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Seroprevalence of *Campylobacter jejuni* infection in common subtypes of Guillain–Barre syndrome in Kashmiri population

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Abstract

Background Guillain–Barre Syndrome (GBS) is a common differential diagnosis of acute-onset flaccid quadriparesis with or without bulbar involvement. Various illnesses precede GBS, respiratory illness being the most common. *Campylobacter jejuni* is the single most common organism found associated with GBS. The aim of the present study was to determine the prevalence of *Campylobacter jejuni* positivity in different subtypes of GBS.

Methods Sixty patients of GBS were tested for serological evidence of *Campylobacter jejuni* and compared with 60 age and sex matched controls.

Results Mean age of cases was 39.58 (± 14.76 years) and that of controls was 35 (± 12.31 years). Preceding illness was present in 38% cases. Respiratory tract illness was present in 9 (15%) cases, while as GI illness was present in 8 (13%) cases. AIDP was the most common variant accounting for 65% of cases, followed by AMAN (18.3%). Among cases, 24 (40%) tested positive for *Campylobacter jejuni* antibody whereas only 12 (20%) tested positive for antibody among controls. The difference was statistically significant between cases and controls (p value = 0.003). There was no statistically significant difference in antibody positivity and preceding illness among different variants of GBS (p value = 1.0).

Conclusion *Campylobacter jejuni* infection is a frequent preceding illness in GBS patients, although it may be asymptomatic. There is however no relation between different subtypes of GBS and *C. jejuni* infection.

Keywords *Campylobacter jejuni*, GBS, AIDP, Quadriparesis, IVIg, Plasmapheresis

Introduction

Guillain–Barre syndrome (GBS) is a fulminant polyradiculoneuropathy characterized by a rapidly evolving ascending areflexic quadriparesis associated with weakness of facial, bulbar, and respiratory muscles [1]. Commonly encountered subtypes of GBS include acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor and sensory neuropathy (AMSAN) and the Miller Fisher syndrome [2]. While AIDP is the most common form of GBS in North America and Europe, accounting for around 90% cases; in Asia, South and Central America, the axonal form of GBS is much more common and constitutes 30% to 66% of cases [3]. GBS has been described as a post-infectious immune-mediated disease that occurs as a result of molecular mimicry wherein immune mechanisms, both cellular as well as humoral, normally directed against microbes, recognize body's own antigens as foreign [4]. Preceding infections occur in around two-third of GBS cases, especially an upper respiratory tract infection or gastroenteritis [5]. The most common antecedent infectious agent causing GBS is *Campylobacter jejuni* [6]. Other infectious agents include

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cytomegalovirus, Epstein–Barr virus, *Mycoplasma pneumoniae*, and *Haemophilus influenzae* [7–11]. GBS has also been reported after vaccination, surgery, and head trauma [12–14].

Campylobacter jejuni is a Gram-negative bacillus and is the most common cause of bacterial gastroenteritis in the world. The diarrheal illness is usually self-limited and is caused due to intake of uncooked animal products and contaminated water [15]. *Campylobacter jejuni* infection can be diagnosed by stool culture, enzyme immune assay (EIA) or polymerase chain reaction (PCR). The organism was first isolated from the stools of a GBS patient in 1982 by Rhodes and Tattersfield [16]. This bacterium is continuously excreted in stools in affected patients for an average of 16 days [17]. Since GBS occurs on an average 3 weeks after the diarrheal illness, stool cultures at the onset of GBS in such patients may be negative. Therefore, studies based on stool culture alone underestimate the frequency of previous *C. jejuni* infections in GBS. Studies in Northern China report more frequent Campylobacter infections in patients with AMAN than AIDP [18]. However, several studies on GBS patients from Europe and North America were unable to demonstrate an association between *C. jejuni* infections and this clinical and electrodiagnostic pattern [19]. Due to scarcity of data regarding *Campylobacter jejuni* in GBS from our part of the world, this study was done.

Aims and objectives

To determine the prevalence and significance of serological evidence of *Campylobacter jejuni* in different subtypes of GBS.

Methods

Study design

The present study was a prospective case–control study. The study was carried out in the department of Neurology, Sheri Kashmir Institute of Medical Sciences, an advanced tertiary care institution that cares to the medical needs of more than 15 million people of northernmost union territory of India. The present study was done over a period of two years from August 2017 to July 2019. Sixty consecutive patients admitted to our department with the diagnosis of GBS were included in this study. The control group consisted of 60 age and sex-matched normal subjects.

Inclusion criteria

1. Patients who fulfilled Asbury and Cornblath [20] diagnostic criteria for GBS were included in this study.
2. Miller Fisher syndrome, which is not covered by these criteria was diagnosed as a triad of acute onset of ophthalmoplegia, areflexia, and ataxia after ruling out other aetiologies.

Exclusion criteria

1. Patients where diagnosis of GBS was equivocal.
2. Patients where *Campylobacter jejuni* serology was not available.
3. Patients with other causes of polyradiculoneuropathy.

Clinical assessment

Complete history including age, sex, first neurological symptom, day of illness on which patient was admitted, the season of presentation, history of antecedent illness and duration between preceding illness and onset of symptoms was obtained. Detailed nervous system evaluation was done including cranial nerve examination, power, and reflexes. Duration of hospital stay, need for ventilatory support, development of autonomic dysfunction, and time required to attain peak deficit was also noted in days (time from onset of symptoms to intubation in those patients who required mechanical ventilation and time from onset of symptoms to peak disability in non-ventilated patients). Hughes functional grading scale was used to assess outcome ranging from 0 (normal), 1 (being able to run), 2 (able to walk at least 5 m, but unable to run), 3 (able to walk 5 m with walker or support), 4 (bedridden), 5 (ventilated), to 6 (dead) [21].

Laboratory assessment

The routine investigations including complete blood count, blood sugar, and biochemical parameters including kidney and liver function tests were done in all patients. Cerebrospinal fluid (CSF) examination was done after one week of illness in all patients and was examined for microscopic and biochemical parameters including total cell count, differential cell count, protein, and sugar.

Serological diagnosis of *Campylobacter jejuni* was achieved by measuring IgG antibodies by ELISA in the serum obtained from patients on the day of admission before any form of therapy was administered. All blood samples were collected prospectively in polypropylene tubes, centrifuged, and stored at − 80 °C within 2 h of sampling in 1.5–2 ml Eppendorf tubes until analysis. The ELISA kit assay used measures human PEB 1 (Phosphatidylethanolamine-binding protein 1) level in the samples. Microtiter plate wells were coated

with purified PEB1 antibody to which serum samples obtained from cases and controls were added. The antigen–antibody complex was labelled with horseradish peroxidase (HRP) which releases a blue colour when tetramethylbenzidine (TMB) substrate solution is added and the colour changes to yellow when sulphuric acid stop solution is used. The intensity of the colour was measured at 450 nm using a spectrophotometer. The concentration of PEB 1 was then measured by comparing the optical density of the samples to the standard curve. The standard curve (Fig. 1) was constructed by plotting the mean absorbance (Y) against known concentration in linear regression using the four-parameter algorithm. Results were reported as concentration of PEB 1 ng/ml in samples. The measured concentration of samples calculated from the standard curve was multiplied by their respective dilution factor because samples had been diluted prior to assay.

Neurophysiological assessment

All patients underwent nerve conduction studies (NCS) at the onset and after one week of illness using Medelec Synergy equipment (TECA Synergy T5EP, Oxford Instruments, United Kingdom). Nerve conduction studies done after one week of illness were used for the diagnosis and subtyping of GBS in this study. Motor NCS was done by stimulating the common peroneal nerve (CPN), posterior tibial nerve (PTN), median and ulnar nerves. Neurophysiologic parameters obtained were compound muscle action potentials (CMAP) amplitude, distal latency, duration and conduction velocity. A sensory NCS was done to assess sural, median, and ulnar sensory nerve action potentials (SNAPs). Minimum F wave latency was also measured. Albers and Kelly electrophysiological criteria were used for the diagnosis of AIDP [22].

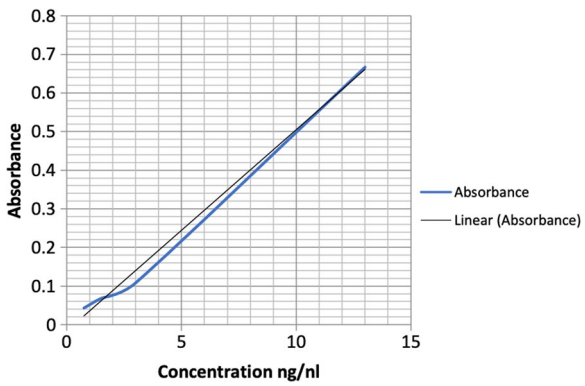

Fig. 1 Standard curve

Statistical analysis

SPSS (Version 23.0; 2015, IBM, USA) was used for statistical analysis. Continuous variables were summarized as mean ± SD and categorical variables were expressed as frequencies and percentages. Chi-square test or Fisher's exact test, whichever appropriate, was employed to compare categorical variables. Mann–Whitney U test was used for subgroup analysis among GBS cases. Spearman correlation was used to assess the correlation between CSF protein levels and antibody positivity. A p-value of less than 0.05 (< 0.05) was considered statistically significant.

Results

The mean age of cases was 39.58 (± 14.76 years) with a minimum of 14 years and a maximum of 70 years. Most of the patients (75%) were in the age group of 20–50 years. The mean age of controls was 35 (± 12.31 years). There was no significant difference in mean age between cases and controls (p value = 0.313). Out of 60 patients of GBS, 36 were male and 24 female. There was a slight male preponderance. However, there was no significant age difference between males and females. Cases and controls were also compared for gender difference; however, no significant difference was found (p value = 0.61). The frequency of admissions was more in autumn season.

The preceding illness was present in 38% of cases. Respiratory tract illness was present in 9 (15%) cases, while gastrointestinal illness was present in 8 (13%) cases. Six (10%) females were either pregnant or in puerperium (Fig. 2). Mean hospital stay was 9.15 (± 3.23) days. Most of the patients stayed for 5–9 days (Fig. 3). Patients were observed in hospital till progression stopped and were discharged once in recovering or plateau phase. Functional status was assessed using Hughes grading [21]. Mostly patients progressed to Hughes grade 3 and 4. Among GBS variants, AIDP was the most common variant accounting for 65% of cases, followed by AMAN (18.3%). Other variants observed were AMSAN (10%), Miller Fisher syndrome (3.3%), and polyneuritis cranialis (3.3%) (Fig. 4).

Around 80% of patients showed albuminocytologic dissociation. Mean CSF protein concentration was 82.64 (± 74.54) mg/dl. The difference in CSF concentration among different variants was statistically not significant (p value = 0.261) (Table 1). Antibody testing was done using ELISA in all 60 cases and 60 age and gender-matched controls. Among cases 24 (40%) tested positive for *Campylobacter jejuni* antibody. Compared to cases, only 12 (20%) tested positive for antibody among controls. The difference in antibody positivity was statistically significant between cases and controls

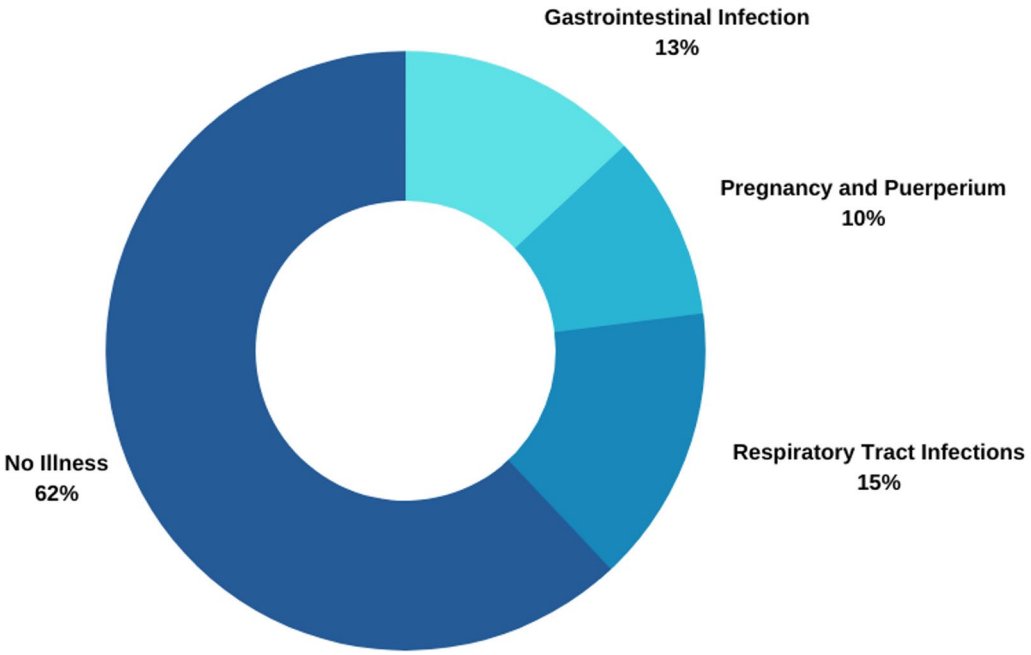

Fig. 2 Distribution of preceding illnesses in our GBS cohort

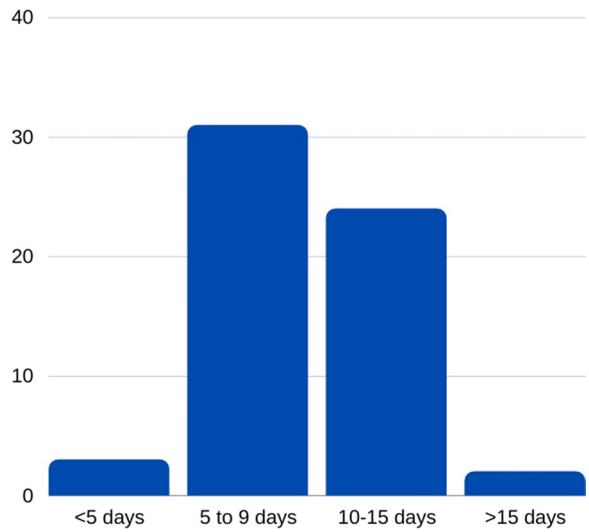

Fig. 3 Duration of hospital stay (in days) of GBS patients

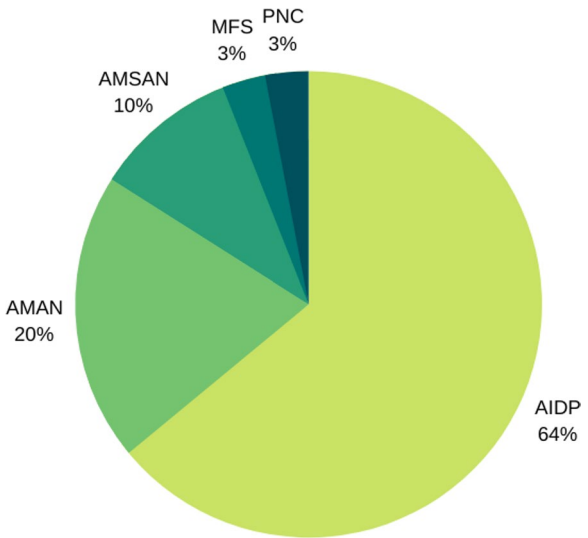

Fig. 4 Distribution of various GBS variants seen in our study

($p = 0.003$) (Fig. 5). There was no effect of preceding illness on antibody positivity (p value = 1.0). There was no statistically significant difference in antibody positivity among different variants of GBS (p value = 1.0) (Fig. 6). Out of 60 patients, 11 patients received immunotherapy while 49 received only supportive care. Out of 11 patients 8 received IVIg, 2 plasmaphereses, and 1 was managed with a combination of plasmapheresis and IVIg. Twelve patients required ventilatory support; out of which, three died.

Discussion

Preceding infections occur quite commonly in Guillain–Barre syndrome (GBS) and may differ depending upon the subtype of the clinical syndrome. *Campylobacter jejuni* infection is the most common preceding infection, seen in around 30% patients [23]. However, certain

Table 1 CSF protein concentration in different GBS variants

GBS variant	No. of patients (n)	Mean protein concentration (mg/dl)	SD
AIDP	38	98.00	88.576
AMAN	12	58.00	20.425
AMSAN	6	52.17	17.589
MFS	2	37.00	7.071
PNC	2	63.50	9.192
Total	60	82.64	74.540

AIDP acute inflammatory polyradiculoneuropathy, *AMAN* acute motor axonal neuropathy, *AMSAN* acute motor and sensory axonal neuropathy, *MFS* Miller Fisher syndrome, *PNC* polyneuritis cranialis

studies using highly specific ELISA based on two recombinant outer antigens encoded by *C. jejuni* genes, Cj0017 (P39) and Cj0113 (P18), found serological evidence of a preceding *C. jejuni* infection in 80.6% of the patients [24]. *Campylobacter jejuni* infection is one of the leading causes of bacterial gastroenteritis worldwide. It is a common cause of self-limited diarrheal illness particularly in developed world. Though many local and extraintestinal complications can follow Campylobacter infection, the most important is Guillain–Barre syndrome [25, 26]. Although Campylobacter infection is the most common preceding illness in GBS, the risk of developing GBS after Campylobacter infection is less than one per thousand *Campylobacter jejuni* infections [27].

The exact mechanism of development of this neurological illness post-Campylobacter or other infections is not known, but possibly involves molecular mimicry between peripheral nerve proteins or glycolipids and lipopolysaccharide of bacterial cell wall [28]. The risk of developing GBS post-infection may vary with the serotype that caused the infection as well as the area where infection has occurred. For example, the risk of developing GBS may be higher after infection with *C. jejuni* type O:19, a finding seen particularly in Japanese population [29].

GBS usually follows Campylobacter infection by a time lag that may be 1–3 weeks in duration. Since Campylobacter may be excreted continuously in stools for around two weeks after infection, isolation of the bacterium from the stools of many GBS patients who have a time lag of more than two weeks will not be possible. In addition, many Campylobacter infections may be asymptomatic and may not result in any diarrheal illness. Thus, stool culture to detect the presence of Campylobacter in stool specimens of GBS patients may underestimate the prevalence of preceding Campylobacter infections. Hence, serological studies have been used to document the presence of preceding Campylobacter infection in GBS patients [30].

Many studies report that GBS that follows Campylobacter infection often is severe, often requires ventilatory assistance and is associated with sever axonal damage. For example, in China preceding Campylobacter infection is associated with AMAN variant of GBS, which is associated with greater axonal injury than usual AIDP variant [31]. However, in Western countries, AMAN variant is rare though Campylobacter infections are quite common [32]. Still certain studies from Western societies report that preceding Campylobacter infection in GBS

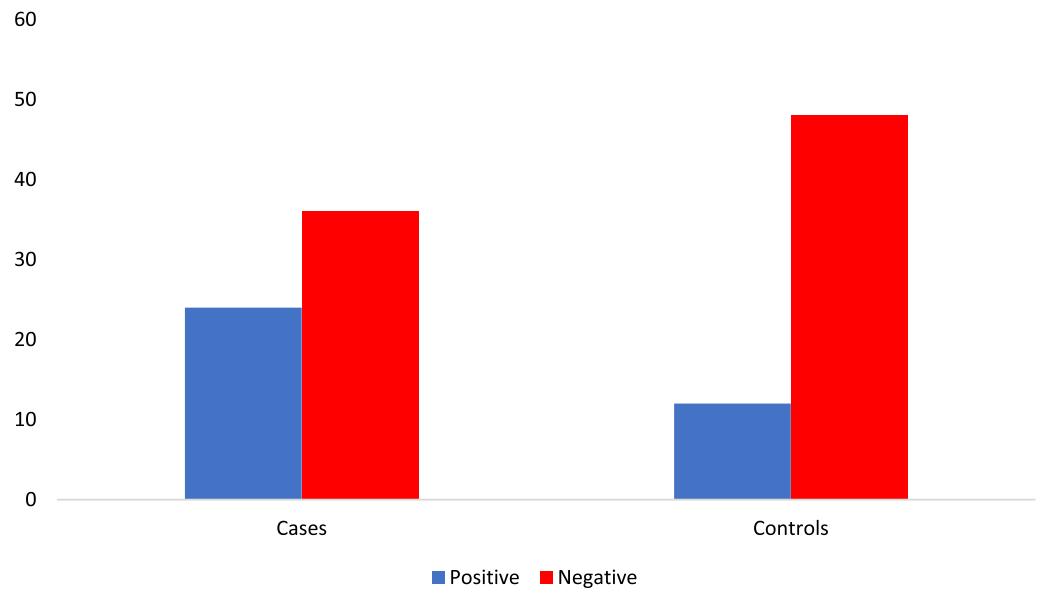

Fig. 5 Campylobacter antibody positivity in cases vs controls

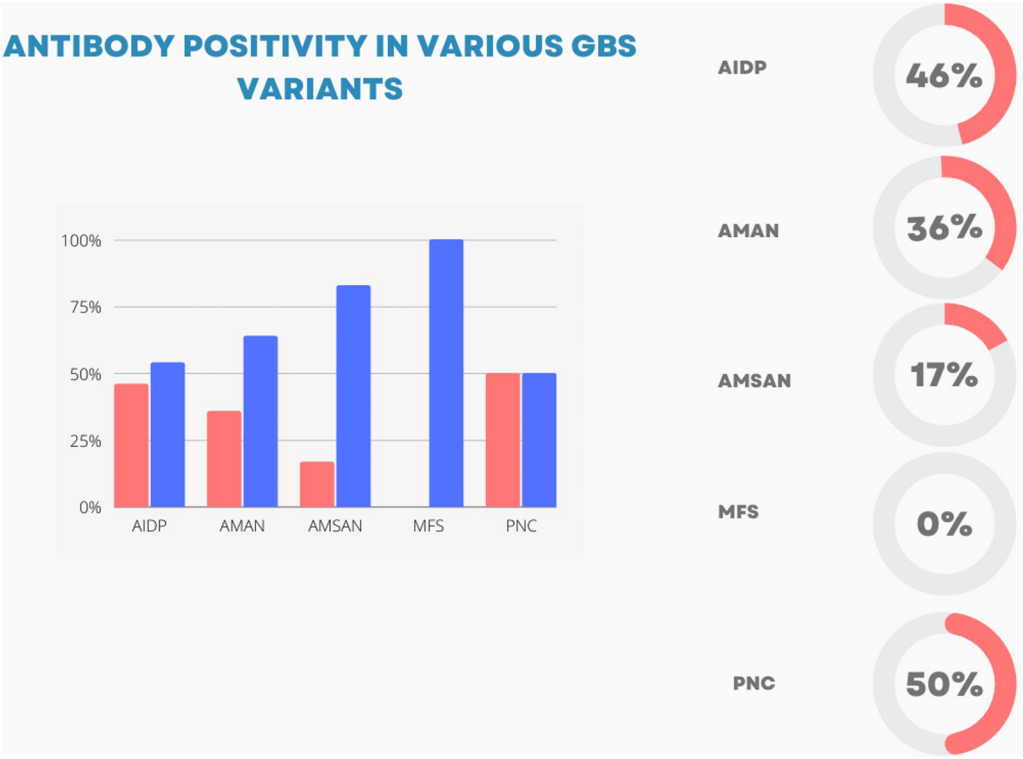

Fig. 6 Distribution of Campylobacter antibody positivity in various GBS variants in our study

is associated with axonal degeneration, slow recovery, and severe residual disability [33]. Thus, the relationship between preceding Campylobacter infection and AMAN variant of GBS may not hold true. In our study also, though serological evidence of preceding Campylobacter infection was seen in 40% GBS patients, no special relationship between preceding Campylobacter infection and subtypes of GBS was seen. However, in many other parts of India, axonal form has been reported more commonly in association with prior *Campylobacter jejuni* infection [34].

The response of GBS to therapy like IVIG and plasmapheresis is usually excellent. However, in our study, only 11 patients received immunotherapy while 49 received only supportive care. Out of 11 patients 8 received IVIg, 2 received plasmapheresis and 1 was managed with a combination of plasmapheresis and IVIg. This represents the treatment gap because of the poor economy and lack of health insurance policy in our part of the world.

Conclusions

GBS is an immune (especially post-infectious) mediated acute polyradiculoneuropathy. The most common subtype of GBS in our part of the world is AIDP followed by AMAN. Most of the cases occur in the autumn season and respiratory illness is the most common preceding illness. *Campylobacter jejuni* infection is one of the common preceding illness and serological evidence of infection is present in 40% cases compared to 20% in controls. The disease mostly affects the younger age group with a mortality of 5% and ventilatory assistance required by 20%.

Abbreviations

GBS	Guillain–Barre syndrome
AIDP	Acute inflammatory demyelinating polyradiculoneuropathy
AMAN	Acute motor axonal neuropathy
AMSAN	Acute motor sensory axonal neuropathy
MFS	Miller Fisher syndrome
PNC	Polyneuritis cranialis

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Author contributions

FM, AY collected data; WD wrote the manuscript; MuW screened manuscript for errors and did corrections; RA, MaW supervised the study.

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Availability of data and materials

Available with the corresponding author.

Declarations

Ethics approval and consent to participate

The study was approved by Institutional Ethics Committee (IEC) of Sheri Kashmir Institute of Medical Sciences (SKIMS) Srinagar on 6th Nov 2019.

Consent for publication

An informed consent was obtained from all the patients/ and/or their family members (where patient was not able to give the consent) before entering study. Identity of none of the patients is revealed in the study.

Competing interests

The authors declare that they have no competing interests.

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