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RESPONSE OF ANTI-CARDIOLIPIN ANTIBODIES TO VARIOUS TREATMENT MODALITIES IN GUILLAIN BARRE SYNDROME

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ABSTRACT

This study was undertaken to investigate the presence of anti-cardiolipin antibodies in serum samples of Guillain Barre patients not previously diagnosed with autoimmunity disorder and to examine the fluctuation in ACA in serum samples of GBS patients in response to the course of treatment. Twenty one patients were recruited in this study and were divided into 2 main groups based on the treatment method, the first group received plasma exchange and the second group received intravenous immunoglobulin treatment. Anticardiolipin antibodies were assessed in all our patients and were compared to the normal control group to establish a cut-off value of 10 U/ml. The results showed a significant reduction in serum Anticardiolipin antibodies in all our patients after starting of the treatment. In addition, patients received plasma exchange therapy had a more significant reduction in this marker compared to the patients received intravenous immunoglobulin therapy at $p<0.05$. The current study illustrate the beneficial use of anti-cardiolipin antibodies titre in monitoring patient's response to treatment in Guillain Barre syndrome and favours the plasma exchange

to the intravenous immunoglobulin as the preferred method of treatment which has the best outcome.

Keywords: Anti-cardiolipin antibodies, Guillain Barre Syndrome, Plasma exchange, Intravenous immunoglobulin.

INTRODUCTION

Guillain Barre Syndrome (GBS) is a clinical disorder characterised by the combination of a rapidly progressive symmetrical weakness in the arms and legs with or without sensory disturbances, hyporeflexia or areflexia, in the absence of a CSF cellular reaction[1]. The proposed mechanism in GBS is that an antecedent infection evokes an immune response, which in turn cross-reacts with peripheral nerve components because of the sharing of cross-reactive epitopes (molecular mimicry) [2]. The end result is an acute polyneuropathy. This immune response can be directed towards the myelin or the axon of peripheral nerve [2].

Immune reactions directed against epitopes in Schwann cell surface membrane or myelin can cause acute inflammatory demyelinating neuropathy (AIDP)[2]. The pathology is that of multifocal inflammatory demyelination starting at the level of the nerve roots. Both the cellular and humoral immune responses participate in the process. Immune reactions against epitopes contained in the axonal membrane cause the acute axonal forms of GBS: acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN) [2].

Treatment of GBS is subdivided into techniques for managing severely paralysed patients requiring intensive care and ventilator support [3], and specific therapy to lessen the nerve damage. Immunomodulating treatments such as plasma exchange (PE) and intravenous immunoglobulin (IVIg) are indicated for patients who are unable to walk independently [4,5].
Antiphospholipid protein antibodies (APA) are a family of autoimmune and alloimmune immunoglobulins (IgG, IgM, IgA, or mixtures) that recognize protein phospholipid complexes in vitro laboratory test systems [6,7]. Originally, these antibodies were thought to have specificity for anionic or neutral phospholipids [8].

Venous or arterial thrombosis in women and the laboratory evidence of antiphospholipid antibodies (APA) or phospholipid-binding protein cofactors are essential criteria determining the antiphospholipid syndrome (APS) [9]. APS is considered an autoimmune disease with

unpredictable occurring episodes of thrombo-embolism. Deep vein thrombosis (DVT) and pulmonary embolism are the most common venous events, whereas the cerebral system is most commonly affected on the arterial site. Nearly any neurological manifestation may occur in patients with APS [10]. Non-thrombotic manifestations were described in relation to the presence of APA like epilepsy, chorea, transverse myelitis, multiple sclerosis, GBS, dementia, and psychiatric disease [11].

Aim of the Study

This study was undertaken to investigate the presence of anti-cardiolipin antibodies (ACA) in serum samples of GBS patients not previously diagnosed with autoimmunity disorder and to examine the fluctuation in ACA in serum samples of GBS patients in response to the course of treatment.

MATERIALS AND METHODS

Study Protocol and Cases selection

The study protocol was reviewed and approved by the Scientific and Ethical Committee of the College of Medicine, Al-Nahrain University. Informed consent was obtained from each subject. Patients were drawn from the Neurology Department in Al-Kadhimiya Teaching Hospital, Baghdad, Iraq for the period from February 2010 to November 2011.

Suitable patients who were included in this study should fulfil the following eligibility criteria:

- Patients are examined & diagnosed with GBS according to the Asbury and Cornblath criteria [12]. The features which allow the diagnosis include clinical, laboratory, and electrodiagnostic criteria.
- Patients are not improving to the supportive treatment.
- The period from the appearance of first symptoms of GBS till time of blood sampling is less than two weeks.
- The patients didn't receive steroid therapy, IVIg or had a PE since the appearance of first GBS symptoms.

The exclusion criteria from the study include previous history of autoimmune disorder & diabetes mellitus.

Blood sampling

Ten milliliters (10ml) of venous blood sample were drawn from our participants using plastic

disposable syringes. Samples were separated by centrifugation at (3000 rpm) for 15 minutes. the sera were stored frozen at (-20 °C) until assayed. After starting the treatment, blood samples were also collected after the 2nd and 5th dose of IVIg or after 2nd and the last session of PE. Sera were collected and stored as above till analysed.

Measurement of ACA

The presence of ACA antibodies were investigated by using (Anti- Cardiolipin Screen kit) which is an indirect solid phase enzyme immunoassay (ELISA) for the simultaneous quantitative measurement of IgG, IgM and IgA class auto antibodies against cardiolipin in human serum or plasma, using (Biotek ELISA, Winooski, VT 05404, USA). A cut-off value of (10 U/ml) was established through measuring ACA in normal healthy controls.

Statistical analysis

The data was analysed using Statistica (Version 8) and Microsoft Excel (Office2007, Microsoft). All values were expressed as mean ± standard deviation (M±SD). Statistical analysis was performed using a one-way ANOVA followed by paired t-test between group comparisons. In all tests, $p < 0.05$ was considered to be statistically significant.

RESULTS

Table 1: Characteristics of the patients included in the study

Case no.	Sex	Age	Blood sampling day	Systemic infection	Mechanical ventilation	Rx Type	ACA titre (U/ml) Pre Rx	ACA titre (U/ml) Post Rx Day 2	ACA titre (U/ml) Post Rx Day 5
1.	M	8	5th	RTI	Yes	IVIg	14.6	13.8	12.3
2.	M	41	10th	RTI	No	PE	5	4	1.6
3.	M	62	13th	RTI	No	PE	4.6	3	5.1
4.	M	21	7th	RTI	No	PE	21.8	19	14.4
5.	M	53	13th	RTI	No	PE	8.6	5.4	3.2
6.	F	7	11th	RTI	No	IVIg	11.3	10.8	9
7.	M	9	10th	RTI	Yes	IVIg	2.7	2.2	2.2
8.	F	4	5th	GITI	No	IVIg	15.8	11	9
9.	M	4	12th	RTI	No	IVIg	4.1	3	2.6
10.	M	6.5	5th	RTI	No	IVIg	32.6	20.8	18
11.	F	20	6th	RTI	No	PE	38.2	35.6	25.6
12.	F	7	13th	RTI	Yes	IVIg	6	5.3	3
13.	F	5	3rd	RTI	No	IVIg	40.6	34.3	25
14.	M	27	4th	RTI	No	PE	39.1	18.6	15

15.	F	40	4th	RTI	No	PE	12.5	9.4	7.1
16.	F	43	11th	RTI	Yes	PE	7.5	6.4	4.3
17.	M	32	7th	RTI	No	PE	42.6	35.8	22.6
18.	M	3	4th	GITI	No	IVIg	12.6	11	6
19.	M	18	5th	RTI	No	PE	14.8	8.9	5
20.	F	53	2nd	RTI	No	PE	10.2	5.2	2.5
21.	M	57	14th	RTI	No	PE	6.8	4.4	3.3

RTI: Respiratory tract infection; GITI: Gastro-intestinal tract infection; Rx: treatment; IVIG: Intravenous immunoglobulin; PE: plasma exchange; ACA: Anti-cardiolipin antibodies.
The study showed that 13 patients had positive ACA titre (cut-off values: 10 U/ml). In all the 21 GBS patients, ACA level was significantly higher in the pre-treatment group (16.8+13.4 U/ml) compared to the day 2 post treatment group (12.8±10.8 U/ml). The level of ACA was significantly lower in day 5 post treatment group (9.4±7.8 U/ml) comparing to the pretreatment and day 2 post treatment groups at a p value less than 0.05, (Table 2).

Table 1 illustrates the 21 GBS patients included in the study. History details and findings of the medical examination were included in the study questionnaire. Thirteen patients were male, 8 were female; age range from (3-62) years, 4 patient needed mechanical ventilation. Two patients had preceded gastrointestinal infection while the remaining 19 patients were complaining from respiratory infection. Twelve patients present in 1st week of the disease and 9 patients in 2nd week, also 9 patients treated with IVIg, while 12 patients treated with PE. In the treatment we followed the Guideline recommendations and Choice of therapy using either IVIG or PE, according to local availability and on patient-related risk factors, contraindications, and preference.

Table 2: Anti-cardiolipin antibodies (ACA) titer levels (Mean±SD) of the GBS patients before and after treatment.

ACA (U/ml)	Pre treatment	Day 2 & 2nd session	Day5&last session	P value
All Treatment Groups N=21	16.8±13.4	12.8±10.8	9.4±7.8	0.001 [a]
				0.001 [b]
				0.0001 [c]
IVIg Treatment Group N=9	15.6 ± 12.91	12.5± 10.0	9.7 ±7.71	0.04 [a]
				0.01 [b]
				0.01 [c]
PE Treatment Group N=12	17.64 ± 14.3	12.98 ±11.8	9.14± 8.2	0.01 [a]
				0.001 [b]
				0.001 [c]

[a] paired t-test: comparison of patients before treatment and day 2 after treatment.

[b] paired t- test: comparison of patients treatment in day 2 and day 5 post treatment.

[c] paired t-test: comparison of patients before treatment and day 5 after treatment.

ACA: Anti-cardiolipin antibodies; Rx: treatment; IVIG: Intravenous immunoglobulin; PE: plasma exchange

In addition, patients treated with PE showed a more reduction in the level of ACA after the second session (12.9±11.8 U/ml) and after last session (9.14+8.2 U/ml) compared to the IVIg treated patients (12.5±1.0 U/ml and 9.7±7.7 U/ml, respectively), $p<0.05$ (Table 2, Figure 1).

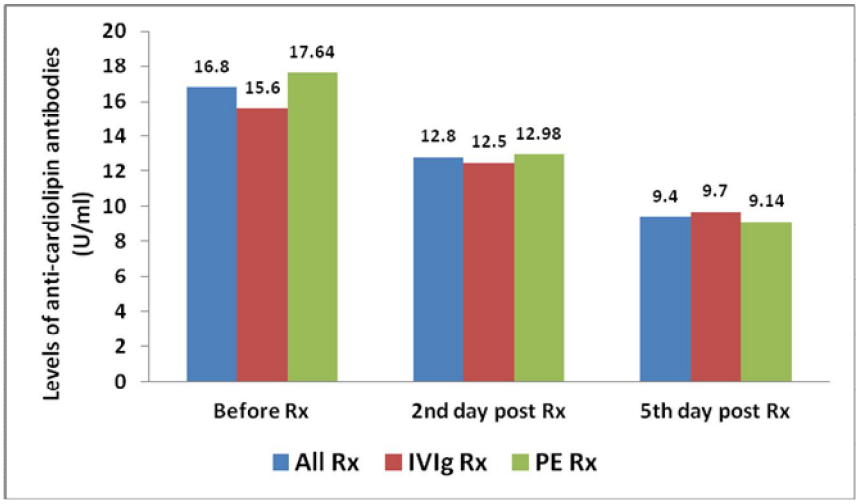

Figure 1: Mean concentrations of anti-cardiolipin antibodies in GBS patients. Levels are expressed for all patients (All Rx); intravenous immunoglobulin treatment group (IVIg Rx) and plasma exchange treatment group (PE Rx).

DISCUSSION

This research has demonstrated the benefits of using ACA titer in monitoring GBS patient response to the treatment using (Anti-Cardiolipin Screen kit) which is a simultaneous quantitative measurement of IgG, IgM and IgA class autoantibodies against cardiolipin, with a cut-off value:10 U/ml. In addition, this parameter can be used to evaluate the efficacy of the methods of treatment used in GBS.

Anti-phospholipid antibodies were measured by several previous studies [13-15] trying to explain the pathogenesis of the associated infections (respiratory infection and GIT infection) that usually occur within two weeks before the onset of GBS. A recent study identified the presence of elevated APA as a common phenomenon in patients with common infections,

independent on the type of infection, and explained that the existence of these APA occur as a part of the immune-reaction developed against the inoculated pathogen [16].

It is not well understood whether the infections associated with ACA may play a role in the pathogenesis of the polyneuropathy or represent only reactionary antibodies to the infection preceding the GBS [15]. But the immune-pathology findings in autopsies suggest that antibody-mediated injury is a predominant disorder in the demyelinating form of GBS [6].

Also the association of GBS and certain autoimmune diseases, including systemic lupus erythematosus, is well recognized [17]. High levels of APA were expressed in patients with lupus like syndrome who also developed secondary GBS [18]. It is thought that whenever polyneuropathy occurs in the context of autoimmune diseases, mainly in systemic lupus erythematosus, where anti-phospholipid activity already exists, these antibodies can cross-react with phospholipids and mediate damage in neural structures containing the particular phospholipids [18]. It has been reported that 15% of patients with inflammatory demyelinating polyradiculoneurophathy also suffer from autoimmune diseases. Many studies have evaluated the clinical relevance of the anti-phospholipid antibodies but with conflicting results [14,19,20]. In our study polyneuropathy should be considered as a primary disorder since none of the patients suffered from any kind of autoimmune disease, nor did any clinical or laboratory findings indicate anti-phospholipid syndrome.

Some studies tested the response of APA in GBS patients to treatment; one study used PE in treatment, showed 10 out of 15 cases had a significant reduction in APA titre while the remaining 5 cases had persisting high APA titre after PE treatment [14]. Another study used IVIg in the treatment and showed a significant reduction in APA titre after treatment, p-value<0.01 [16]. In our study we compared the effect of both treatments on ACA titre by dividing the patients in 2 groups depending on the course of treatment (PE and IVIg groups). Interestingly, our results showed that the reduction in ACA titre was more significant in the PE treated group compared to the IVIg treated group (Table 2, Figure 1. This may provide some information about the effectiveness of the treatment regimen used in GBS which may affects the disease progression and prognosis and need more studies to confirm the outcome in correlation with the clinical aspects.

CONCLUSION

The current study illustrate the beneficial use of anti-cardiolipin antibodies titre in monitoring patient's response to treatment in Guillain Barre syndrome and favours the plasma exchange to the intravenous immunoglobulin as the preferred method of treatment which has the best outcome.

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