
- اخذ التاريخ المرضي بالتفصيل و الفحص الإكستيرميك الشامل و قياس نسبة العجز بمقياس العجز العادي
- تم عمل اختبار الكهروفسيولوجى للأعصاب وقياس نسبة (تي أن أف ألفا) عند دخول المستشفى و بعد تناول العلاج (أي في أي جي).

أسفرت هذه الدراسة عن: وجد أن نسبة العجز بمقياس العجز العادي عند دخول المستشفى أكبر من نسبة العجز بعد العلاج باستعمال (أي في أي جي) وكان الفرق ذو دلالة إحصائية. وجد أيضاً أن نسبة (تي أن أف ألفا) في مصل مريض متلازمة جيلان باري قبل العلاج (أي في أي جي) كانت أكبر منها في مصل المريض بعد العلاج وكان الفرق ذو دلالة إحصائية. كذلك وجد أن هناك تناسب طردي بين نسبة (تي أن أف ألفا) ودرجة الإعاقة في كل مريض متلازمة جيلان باري وكان الناتج ذو دلالة إحصائية.

الخلاصة: ترتفع نسبة (تي أن أف ألفا) في مصل مريض متلازمة جيلان باري بعد تناول علاج (أي في أي جي) في هؤلاء المرضى.