

# Role of Intravenous Immunoglobulin in Neurological Recovery and its effect on Circulating TNF- $\alpha$ Levels in Guillain-Barré Syndrome

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**Abstract:** Study was undertaken to determine the role of TNF- $\alpha$  in pathogenesis of Guillain-Barré Syndrome (GBS) and to study the role of Intravenous Immunoglobulin (IVIg) in neurological recovery and its effect on TNF- $\alpha$  levels. 28 patients of GBS were divided into Group I, where IVIg (400mg/kg body weight/day for 5 days) was given, and Group II where IVIg was not given. TNF- $\alpha$  levels were measured at admission and after 5 days. Mean TNF- $\alpha$  level in Group I was 34.01pg/ml $\pm$ 32.91, which decreased to 12.61 $\pm$ 7.53 after IVIg therapy. In Group II the mean TNF- $\alpha$  at admission was 15.92 $\pm$ 7.76 while the mean at day 5 was 22.91 $\pm$ 17.41. Neurological improvement was seen in 16 out of 20 patients following IVIg therapy, and 13 out of these 16 patients also registered a fall in TNF- $\alpha$  levels. Overall neurological recovery was seen in 19 patients, out of which 12 registered a fall in TNF- $\alpha$  levels at day 5. Out of the 9 patients who did not show any neurological recovery, 1 patient registered fall in TNF- $\alpha$  levels. **Conclusion:** TNF- $\alpha$  levels are raised in Guillain-Barré Syndrome, indicating its role in pathogenesis. Intravenous immunoglobulin seems to have a beneficial effect on the neurological outcome, which corresponds to the fall in TNF- $\alpha$  levels.

## INTRODUCTION

Guillain-Barré syndrome (GBS) may be described as a collection of clinical syndromes that manifests as an acute inflammatory polyradiculoneuropathy with resultant weakness and diminished reflexes. With poliomyelitis well under control, GBS is now the most important cause of acute flaccid paralysis. GBS has an annual incidence of about 1-3/100,000 and has been diagnosed in patients as young as 2 months and as old as 95 years<sup>1</sup>.

The etiology and pathogenesis of this disorder and its variant remains obscure but an immune mediated destruction of peripheral nerve myelin is suspected. Cellular and humoral immune mechanisms probably play a role in its development.

GBS remains a diagnosis made primarily through the assessment of clinical history and examination findings. Albuminocytological dissociation in cerebrospinal fluid and nerve conduction studies only play a contributory role.

Cellular interactions mediated through release of cytokines (TNF- $\alpha$ , IL-1) play a role in the pathogenesis of GBS<sup>2</sup>. The inflammatory cells infiltrating the peripheral nervous system (PNS) in GBS are composed of lymphocytes and cells of the macrophage lineage, exerting most of their effects through immuno-regulatory cytokines<sup>3</sup>. Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) is regarded as one of the immune factors that can induce demyelination of peripheral nerves in patients with GBS, which in an immune mediated demyelinating disease<sup>4</sup>. It is thought to significantly contribute to disease development by recruiting effector cells to the PNS and by enabling in situ release of other products toxic for Schwann cells (SCs) and myelin such as free radicals, oxygen intermediates and nitric oxide (NO)<sup>5</sup>.

A water-shed event for the management of GBS has been the demonstration of the benefits of Intravenous Immunoglobulin (IVIg). Treatment with IVIg hastens the recovery from GBS<sup>6</sup> but its precise mechanism of action is not known. Experimental studies suggest that it exerts multiple effects on induction, proliferation and effector phase of immune response. IVIg suppress antibody dependent cellular toxicity, decreased natural killer cell function, inhibit auto antibodies production, neutralises circulating pathological antibodies and interferes with complement activation<sup>7</sup>.

There have been studies that showed that treatment with IVIg therapy reduces the levels of TNF- $\alpha$  in pts with GBS<sup>2</sup>. IVIg is as effective as

plasma exchange in reducing duration of disability and hospitalization<sup>6</sup>. This treatment offers an additional advantage of being easier to administer and not requiring specialized equipment or personnel.

In a study<sup>4</sup> of 36 pts with GBS in progressive stages of motor weakness were included. The serum levels of TNF- $\alpha$  and soluble TNF receptors were measured in the serum samples of these patients before and after IVIg therapy by a sandwich ELISA. Of the 36 patients with GBS, 26 showed elevated serum TNF- $\alpha$  level prior to IVIg therapy. Following a complete course of IVIg therapy, there was a progressive decrease in concentration in these 26 patients. On the other hand, the soluble TNF receptors showed an increase in the serum of GBS patients following IVIg therapy.

During the past few decades, 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 immunomodulatory mechanisms of the action of IVIg have been described<sup>8</sup>. Present study was undertaken to underline the role of TNF- $\alpha$  in the pathogenesis of GBS and to study the role of IVIg in neurological recovery and its effect on circulating TNF- $\alpha$  levels.

## MATERIAL AND METHODS

The study was undertaken at a very busy medical college and hospital in a North Indian city. A total of 28 patients presenting in the hospital over a period of 12 months and fulfilling Asbury's diagnostic criteria<sup>9</sup> for GBS were studied.

Patients who were excluded were those taking corticosteroids, had symptoms for more than 2 weeks, had hypokalemia or hypermagnesemia. The patients suffering from diabetes mellitus, poliomyelitis, myasthenia gravis or any other concurrent neurological disorder were also excluded.

All the patients were subjected to detailed history and clinical examination, cerebrospinal fluid examination and nerve conduction velocity study. Other investigations included routine blood examination, serum magnesium, phosphate, fasting sugar and complete urine analysis.

The patients were divided into 2 groups. Group I included patients who were given IVIg at a dose of 400mg/kg body weight every day for 5 consecutive days within first 2 weeks of illness. Group II included patients who were either not suitable for IVIg therapy or who could not afford the treatment.

For clinical assessment of these patients a six point functional grading scale devised by Hughes et al<sup>10</sup> was adopted. The disability scale was applied to all patients of both groups at admission, at 4 weeks and at 12 weeks.

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TNF- $\alpha$  level in blood was measured in all patients at time of admission, after 5 days of treatment with IVIg (Group I) or at day 5 in patients who were not given IVIg (Group II).

For test, 2 ml sample was taken in a serum separator tube and sent to lab within 2 hours. In case of delay, sample was to be refrigerated upto maximum of 48 hours. A sandwich ELISA using TNF- $\alpha$  immunoassay kits (from Sigma, USA) was used to determine the serum level of TNF- $\alpha$  in the test. The reference value for TNF- $\alpha$  was taken as  $<8\text{pg/ml}$ . A standard was run on each plate, using the recombinant human TNF- $\alpha$  standard in serial dilution (1000-0 pg/ml).

For statistical analysis paired Student's 't' test was used to compare data in Group I and II. 'p' values were calculated to determine statistical significance.

## RESULTS

A total of 28 patients admitted with the diagnosis of GBS were taken up for study. Among these, 20 patients were given IVIg (Group I) and 8 patients could not be given IVIg (Group II). General characteristics and demographic data of all the patients in Group I and Group II are shown in Table 1.

Table 1: Demographic characteristics of Group I and Group II

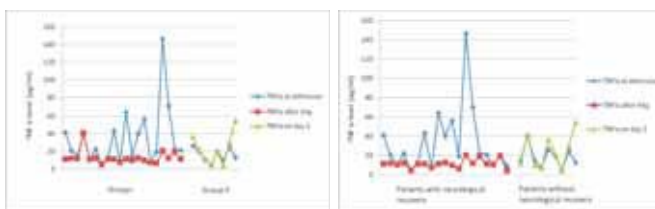
Characteristics	Overall (n=28)	Group I (n=20)	Group II (n=8)
Age	36.79 $\pm$ 16.39	35.55 $\pm$ 16.92	40.38 $\pm$ 15.47
Male	17(60.7%)	14(70%)	3(37.5%)
Female	11(39.3%)	6(30%)	5(62.5%)
Antecedent infection	14(50%)	11(55%)	3(37.5%)
Raised CSF proteins (>45mg/dl)	15(53.6%)	12(60%)	3(37.5%)
Normal CSF proteins	13(46.4%)	8(40%)	5(62.5%)
Sensory motor neuropathy	15(53.6%)	10(50%)	5(62.5%)
Pure motor neuropathy	13(46.4%)	10(50%)	3(37.5%)
Disability score			
Stages 0, 1, 2, 3	0	0	0
Stage 4	16	10	6
Stage 5	12	10	2

TNF- $\alpha$  was measured in all the patients at admission and 85.7% had high TNF- $\alpha$  at admission with a mean of 32.69 $\pm$ 26.76.

The mean TNF- $\alpha$  level of patients in Group I before starting IVIg therapy was 34.01pg/ml $\pm$ 32.91, whereas TNF- $\alpha$  after IVIg therapy was 12.61 $\pm$ 7.53 (p value 0.006). In Group II the mean TNF- $\alpha$  at admission was 15.92 $\pm$ 7.76, while the mean at day 5 of admission was 22.91 $\pm$ 17.41 (p 0.230 - not statistically significant), as shown in Table 2 and Figure 1.

Table 2: TNF- $\alpha$  levels in Group I and Group II

	Group I		Group II	
	Before IVIg	After IVIg	At admission	Day 5
No. of patients (n)	20	20	8	8
Mean TNF- $\alpha$ (pg/ml)	34.01	12.61	15.92	22.91
S.D.	32.91	7.53	7.76	17.41
P value	0.006	0.230		



Each marker represents an individual patient.

Figure 1: TNF- $\alpha$  levels in Group I and II  
Figure 2: TNF- $\alpha$  levels in relation to neurological recovery.

Neurological improvement (decline of one or more stage in clinical disability scale) was seen in 16 out of 20 patients (80%), following IVIg therapy, and 4 patients (20%) did not show any neurological improvement

at 4 weeks. 13 out of these 16 patients (81.25%) who showed neurological improvement after IVIg therapy, also simultaneously registered a significant fall in TNF- $\alpha$  levels, whereas the remaining 3 showed either no change or a slight rise in TNF- $\alpha$  levels.

Table 3: Neurological recovery in Group I and Group II

	Group I		Group II	
	Neurological improvement	No Neurological improvement	Neurological improvement	No Neurological improvement
No. of patients (n)	16	4	3	5
Percentage improvement	80%	20%	37.5%	62.5%

In the Group II where IVIg was not given, 5 patients did not show any neurological improvement at 4 wks, while 3 patients showed improvement at 4 wks, as shown in Table 3.

Overall neurological recovery was seen in 19 out of the 28 patients (67.85%) enrolled in the study. 12 out 19 patients (63.15%) showing neurological recovery registered a significant fall in TNF- $\alpha$  levels after IVIg therapy or at day 5. On the other hand, out of the 9 patients who did not show any neurological recovery, only 1 patient (11.11%) registered a fall in TNF- $\alpha$  levels and in rest of them TNF- $\alpha$  levels either remained same or registered a rise (Figure 1).

## DISCUSSION

TNF- $\alpha$  is produced by inflammatory cells in response to diverse infectious stimuli and tissue injury. It induces a cascade of endogenous mediators that direct host immunologic function and it has been found to be raised in the sera of patients of GBS in early stages of the disease. It was also thought that TNF- $\alpha$  may have a role in pathogenesis of the disease. Furthermore the TNF- $\alpha$  level were reported to decrease following IVIg therapy; this correlated with neurological recovery<sup>11</sup>. In view of this it was decided to study the levels of TNF- $\alpha$  in plasma and to correlate them with IVIg therapy and neurological recovery in patients of GBS presenting in our hospital in a format of two groups where patients of one group would receive IVIg therapy whereas the other group would comprise of the patients who could not receive the IVIg for various reasons. All pts were in progressive stages of weakness and disease duration was less than 2 wks.

TNF- $\alpha$  levels were done in all subjects at baseline on admission and after IVIg therapy in Group I and on day 5 in the patients of Group II who were not given IVIg. Out of the total of 28 pts of GBS, 24 (85.7%) showed high levels of TNF- $\alpha$  at admission. Earlier studies<sup>3,3,12</sup> have also documented 61-72% of patients to have high TNF- $\alpha$  levels, though the incidence in our study is slightly higher. Among the 20 pts who were given IVIg (Group I), 17 had raised levels of TNF- $\alpha$  and 15 out of those 17 registered a statistically significant fall in levels of TNF- $\alpha$  in response to IVIg therapy implying that IVIg administration had significant effect on fall of TNF- $\alpha$  concentration (Figure 1). On the other hand, all of the 8 patients (100%) who were not given IVIg (Group II) instead registered a rise in TNF- $\alpha$  levels after 5 days, though the rise was not statistically significant. It suggests that in the patients who were not given IVIg there was either no significant change in TNF- $\alpha$  levels after 5 days, or there was slight rise which was not statistically significant. Similar findings were also observed in an earlier study by Reuben et al which showed significant fall in serum TNF- $\alpha$  when the sera of GBS patients was incubated with IVIg for 24 hours, which also correlated well with neurological improvement<sup>12</sup>. Similar results were reflected in a study by Sharief<sup>2</sup> in which circulating levels of proinflammatory cytokines (including TNF- $\alpha$ ) were assayed and the fall in their levels was compared between patients receiving IVIg and plasma exchange. The results showed that circulating levels of proinflammatory cytokines decreased after treatment with IVIg but remained relatively high in untreated patients as well in those treated by plasma exchange.

In the present study, 16 patients out of the 20 (80%) given IVIg (Group I) showed neurological improvement and the remaining 4 patients (20%) did not record any neurological improvement with IVIg therapy. Among 16 pts who showed neurological recovery following IVIg therapy, 10 had clinical disability scale IV and 6 had disability stage V. all pts with stage IV disability showed neurological recovery at 4 wks following IVIg therapy. As discussed earlier, this is the same group of patients (Group I) that showed significant fall in serum TNF- $\alpha$  levels after IVIg therapy. All the non-responders were advanced cases of GBS. One patient died because of ventilator related respiratory complication, while the remaining 3 patients were at Stage 5 (ventilator assisted patients) of disability score. In a study by Visser et al<sup>13</sup> which included 25 ventilator dependent patients, the authors recorded slow clinical recovery after treatment with IVIg, 56% were unable to walk independently at 8 weeks following treatment. Our study, like the prior study, also showed that patients who required assisted ventilation at earlier stages of the disease had poor prognostic outcome.

However, limitation of this study was a rather small number of subjects in group II and the significant difference in mean TNF- $\alpha$  levels of both the groups which can be attributed to small number of patients in group II. The very high TNF- $\alpha$  levels in group I underline the patients' and their attendants' psychology to opt for a costly treatment like IVIg only when the disease is severe or the disability score is high. Therefore the authors feel that a similar study with institutional support can mitigate this limitation.

In conclusion, TNF- $\alpha$  levels were raised in patients of Guillain-Barré Syndrome, indicating its role in the pathogenesis of the disease. Intravenous immunoglobulin therapy seems to have a beneficial effect on the neurological outcome of Guillain-Barré Syndrome, which corresponds to the fall in TNF- $\alpha$  levels in such patients.

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