RESEARCH ARTICLE

Study of Guillain-Barre syndrome etiology in Pakistani patients

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Abstract

Objective: To examine clinical features, biochemical markers, demographic features, antecedent infections, frequency and treatment strategies related to Guillain-Barré syndrome.

Methods: The case-control study was conducted at the Pakistan Institute of Medical Sciences, Islamabad, Pakistan, and the District Headquarters Hospital, Rawalpindi, Pakistan, from 2018 to 2020, and comprised Guillain-Barré syndrome patients in group A and healthy controls in group B. The patients were diagnosed on the basis of clinical presentation, nerve conduction study, electromyography, cerebrospinal fluid analysis and biochemical profile. Data was analysed using SPSS 23.

Results: Of the 167 subjects, 90(54%) were in group A and 77(46%) were in group B. The mean age of group A was 40.20±14.90 years, while there were 61(67.7%) males and 29(32.2%) females compared to 50 (64.93%) males and 27 (35.06%) females with mean age 38.40±12.34 years in group B. Acute inflammatory demyelinating polyneuropathy was the most common electrophysiological variant of Guillain-Barré syndrome 41(46%). There was significant difference in mean interleukin-17 levels between group A 23.12±3.41 pg/ml and group B 8.82±2.49 (p<0.05). Gastrointestinal infection was the most common preceding infection 51(56.66%). The mean cerebrospinal fluid protein was 100.83±51.32g/dl and albumiocytologic dissociation was found in all the four variants (p= 0.005).

Conclusion: Guillain-Barré syndrome affected patients regardless of age, while males were more affected than females. Majority of the patients had an antecedent infection before disease onset. Increased levels of interleukin-17 showed involvement of autoimmunity. Albuminocytologic dissociation differentiated it from poliomyelitis.

Keywords: Guillain-Barré syndrome, Cerebrospinal fluid, Albuminocytologic dissociation, Nerve conduction studies, Variants, Electromyography. (JPMA 71: 2539; 2021) **DOI: https://doi.org/10.47391/JPMA.202**

Introduction

Guillain-Barré syndrome (GBS) is an autoimmune disease that was first reported in 1916. It affects the peripheral nervous system (PNS). Ascending weakness resulting in acute flaccid paralysis is the striking feature of this polyneuropathy. About one-third of patients require long-term follow-up in an intensive care unit (ICU) with or without mechanical ventilation.[1]

There are four common GBS variants: acute motor axonal neuropathy (AMAN), acute inflammatory demyelinating polyneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAN) and Miller Fischer syndrome (MFS). GBS is a global disease having an annual incidence of 0.81-1.89 per 100,000 persons. In Western countries, it is reported to be increasing exponentially with growing age, and males are more affected than females.[2]

Albuminocytologic dissociation (ACD) in cerebrospinal fluid (CSF) is one of the diagnostic markers of GBS along with nerve conduction study (NCS) and electromyography (EMG).[3]

The exact aetiology of GBS is still unknown, but there is evidence of some preceding infection in approximately two-thirds of patients, mostly gastrointestinal (GI) infection and upper respiratory tract infection (URTI), and GBS follows after three to four weeks of preceding infections. The most common antecedent infection-causing agent is Campylobacter (C.) jejuni.[4,5]

Clinical presentation includes symmetrical ascending weakness, acute flaccid paralysis, areflexia, autonomic and brainstem abnormalities, numbness and tingling. The involvement of the cranial nerve may affect facial muscles, swallowing and eye movements.[6]

As GBS is an autoimmune disease, both humoral and cellular immunity are involved in pathogenesis. Plasma antibodies to gangliosides are found in about half of GBS patients. There is also evidence of lymphocytes infiltration in peripheral nerves and spinal roots, followed by macrophage-mediated stripping of myelin.[7]

Cellular immune response against GBS is increased by the release of cytokines by lymphocytes. T-helper-17 (Th-17) lymphocytes release interleukin-17 (IL-17) cytokine which is the main trigger of this autoimmunity. Both Th-17 and IL-

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17 play a crucial role in host defence mechanism and inflammatory responses.[8]

Th-17 cells are involved in autoimmune diseases of mice and humans, such as multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). Six isoforms of IL-17 are known, from IL-17A to IL-17F, but only IL-17A and IL-17F are produced by Th-17 cells. Both these cytokines are involved in inflammation production. The roles and characteristics of Th-17 and IL-17 in GBS have been poorly defined. Increased concentrations of IL-17 in CSF and plasma of GBS patients have been reported.[9]

GBS is treated by intravenous immunoglobulin (IVIG) or plasma exchange (PE), or both. Persistent severe morbidity is seen in 10-20% of cases, while the mortality rate of GBS is 3-10%.[10]

The current study was planned to explore clinical features, biochemical markers, antecedent infections and treatment of GBS, and to assess its association with demographic and biochemical features.

Patients and methods

The case-control study was conducted at the Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan, and the District Headquarters (DHQ) Hospital, Rawalpindi, Pakistan, from 2018 to 2020. The study was reviewed and approved by the University Ethics Committee of Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi during its 18th meeting held on 27th September, 2018. Demographic data was collected from the medical record of the patients, while biochemical analyses were performed from CSF and blood samples. Clinical examination was performed at the Biotechnology Laboratory, Department of Biochemistry and Biotechnology, PMAS, Arid Agriculture University, Rawalpindi. The sample comprised consecutive patients of GBS and healthy controls randomly enrolled from the community and did not have GBS or any other known autoimmune disease. Patients were included irrespective of gender and age who had been diagnosed with GBS by physicians. Patients having any autoimmune disease other than GBS were excluded. Apart from routine examination (R/E), CSF, NCS and EMG were the diagnostic tools. A questionnaire was generated to collect detailed history of the patients. Neurologists were involved in conducting NCS, EMG and physical examination of all the patients.[11]

A widely-accepted disability score[12] was used to determine the functional status of the patients. The score range was 0-6; 0 = normal functions of the body, 1 = little signs and symptoms present but able to run, 2 = having ability to walk independently for 10 meters but not able to run, 3 = having ability to walk 10 meters with assistance, 4 = bound to chair or bed, 5 = requiring assisted ventilation, 6 = death.[12]

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and serum separation tube (SST). After centrifugation, plasma was stored at -20 degrees centigrade. CSF samples were collected by lumbar puncture technique after two weeks of GBS onset. The clinical chemistry profile was performed on AU-480 automated chemistry analyser (Beckman coulter, USA), haematology profile was performed on Mindray BC-2800 automated haematology analyser (Mindray, China). IL-17 levels were measured by enzyme-linked immunosorbent assay (ELISA), using commercially available kits (PromoCell, Germany) according to manufacturer's instructions.[13]

Data was analysed using SPSS 23. Mean values and standard deviations as well as percentages and frequencies were calculated. Data was subjected to Student's t-test, chi-square test and analysis of variance (ANOVA). P<0.05 was considered statistically significant.

Results

Of the 167 subjects, 90(54%) were in group A and 77(46%) were in group B. The mean age of group A was 40.20±14.90 years, while there were 61(67.7%) males and 29(32.2%) females compared to 50 (64.93 %) males and 27 (35.06 %) females with mean age of 38.40±12.34 years in group B. Among the male patients, there were 24(39.3%) with AIDP,

Table-1: Cerebrospinal fluid (CSF) analysis in Guillain-Barré syndrome (GBS) patients.

GBS variant (n)	Mean±SD	Minimum	Maximum	p- value
AIDP (41)	119.65±51.25	25.0	186.0	0.005
AMAN (33)	91.62±45.55	30.0	198.0	
AMSAN (14)	68.28±48.58	18.0	197.0	
MFS (2)	92.50±3.53	90.0	95.0	
Total (90)	100.80±51.32	18.0	198.0	

AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, AMSAN: Acute motor and sensory axonal neuropathy, MFS: Miller Fischer syndrome. Significant difference of CSF protein between AIDP and AMSAN, (p=0.001, followed by AIDP and AMAN (p=0.015).

Table-2: Comparison of IL-17 levels between cases and controls.

Subjects (%)		Mean±SD	Minimum	Maximum	p- value
GBS variants	AIDP (46)	23.04±4.27	15.3	29	0.031
	AMAN (37)	19.89±3.91	16.4	27.8	
	AMSAN (16)	20.64±4.28	15.0	25.5	
	MFS (2)	24.45±1.48	23.0	24.45	
Cases	GBS	23.13±3.42	15.00	29.00	<0.001
Controls	HC	8.82±2.49	5.00	13.00	

AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, AMSAN: Acute motor and sensory axonal neuropathy, MFS: Miller Fischer syndrome, SD: Standard deviation, HC: Healthy controls.

Table-3: Antecedent infections and demographic features of GBS patients.

Parameter	AIDP	AMAN	AMSAN	MFS	Total	*p*-value
Gender, n (%)						0.034
Male	24 (58.53)	21 (63.23)			61 (67.77)	
female						
	17 (41.46)	12 (36.36)	14 (100)	2 (100)	29 (32.22)	
Antecedent infection, n (%)						0.013
GI	30 (73.17)	15 (45.4)	5 (35.71)	1 (50)	51 (56.66)	
URTI	7 (17.07)	3 (9.09)	2 (14.28)		12 (13.33)	
Fever		7 (21.2)	1 (7.14)		8 (8.88)	
None	4 (9.75)	8 (24.2)	6 (42.85)	1 (50)	19 (21.11)	
Seasonal trends, n (%)						<0.001
Summer	41 (100)	14 (42.4)			55 (61.11)	
Winter		3 (9.09)			3 (3.33)	
Autumn		9 (27.2)	5 (35.71)		14 (15.55)	
Spring		7 (21.02)	9 (64.28)	2 (100)	18 (20)	
Residence, n (%)						<0.001
Urban	30 (73.17)				30 (33.3)	
Rural	11 (26.82)	33 (100)	14 (100)	2 (100)	60 (66.6)	

AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, AMSAN: Acute motor and sensory axonal neuropathy, MFS: Miller Fischer syndrome, GI: Gastrointestinal infection, URTI: Upper respiratory tract infection.

21(34.4%) with AMAN, 14(23%) with AMSAN and 2(3.3%) with MFS. Among the female patients, 17(58.6%) had AIDP and 12(41.4%) had AMAN.

The mean CSF protein was 100.83±51.32g/dl and ACD was found in all the four variants (p= 0.005) (Table 1). There was significant difference in mean IL-17 levels between group A 23.12±3.41pg/ml and group B 8.82±2.49pg/ml (p<0.05). AIDP was the most common electrophysiological variant of GBS 41(46%) (Table 2).

Gastrointestinal infection was the most common preceding infection 51(56.66%), followed by URTI 12(13.33%) and fever 8(8.88%). Also, 19 (21.11) GBS patients had no antecedent infection (Table 3).

The incidence of GBS during the summer, from May till September, was 55(61%) patients, followed by 18(20%) during spring, from March to April, 14(13.72%) during autumn, from October to November, and 3(3.33%) during the winter, from December till February).

Disability scoring showed there was no patient with score 0-1, 10(11.1%) patients had score 2, 30(33.3%) had score 3, 26(28.9%) had score 4, 23(25.5%) had score 5 and 1(1.1%) patient died at score 6.

Discussion

GBS is an autoimmune polyradiculoneuropathy. Activation of adaptive immunity plays an important role in this autoimmunity. There are several aetiological agents of GBS. The most widely accepted theory is that GBS is caused by molecular mimicry between lipopolysaccharides in the cell wall of C. jejuni and ganglions of human nerves. C. jejuni causes diarrhoea in humans and is found to be the causative agent of more than 30% of GBS cases. Other known aetiological agents are cytomegalovirus and Epstein-Barr virus.[14] IL-17, a cytokine released by Th-17, promotes migration of neutrophils and plays an inflammatory role in the development of the disease. The current study showed that IL-17 levels were markedly increased in GBS patients when compared to the healthy population samples. CSF is considered to reflect events in the blood-nerve barrier. ACD was seen in all GBS variants with increase in AIDP. This may be due to myelin sheath degeneration in AIDP.[15] ACD differentiated paralysis caused by GBS from paralysis caused by poliomyelitis. CSF analysis in GBS showed increased protein concentration with normal or fewer ACD cells, while CSF analysis in poliomyelitis had normal protein concentration with increased cell count. Autonomic symptoms, such as vasomotor disturbances, urinary retention and constipation, are also very frequent in GBS.[16] Very little data on GBS is available in Pakistan because of being a rare disease, and remote areas of Pakistan have very poor health facilities and data maintenance. Many patients remain undiagnosed due to a lack of health facilities. The current study had 90 GBS patients having males more infected than females showing a gender ratio of 2.1:1. This ratio was in line with 2.1:1, and higher than 1.6:1 reported earlier in Karachi.[17] CSF with increased protein level and white blood cell (WBC) count ≤10/cmm was taken as an indicatin of ACD. In the current study, mean CSF protein of GBS patients was 100.80±51.32mg/dl ranging 18-198mg/dl. Another study showed raised mean level of CSF proteins 131.1±110mg/dl.[18] AIDP was found as the most common subtype of GBS, followed by AMAN and AMSAN. A study also reported AIDP as the most common GBS variant.[19] The most striking feature of GBS is ascending weakness followed by a tingling sensation, while several patients face walking difficulty, paresthesia and numbness, and some patients suffer from respiratory distress and require assisted ventilation.[20,21] The patients in the current study were offered treatment by intravenous immunoglobulin (IVIG), plasma exchange (PE), or IVIG preceded by PE in line with literature, the results suggested that both therapies are equally effective.[22] Most of the patients in the current study had complained of diarrhoea which was the commonest preceding event. URTI was the second most common antecedent infection. The mean age of GBS patients was 40.20 years. Age >50 years was more susceptible to GBS as 36% patients fell in that age group. Young age group was comparatively less affected.[13,24]

Conclusions

ACD helped in GBS diagnosis. Increased level of IL-17

underlined the status of autoimmune disease. Males were more affected than females, while most of the patients had GBS during summer months.

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Conflict of Interest: None.

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