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Antiganglioside Antibodies in Sub Types of Guillain-Barre Syndrome in an Indian Population

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Antiganglioside antibody tests for immune-mediated neuropathy are now widely available. They can identify subsets of patients within the large group of idiopathic neuropathies that have lacked specific clinical definition. The aim was to study the subtypes of Guillain Barre Syndrome based on the presence of type of antiganglioside antibodies and to correlate these with the clinical features. Sera from 73 patients with GBS which included the subtypes of Acute Inflammatory Demyelinating Polyneuropathy (AIDP), Acute Motor Axonal Neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN) and Miller Fisher Syndrome (MFS) were examined for the presence of IgG and IgM (GM1, GM2, GM3, GD1A, GD1B, GT1B, GQ1B) antiganglioside antibodies using qualitative *in vitro* kit. In GBS, IgG GM1 (59%) and IgM GM1(25%) were positive in maximum number of cases. In subgroup AMAN, IgG (GM1 and GD1B) were positive in 53% of cases, IgM GM1 was positive in 50% of cases. Two patients of AMAN who had a previous history of gastroenteritis were positive for all IgG antibodies. In the subgroup AIDP, IgG GT1B and IgM GM1 were positive in 28 and 18%, respectively. Multiple types of antiganglioside antibodies were associated with the subtypes of GBS in the population from South India. AMAN variant was commonly associated with antibodies as compared to other variants. Antibodies associated with subtype AMAN were IgG GM1, GD1B and IgM GM1. AIDP variant was associated with IgG GT1B and IgM GM1. The assay may be useful for rapid screening of GBS sera for antibodies to multiple gangliosides.

Key words: Neuropathy, miller fisher syndrome, acute inflammatory demyelinating polyneuropathy

INTRODUCTION

Guillain-Barré Syndrome (GBS) is a common cause of acute flaccid paralysis, with an annual incidence of 1-2 cases/100 000 population (Pritchard and Hughes, 2004). This is an immune mediated polyradiculo-neuropathy characterized by the acute onset and rapid progression of flaccid, hyporeflexi quadriparesis with variable sensory and autonomic involvement. The disease process has been characterized as an immune response triggered by a prodromal syndrome, which may be an infection, immunization or surgical procedure. GBS had been considered to be almost synonymous with Acute Inflammatory Demyelinating Polyneuropathy (AIDP) after the landmark report of Asbury *et al.* (1969), which emphasized the role of inflammatory demyelination in the pathogenesis of the disease. Feasby *et al.* (1986) described an axonal variant of GBS and studies of the motor paralysis affecting Chinese children in the summer months led to the description of the Acute Motor Axonal Neuropathy (AMAN) (McKhann *et al.*, 1991). These conditions may clinically be indistinguishable from AIDP and are now considered part of the spectrum of GBS. Of two predominant GBS subtypes, a demyelinating subtype AIDP predominates in the United States and Europe and axonal subtype AMAN is the predominant form in China and Mexico (Nachamkin *et al.*, 2007). As experience with GBS accumulates, it appears that the incidence of several subtypes that make up GBS varies markedly from continent to continent and from culture to culture. GBS in the Western world (Europe, United States, Canada and Australia) approximates 85 to 90% AIDP, with the remainder composed of Miller Fisher syndrome, AMAN and AMSAN as well as indeterminate cases. In contrast, in North China, studies indicate that approximately 70% of GBS is of the AMAN type and less than a quarter AIDP and other types (Hughes and Cornblath, 2005). What determines the mix of subtypes in a given population is not understood, but appears to involve both host and environmental factors. The distribution in the Indian population remains unclear.

Gangliosides are a family of acidic glycolipids that are composed of lipid and carbohydrate moieties. They are generally situated in the outer layer of the neuronal plasma membrane. Four ganglioside are especially abundant in the brain-GM1, GD1a, GD1b and GT1b. In the peripheral nerve a fifth ganglioside, LM 1 also occurs in plenty. Several minor gangliosides are also present (Misra *et al.*, 2008). Heterogeneity of ganglioside expression in the peripheral nervous system may underlie the differential clinical manifestation of GBS variant (Kenichi *et al.*, 2009).

The aim was to study the subtypes of Guillain Barré syndrome based on the qualitative presence of the different antiganglioside antibodies in the serum and to correlate these with the clinical features. Since, the pattern of infection in the Indian population differs from the Western population, this data represents the first study on the pattern of Ig G and Ig M class of antibodies in GBS in the Indian population.

MATERIALS AND METHODS

We prospectively studied 73 patients admitted to Neurology ward at hospitals in Hyderabad City from 2005 to 2009. Clinical histories were obtained. All protocols were approved by the hospital institutional review boards. Sera were collected from patients and stored at -70°C until testing.

Criteria for AIDP included features of an acquired demyelinating polyneuropathy such as prolonged distal and F wave latencies and reduced conduction velocities. Criteria for AMAN included normal sensory studies, normal motor conduction velocities for amplitude and reduced distal motor CMAP amplitudes. The degree of illness was assessed on admission and daily thereafter.

The GBS patients were defined according to criteria described previously (Asbury *et al.*, 1969) and were further subdivided into GBS subtypes (Hadden *et al.*, 2008). A total of 73 patients were enrolled in the study of which 46 patients had AIDP, 18 patients had AMAN, 6 patients had AMSAN and 3 patients had MFS. Statistical analysis was performed by using SPSS software version 10.

Blood was drawn from patients who were clinically diagnosed with GBS. Sera from these patients were examined for the presence of IgG (GM1, GM2, GM3, GD1A, GD1B, GT1B, GQ1B) and IgM (GM1; GM2, GM3, GD1A, GD1B, GT1B, GQ1B) antiganglioside antibodies using a qualitative *in vitro* assay kit from Euroline, Euroimmun, Medizinische Labordiagnostika AG.

RESULTS

Of the 73 patients, 46 had AIDP, 18 had AMAN, 6 had AMSAN and 3 had MFS (Table 1), most of the sera reacted positively for antibodies against more than one ganglioside. 22 patients showed positive reaction for the antibodies against single ganglioside, 7 patients showed positive reaction for the antibodies against two gangliosides, 12 patients showed positive reaction for the antibodies against more than two gangliosides, 4 patients showed positive reaction for the antibodies against all the

Table 1: Population characteristics of Indian population in the study

| Characteristics | AIDP | AMAN | AMSAN | MFS |
|-----------------|-------|------|-------|-------|
| No. | 46 | 18 | 6 | 3 |
| M/F | 34/12 | 11/2 | 3/2 | 4/2 |
| Mean age (F) | 35.4 | 31.6 | 35.5 | 18 |
| Mean age (M) | 39.9 | 40.0 | 34.36 | 47.25 |

Table 2: Positive reactivity of sera for antibodies in GBS patients

| | GM1 | GM2 | GM3 | GD1a | GD1b | GT1b | GQ1b |
|---------|-----|-----|-----|------|------|------|------|
| IgG (%) | 59 | 26 | 22 | 21.0 | 25 | 34.0 | 22.0 |
| IgM (%) | 25 | 19 | 8 | 12.4 | 15 | 16.6 | 16.6 |

gangliosides tested and 28 showed negative reaction for all the antibodies against gangliosides tested. The patients who showed positive reaction for the antibodies against gangliosides belonged to both IgG and IgM class.

Presence of positive reactivity of sera for antibodies in GBS patients especially IgG highly expressed comparatively IgM, percentage of expression clearly shown in Table 2, in GBS IgG for GM1 was positive in maximum number of cases (59%) in combination with other anti ganglioside antibodies, while GD1A (21%) was positive in the minimum number of cases. Similarly, among the IgM class, GM1 was positive in 25% while GM3 was positive in 8% of cases.

In subgroup AMAN, GM1 and GD1B of the IgG class were positive in 53% of cases, while GD1A was positive only in 27% of cases. Similarly, IgM GM1 was positive in 50% of cases. Two patients of AMAN who had a previous history of gastroenteritis were positive for all IgG antibodies.

In the subgroup AIDP, IgG GT1B and IgM GM1 were positive in 28 and 18%, respectively while GM3 of IgG and IgM class was positive only in 6.5 and 3%, respectively.

There was no correlation between any specific clinical features and the antiganglioside antibodies among the subgroups of GBS. Absence of antiganglioside antibodies was noted in all the patients requiring mechanical ventilation irrespective of GBS subtypes except in 2 cases of AIDP which were positive for IgG (GT1B).

All the patients were on IVIG treatment and were followed for 9 months. Three patients died and 70 patients improved clinically. The three patients who died (mean age of 51.2 years) included two females and one male patient. All the 3 belonged to AIDP sub group of GBS and presented with gastroenteritis (GE), Upper Respiratory Tract (URI) Infections, Sensory Involvement (SI) and all three were on mechanical ventilator; an important observation was that there was absence of antiganglioside antibodies in all the three patients and Percentage representation of each type with clinical features shown in Table 3.

Table 3: Percentage representation of each type with clinical features

| Features | AMAN | AIDP | AMSAN | MFS |
|--|------|------|-------|-----|
| Gastroenteritis (n = 10) (%) | 50.0 | 50.0 | -- | -- |
| Upper respiratory tract infection (n = 20) (%) | 10.0 | 80.0 | 5.0 | 5.0 |
| Sensory involvement (n = 35) (%) | 5.7 | 77.2 | 11.4 | 5.7 |
| Patients requiring mechanical ventilation (n = 10) (%) | 10.0 | 80.0 | 10.0 | -- |

DISCUSSION

Guillain-Barré Syndrome (GBS) is an acute polyneuropathy consisting of different subtypes. Acute inflammatory demyelinating polyradiculoneuropathy, the classic demyelinating form of GBS, accounts for 90% of all GBS cases in the Western world. Acute Motor Axonal Neuropathy (AMAN) and Acute Motor and Sensory Axonal Neuropathy (AMSAN) are axonal forms of GBS that are more prevalent in Asia, South and Central America, often preceded by infection by *Campylobacter jejuni*. Acute Inflammatory Demyelinating Polyneuropathy (AIDP), Acute Motor Axonal Neuropathy (AMAN), were the more prevalent forms of GBS in present study. Gangliosides along with other components as cholesterol are known to form lipid rafts, in which the carbohydrate portions of two different gangliosides may form a new conformational epitopes. Within the rafts, gangliosides are considered to interact with important receptors or signal transducers. The antibodies against ganglioside complexes may therefore directly cause nerve conduction failure and severe disability in GBS (Kusunoki *et al.*, 2008).

The AMAN and AMSAN may be mediated by specific anti-ganglioside antibodies that inhibit transient sodium ion (Na⁺) channels. The efficacy of plasmapheresis and intravenous immunoglobulin has been established in large international randomized trials, with corticosteroids proven ineffective. Although, axonal demyelination is an established pathophysiological process in GBS, the rapid improvement of clinical deficits with treatment is consistent with Na⁺ channel blockade by antibodies or other circulating factors, such as cytokines.

Most studies have shown the age-specific incidence rises approximately four fold between the under 20 age group and the over 60 age group (Winner and Grimley, 1990). Guillain-Barré syndrome occurs at all ages, but a bimodal distribution with peaks in young adulthood (15-35 years) and in elderly persons (50-75 years) appears to exist. Rare cases have been noted in infants (Lo *et al.*, 2007). In our study, 71.23% were male and 28.76% were female. In our study (13.69%) were below 15 years of age, (24.65%) were between the age group of 15-35 years, (46.57%) between 36-55 years, (13.69%) between 56-75 years, (1.3%) >75 years contradictory to (Winner

and Grimley, 1990; Hartung *et al.*, 1995) with the peak incidence between 15-35 years of age. The GBS is encountered in all regions of the world. The incidence of Guillain-Barré Syndrome (GBS) is 1-3 per 100,000 inhabitants, making GBS the most common cause of acute flaccid paralysis in the United States (Seneviratne, 2000; Winer, 2001; Van Koningsveld and Van Doorn, 2005). AMAN and AMSAN occur mainly in Northern China, Japan and Mexico and they comprise 5-10% of GBS cases in the United States (Kinnunen, 1998). The AIDP accounts for up to 90% of cases in Europe, North America and the developed world. Epidemiologic studies from Japan indicate that, in this region, a greater percentage of GBS cases are associated with antecedent *C jejuni* infections and a lesser number are related to antecedent cytomegalovirus infections compared with that in North America and Europe. A study in Iran showed that 47% of pediatric GBS cases had evidence of recent *C jejuni* (*Campylobacter jejuni*) infection (Barzegar, 2008).

Most studies have shown the male-to-female ratio of Guillain-Barré syndrome is 1.5:1 (Winner and Grimley, 1990), while in our study male to female ratio is 2.4:1. A Swedish epidemiologic study indicated that the incidence of Guillain-Barré syndrome is lower during pregnancy and increases in the months immediately following delivery (Jiang *et al.*, 1996).

We did not find any singular distinguishing patterns of anti-ganglioside antibodies among the subtypes of GBS which is in accordance with Alaedini *et al.* (2003) suggesting that ganglioside specific T cells can recognize more than one ganglioside, possibly explaining the presence of antibodies to multiple gangliosides in some of the patients. Anti-GM1 (IgG GM1 and IgM GM1) antibodies were detected in both the AMAN and AIDP groups. Somewhat surprisingly, although anti-GD1a antibodies were found at similar frequency to previous studies of AMAN cases (Ho *et al.*, 1999; Ogawara *et al.*, 2000). They were also present in some AIDP cases, with no significant discrimination between AMAN and AIDP. This is at variance with the widely observed finding that the presence of anti-GD1a antibodies is a good discriminator between AMAN and AIDP (Ho *et al.*, 1999; Ogawara *et al.*, 2000). The presence of IgG antibodies is suggestive of involvement of T cells, which are also implicated in the Guillain-Barré syndrome where multiple ganglioside antibodies have been described (Alaedini *et al.*, 2003; Shamshiev *et al.*, 2000). Antiganglioside antibodies were not seen in all patients with multifocal acquired sensory and motor neuropathy, possibly because the disease in some patients may be mediated by T-cells without antibodies, or antibody levels might fluctuate depending on disease activity, or other

disease mechanisms may be present. In addition, titers of IgG antiganglioside antibodies may be affected by intravenous immunoglobulin, which has been reported to increase autoantibody clearance (Alaedini *et al.*, 2003; Bleeker *et al.*, 2001). The presence of IgG antibodies to gangliosides distinguishes patients with multifocal acquired sensory and motor neuropathy from those with multifocal motor neuropathy, which is associated with IgM anti-GM1 ganglioside antibodies (Alaedini *et al.*, 2003). Autoimmune diseases are often preceded by an infectious illness. Molecular mimicry between microbial antigens and structures in host tissue has been implicated as a mechanism for triggering a cross-reactive immune response after an infection (Godschalk *et al.*, 2004). There is strong but indirect evidence for the pathogenic role of molecular mimicry in Guillain-Barré Syndrome (GBS), an acute peripheral polyneuropathy and the most frequent cause of acute neuromuscular paralysis (Godschalk *et al.*, 2004). Therefore, GBS is an excellent model disease to study both microbial and host factors involved in molecular mimicry.

CONCLUSIONS

In conclusion, multiple types of antiganglioside antibodies were associated with the subtypes of GBS in the population from South India. The AMAN variant was found to be commonly associated with antibodies as compared to other variants. Antibodies associated with subtype AMAN were IgG GM1, GD1B and IgM GM1. Similarly subtype AIDP was associated with multiple gangliosides.

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