C-reactive protein in patients with Guillain Barré syndrome

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ABSTRACT

**Context:** C-reactive protein (CRP) is an acute phase reactant, widely used as a biomarker for various infectious and inflammatory conditions. Guillain-Barré syndrome (GBS) is an acute, autoimmune, polyradiculoneuropathy, triggered by infectious agents such as *Campylobacter jejuni*. GBS is generally precipitated 1-3 weeks following *C. jejuni* infection which suggests a humoral immunopathogenic mechanism. **Aims:** Basal CRP levels were estimated in sera of patients with GBS and compared with adequate controls. **Settings & Design:** The study population was divided into four groups: (i) GBS group included 45 newly diagnosed GBS patients; (ii) Neurological control (NC) group comprised of 59 patients with non-paralytic neurological symptoms/disorders; (iii) Non-neurological controls (NNC) comprised of 43 patients having no neurological symptoms and (iv) Healthy controls (HC) comprised of 101 healthy subjects. **Materials and Methods:** CRP was evaluated using slide latex agglutination test (LAT) and enzyme linked immunosorbent assay (ELISA). **Statistical Analysis:** Statistical analysis was done by the Chi-square test. **Results:** CRP by LAT was positive in 24.4% GBS group, 34% NC group and 44% NNC group. The range of titer in CRP positive samples in the three patient groups (GBS, NC, NNC) was at concentration of 0.6 mg/dl to 19.2 mg/dl. Similar results were also obtained by ELISA in the patient groups. None of the HC subjects was positive for detectable levels of CRP. High basal level of CRP was detected in patients with GBS. **Conclusion:** Autoimmune conditions like GBS can stimulate the production of a high level of inflammation resulting in an increase in the CRP production.

**KEY WORDS:** C-reactive protein, ELISA, Guillain Barre’ syndrome, slide agglutination

INTRODUCTION

C-reactive protein (CRP) is an acute phase reactant produced by the liver in response to factors released by macrophages and adipocytes. Its level rises dramatically during inflammatory processes occurring in the body. It is a critical component of the immune system and is thought to assist in complement binding to foreign and damaged cells, and enhances phagocytosis by macrophages, which express a receptor for CRP.

Neurological disorders can also stimulate the production of a high level of inflammation resulting in an increase in acute phase reactants. Omar *et al.* measured serum CRP by a semi-quantitative method in 50 ischemic stroke patients and found significantly increased CRP in patients compared with controls. Romero *et al.* in a short study of 82 adult patients with a neurological subarachnoid hemorrhage concluded that increased CRP levels were strongly associated with worse clinical prognosis. Song *et al.* from a study of 309 patients with stroke suggested that CRP values on the substrate phase have sufficient value as a predictor of the prognosis of functional disability after first-ever stroke.

Guillain-Barré syndrome (GBS) is an acute, autoimmune, polyradiculoneuropathy affecting the peripheral nervous system and is believed to be triggered by infectious agents such as *Campylobacter jejuni*. GBS is generally precipitated 1-3 weeks following *C. jejuni* infection, which suggests a humoral immunopathogenic mechanism. CRP plays an important role in innate immunity, as an early defense system against infections. Levels of CRP rise when there is a microbial infection or an inflammation without microbes. Thus, the CRP test is complementary and is widely used as a molecular marker during inflammatory conditions. Although an elevated level of CRP has emerged as an important predictor for various infections and inflammatory conditions, no study regarding CRP status is available in GBS patients. In the present study, the basal CRP levels in patients with GBS were estimated because of the immunopathogenic mechanism involved in the precipitation of the disease. Appropriate positive and negative controls were also set up.

MATERIALS AND METHODS

Study population
Patients attending the Neurology Outpatient Department and Emergency Wards of the Postgraduate Institute of Medical Education...
and Research, Chandigarh, formed the basis of our investigation. They included the GBS patients diagnosed by electrophysiological criteria and patients having other neurological symptoms or disorders. Apart from these, patients with no neurological symptoms and healthy subjects were also included in the study. GBS patients and those with neurological symptoms at the time of first reporting were consecutively enrolled in the study regardless of sex, age and duration of disease. However, infants and pregnant women were excluded from the study. The study was approved by the Institute Research Ethical Committee and informed consent was obtained from all the enrolled subjects. This study was carried out between September 2007 and October 2009.

The study population was divided into four groups as follows:

1. **GBS**: This group comprised of 45 GBS patients (cases) who underwent electrophysiological nerve conduction studies to establish the diagnosis.

2. **Neurological controls (NC)**: This group involved 59 consecutive patients with non-paralytic neurological symptoms/disorders such as back pain, migraine, dizziness, muscle weakness, multiple sclerosis, trembling, loss of memory, brain tumor, etc. They served as positive controls to the GBS group.

3. **Non-neurological controls (NNC)**: This group consisted of 43 random patients enrolled from outpatient departments and various wards of the hospital. These patients had no neurological symptoms and acted as positive controls to both the GBS and the NC groups.

4. **Healthy controls (HC)**: This group included 101 random subjects from among the attendants of the enrolled patients. These subjects did not have any ailment at the time of sampling and therefore served as HCs.

**Clinical examination**

All patients enrolled for the study underwent a detailed enquiry along with primary diagnosis were recorded in a pre-printed proforma. The diagnostic criteria by Asbury and Cornblath was taken as the basis for the clinical diagnosis of the GBS patients by the neurologist (SP). Nerve conduction studies involved the motor (median and ulnar) nerves in the upper limbs and the common peroneal and tibial nerves in the lower limb. The sensory nerves examined were superficial peroneal or sural nerves in the lower limbs. The electrophysiological studies involved distal latency, compound muscle action potential and conduction velocity of motor nerves and peak latency, sensory nerve action potential and conduction velocity of sensory nerves. F waves were recorded from the median and tibial nerves and persistence and latencies were taken account of. The GBS patients were classified into sub-groups according to the electrophysiological criteria defined by Hadden et al.

**Collection of blood samples**

Pre-treatment blood samples (5 mL each) were obtained from all patients inclusive of positive control groups. Blood for CRP was collected from GBS patients immediately after the electrophysiological evaluation was performed and the diagnosis was established. Samples from HCs were taken as and when they were available. The sera separated were stored at -20°C for CRP assay, which was performed within 4 weeks of collection.

**Estimation of CRP**

CRP level was determined by the semi-quantitative latex agglutination test (LAT) as well as by an enzyme-linked immunosorbent assay (ELISA). Both negative and positive controls provided in the kits were also set up.

a. **LAT**: The CRP level was determined by the semi-quantitative LAT (Avitex CRP kit, Omega Diagnostic Limited, Omega House, Hillfoots Business Village, Alva, FK12 5DQ Scotland, United Kingdom and Rhexal CRP kit, Tulip Diagnostics (P) Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Baugh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA. Briefly, the test reagents and the serum samples were allowed to come to room temperature. Forty microliters of the patient’s serum was taken on a clean glass slide and 50 μL of anti-human CRP-coated latex reagents was added to it. The specimens were thoroughly mixed with stirring rods and the slides were gently rocked back and forth for 1 min. The serum samples showing clear agglutination were recorded as positive. All positive serum samples were semi-quantitated for CRP by repeating the process on serial doubling dilutions like 1:2, 1:4, 1:8, 1:16 and so on in isotonic saline. The CRP level in the sample was then calculated by multiplying the dilution factor by the detection limit of the reagent to derive the concentration in mg/dL. All negative samples were also semi-quantitated for CRP test to rule out the prozone phenomenon.

b. **ELISA**: CRP levels were also detected in the sera of all patients by ELISA (ZYMUTEST CRP, HYPHEN BioMed, France) as per the manufacturer’s instructions in micro ELISA plate wells. Briefly, 200 μL of immuneconjugate was added to 50 μL of 1:10 diluted serum samples, mixed and incubated for 1 h at room temperature. After five successive washings with 300 μL of wash solution, 200 μL of substrate was added and incubated for 5 min at room temperature. Next, 50 μL of stop solution was added and, after 10 min, mean absorbance values were read at 450 nm and recorded. The values were converted into mg/dL by the help of a CRP calibration standard curve.

**Statistical analysis**

Statistical analysis was carried out by the Chi-square test to compare CRP levels between LAT and ELISA in the different groups.

**RESULTS**

Based on clinical examination and electrophysiological findings, the GBS group was further divided into three sub-groups. Thus, 17 patients belonged to acute inflammatory demyelinating polyneuropathy (AIDP), 21 patients had acute motor axonal neuropathy (AMAN) and seven patients had acute motor sensory axonal neuropathy (AMSAN). The mean CRP levels for AIDP were 8.58 mg/dL, for AMAN were 7.26 mg/dL and for AMSAN were 10 mg/dL. There was no statistical difference in the CRP
levels in the sub-groups and therefore they were all clubbed into the GBS group.

Of the 45 GBS patients, 24.4% were positive for CRP by the LAT method. Campylobacter infection was detected by stool culture in only two of the GBS patients positive for CRP (data not shown). Similarly, 34% of the NC group and 44% of the NNC group were positive for CRP by visual agglutination. None of the HC subjects was positive for detectable levels of CRP by the agglutination method. The range of titer in CRP-positive samples in the three patient groups (GBS, NC and NNC) was at a concentration of 0.6-19.2 mg/dL [Figure 1]. Estimation of CRP by ELISA showed a high level of CRP in the patient population [Figure 2]. Highly significant CRP values were obtained by ELISA with GBS patients \((P < 0.001)\), NC patients \((P < 0.0002)\) and NNC patients \((P < 0.002)\), compared with that obtained by LAT.

**DISCUSSION**

CRP is generally present in trace amounts \(<10\) mg/L in the serum of a normal healthy person, but can increase up to 50,000-fold in response to infectious or inflammatory conditions.\(^{[10]}\) The acute phase develops in a wide range of acute and chronic inflammatory conditions, inclusive of tissue injury. It is a powerful screening test for organic disease and is useful in monitoring known infectious or inflammatory diseases and their response to treatment.\(^{[13]}\) CRP levels have been estimated in almost all kinds of infections and diseases,\(^{[12-17]}\) including the functional psychotic state.\(^{[14]}\) Increased CRP concentrations have also been found in auto-inflammatory disorders like familial Mediterranean fever, which is the most common auto-inflammatory disorder around the world.\(^{[19,20]}\)

Although not well understood, CRP can also bind to auto-antigens and contribute to autoimmune diseases.\(^{[24]}\) The physiological role of CRP is to bind to phosphocholine expressed on the surface of dead or dying cells in order to activate the complement system via the C1q complex.\(^{[22]}\) Thus, it participates in the clearance of necrotic and apoptotic cells. GBS is believed to be an autoimmune condition\(^{[23]}\) precipitated by infectious agents like *C. jejuni*. Interestingly, in the present study, two of the CRP-positive GBS patients had *C. jejuni* in their stool samples. *C. jejuni* has been identified as the most common pathogen precipitating GBS, but as the excretion time of the organisms in the stools is only 2 weeks, and because GBS is a late sequel to *C. jejuni* infection, the patients report much later after the onset of neuropathy.

Measuring for CRP levels help to screen both infections and inflammatory diseases. Dynamic serial measurement of CRP has been widely used to help therapeutic decision making.\(^{[24]}\) An elevated level is a non-specific entity and provides diagnostic support. However, no report hitherto of CRP levels in patients with GBS and very few of those with other neurological disorders are available in the literature, and hence this preliminary study was undertaken.

In a study by Perez-Valdivieso *et al.*\(^{[25]}\) in oncology patients, it was seen that CRP levels \(\geq 8\) mg/dL were significantly associated with an increased in-hospital mortality. In the present study, CRP was detected up to 19 mg/dL by LAT as well as by ELISA. Apart from this, the percentage of CRP positivity by ELISA was also more than that by LAT. Depression is associated with elevated levels of CRP.\(^{[26]}\) A high CRP level could also be attributed to depression in the patient population under study due to the nature of the clinical conditions in them. We did not look for demographic variables like obesity while looking for CRP, and this is the limitation of our study.

The CRP response has no diagnostic specificity, but a high value is an unequivocal evidence of tissue-damaging disease. Serial measurements of CRP by sensitive assays like ELISA can contribute significantly to prognosis and clinical management of a disease. Routine empirical measurement of CRP is a valuable aid to patient management across a broad range of clinical practice. In conclusion, a high basal level of CRP was detected in patients with GBS. Autoimmune conditions like GBS can stimulate the production of a high level of inflammation resulting in an increase in the CRP production. Sensitive CRP assays may become a new tool for monitoring these conditions.

![Figure 1: Semiquantitation of C-reactive protein-positive samples in the different groups](http://www.ijpmonline.org)
risk assessment marker in the future for autoimmune disorders inclusive of GBS.

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