ORIGINAL REPORT

The Role of *Cytomegalovirus, Haemophilus Influenzae* and *Epstein Barr Virus* in Guillain Barre Syndrome

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Abstract- Guillain Barre Syndrome (GBS) is an inflammatory, usually demyelinating, polyneuropathy; clinically characterized by acute onset of symmetric progressive muscle weakness with loss of myotatic reflexes. Thirty five patients with GBS, defined clinically according to the criteria of Asbury and Cornblath, were recruited from three hospital affiliated to Tehran University of Medical Sciences. Controls: As a control group 35 age and sex matched patients with other neurological diseases admitted to the same hospital at the same time, were included in our study. Serum samples were collected before treatment from each patient (within 4 weeks after the disease onset) and controls, and stored frozen at -80ºC until serologic assays were done. Serologic testing of pretreatment serum was performed in all patients. Positive titer of virus specific IgM antibody against cytomegalovirus (CMV) was found in 6 cases and 2 controls. 34 patients and 31 controls had high titer of anti *Haemophilus influenzae* IgG and one patient had serologic evidence of a recent Epstein Barr virus (EBV) infection. The mean titer of IgG antibody against *Haemophilus influenzae* in cases and controls was 5.21 and 2.97 respectively. Although serologic evidence of all these infections were more frequent in cases than in controls, only *Haemophilus influenzae* infection appeared to be significantly related to GBS (P=0.002). Eleven cases and 3 controls had high titers of IgG antibody against *Haemophilus influenzae* type B (titer >8). There is significant association between high titer of IgG antibody against *Haemophilus influenzae* and GBS (P=0.017). Our results provide further evidence that *Haemophilus influenzae* and probably CMV, can be associated with GBS.

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Introduction

Guillain Barre Syndrome (GBS) is an inflammatory, usually demyelinating, polyneuropathy; clinically characterized by acute onset of symmetric progressive muscle weakness with loss of myotatic reflexes (1). Since the near elimination of poliomyelitis throughout the world, GBS ranks as the most frequent cause of acute flaccid paralysis (2). Up to 30% of patients with GBS develop respiratory insufficiency requiring assisted ventilation and between 2 to 5% die of complications. Its pathogenesis remains incompletely defined. In about two thirds of the GBS patients, there is a history of preceding infectious illness, predating the disease by up to 4 weeks. Various organisms, including Cytomegalovirus (CMV), Epstein Barr virus (EBV), Varicella zoster virus, Hepatitis A and B, *Mycoplasma pneumonia*, *Haemophilus influenzae* and *Campylobacter jejuni* (CBJ), have been proposed as being associated with GBS, but apart from CBJ, which has been identified as the most common cause of preceding infection, the causal relationship between infectious agents as prodromal illness and peripheral nerve damage is not known (1,3-9). GBS includes several distinctive subtypes. The critical differences among the subtypes are mainly in terms of the pathological and electrodiagnostic features (4,10,11). The variety of infections seems to contribute to the clinical heterogeneity of GBS (4).

Furthermore, geographical differences (*e.g.* different

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immunogenetic background) may produce different susceptibilities to these organisms in different populations (4). The aim of this study was to investigate the association between GBS and recent infection with CMV, EBV and *H. influenzae* in Iranian patients and to clarify the clinical, electrophysiological and laboratory features of GBS subsequent to these infections.

Patients and Methods

Subjects: Thirty-five patients with GBS were recruited from three general hospitals affiliated to Tehran University of Medical Sciences. The diagnosis of GBS was defined clinically according to the criteria of Asbury and Cornblath. All the subjects were asked about recent infections, using a structured questionnaire, in the preceding 4 weeks. Subjects were also evaluated with respect to the presence of paresthesias, sensory deficits (position sense and tactile function in hands and feet), cranial nerve involvement, arms grade, functional score (Hughes' functional grade) and medical research council (MRC) sum-score at the nadir of the illness (5,7,8). The electro physiologic study and CSF features of GBS patients were also determined.

Controls

As a control group, thirty-five age and sex matched patients with other neurological diseases admitted to the same hospital at the same time were included.

Serum samples were collected before treatment from each patient (within 4 weeks after the disease onset) and controls, and stored frozen at -80^{o}C until serologic assays were done.

Serologic tests: serum anti *H. influenzae* antibody (IgG) titer was measured by the enzyme-linked immunosorbent (ELISA) assay. To determine IgM and IgG antibodies to EBV (VCAp18) and CMV, ELISA was used.

Results

During the two year study period 35 cases and 35 controls were enrolled in the study. The mean age (±SD) in the case group (GBS patients) was 42.45 (±19.89) years (ranging from 16 to 83) and in the controls, 42.37 (±19.53) years (P>0.05). Twenty six (73.3%) were male in each group. GBS cases clearly had a seasonal preponderance, most cases occurring in fall and winter (18 in winter, 9 in fall, 5 in summer and 3 in spring).

Physical examination revealed cranial nerve deficits in 9 (26.5%) of the examined GBS patients. The cranial nerve deficits were bilateral facial palsy in 6, facial and bulbar palsy in 2, and ophthalmoplegia, bulbar and facial palsy in one patient. Sensory involvement occurred in 26 patients, which was mild (sensory grade =<2) in 12 (35.2%), moderate (2<sensory grade≤ 6) in 11 (32/3%) and severe (sensory grade >6) in 3 (8.8%). Eight (23.5%) GBS patients had no clinical sensory involvement and in one patient accurate assessment of severity of sensory loss were not possible.

Sufficient data to evaluate Hughes' disability grade was available only in 31 patients. According to the available data, four patients had Hughes' score 2, nine had 3, seventeen had 4 and one patient had Hughes' score 5. At the peak of the illness, the mean (± SD) of MRC sum-score in upper and lower limbs of the case patients was 25.28 (±8.42) and 21.08 (±8.19) respectively.

Our patients were classified as 27 cases of typical GBS and 8 cases of pure motor type. None of our patients suffered from paraparetic, Miller-Fisher, pure sensory and other variants of the disease. The Arms grade was evaluated in 32 patients. This disability scale was zero (normal) in 3 patients, 1 in 11, 2 in 9, 3 in 6 and 4 in 4 patients.

Lumbar puncture was performed in 25 patients; of these, CSF protein was highly elevated (>=100 mg/dl) in 15 (60%) patients, elevated (45 mg/dl=<CSF protein<100mg/dl) in 5 (16%) and normal in 4 (16%). Based on the results of the electrodiagnostic studies, the AIDP pattern was found in 16 (51.6%) GBS patients, the AMAN pattern in 6 (19.4%), the AMSAN pattern in 5 (16.1%) and the pure sensory pattern in 3 (9.7%). one patient had normal nerve conduction studies (NCS) and in 4 patients the NCS results were not available.

In the 4 weeks preceding the onset of GBS, 29 (82.9%) of the 35 patients reported some forms of infectious illness whereas only 29% of control had positive history of infection [P<0.001 odds ratio (95% confidence interval) = 11.81 (3.65-38.15)]. Positive history of respiratory and gastrointestinal infection was found respectively in 57.1% and 20% of patients as compared with 22.6% and 6.5% in controls. The analysis showed that respiratory infection but not gastrointestinal infection was significantly associated with GBS [P=0.004 odds ratio (95% confidence interval) 4.57 (1.55-13.4)]. The mean (± SD) interval between the infectious illness and neurologic symptoms was 11 (± 8.21) days for respiratory infection and 15.85 (± 9.33) days for gastrointestinal infections.

Serologic testing of pretreatment serum was performed in all patients. Positive titer of virus specific

IgM antibody against CMV was found in 6 (17.1%) cases and 2 controls (5.7%). Thirty-four (97.1%) patients and 31 (88.6%) controls had high titer of anti *H. influenzae* IgG and only one patient had serologic evidence of a recent EBV infection.

The mean titer of IgG Antibody against *H. influenzae* in cases and controls was 5.21 and 2.97 respectively. Although serologic evidence of all these infections were more frequent in cases than in controls, only *H. influenzae* infection appeared to be significantly related to GBS (P=0.002). Eleven cases and 3 controls had high titers of IgG antibody against *H. influenzae* type B (titer>8). There is significant association between high titer of IgG Antibody against *H. influenzae* and GBS (P=0.017).

Of the six patients who had positive anti CMV IgM titer, 4 were men and 2 were women. The median age for these patient was 33 (range 29 to 78). The majority of GBS patients with positive CMV serology had clinical symptoms of preceding infection. Histories of respiratory symptoms were given by 4 patients, one patient diarrhea and only one patient did not have preceding infection. Sensory complaints found in 4 patients, 2 had moderate sensory deficits and one had severe sensory deficit. The average MRC sum-score in upper and lower limbs was 25 to 25.33 respectively (the mean of MRC sum-score in upper and lower limbs of all cases was 25.28 and 21.08).

The mean disability score in 6 CMV positive cases was 3 (all cases: 4.4). Only one patient had cranial nerve deficit. The clinical type of the CMV positive patients was typical in 4 and pure motor in 2. Of the 5 patients with CMV infection who also had an electrophysiologic study, 2 had AIDP, 2 AMSAN and one case had pure sensory pattern (Table 1).

Characteristics of the patients with high titer of IgG antibody against *H. influenzae* type B

The mean age of the 11 GBS patients in this group was 39.9 years. 9 (81.8%) patients were men and 2 (18.2%) were women. of the 11 patients, respiratory tract infection 4 weeks before GBS onset occurred in 8 (72.7%) and gastrointestinal infection occurred in 2 (18.2%).

Cranial involvement was found in 4/11 (36.4%) patients (9/35 in all GBS patients), the average of MRC sum-score of upper and lower limbs were 25 and 15.3 respectively (25.28 and 21.8 respectively in all GBS patients). 9 patients had typical GBS and 2 (18.2 %) had pure motor type. Sensory deficit occurred in 9 patients and included sensory grade 8 in 2 patients, 6 in 2, 4 in 1, 2 in 3 and 3 in one patient. Electrophysiologic examination demonstrated AMSAN in 3 patients, AMAN in 2 AIDP in 3 patients. Nerve conduction study was not carried out in 2 patients.

Illustrative clinical and paraclinical history of the EBV positive patient

The only patient with antecedent EBV infection was a 30 year old man presented with acute flaccid paralysis. There was a history of a viral upper respiratory tract infection 3 days before the onset of neuropathy. He had bilateral facial weakness; motor exam showed MRC sum-score of 26 in his arms and MRC sum-score of 24 in his legs. He was areflexic and had paresthesia in his hands and feet (his sensory grade was 4). The Hughes' score and arms grade were 4 and 2 respectively. CSF analysis on day 7 revealed 10 cells/ml and 13 mg/dl protein. The clinical type of disease was typical GBS and the NCV showed AIDP pattern

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Table 1. Characteristics of the patients with GBS who had CMV infection.

	Patient					
	1	2	3	4	5	6
Age/Sex	31/m	26/m	29/m	36/m	75/f	56/f
Preceding infection	Resp	Resp	Resp	Resp	-	GI
Cranial nerve Involvement	-	-	-	FP	-	-
Clinical type	Classic	Classic	Pure motor	Classic	Classic	Pure motor
Electrophysiological diagnosis	AMSAN	Pure sensory	AIDP	ND	AIDP	AMSAN
CSF pr	108	155	ND	170	85	22
CSF cell	10	0	0	10	0	0
MRC sum-score of upper limbs	32	32	16	22	16	32
MRC sum-score of lower limbs	24	36	26	26	16	24
Hughes grade	3	2	ND	2	4	4
Arms grade	1	1	ND	1	3	1

Resp: Respiratory infection; GI: gastrointestinal infection; FP: facial palsy; ND: Not Determined

Discussion

The purpose of this study was to investigate the relationship between the history of respiratory and gastrointestinal infection, as well as the history of CMV, EBV, and *H. influenzae* infection with GBS. Furthermore, we made attempt to determine the clinical and electrophysiological characteristics of GBS after these infections. The results of this study revealed that the history of recent infection, especially respiratory infection, is clearly related to the development of GBS. History of respiratory infection, and recent infection with CMV and EBV, and also previous infection with *H. influenzae* type b (HIB) (4), in patients with GBS is more common than in patients with other neurologic diseases. Our study may illustrate the probable role of these factors in the development of GBS.

Most of previous studies have demonstrated the relationship between recent infection and GBS (3). In our study, 82.9% of patients in the case group had the previous history of infection, which was 57.12% respiratory infection and 20% gastrointestinal infection.

Our results indicate a strong relationship between previous respiratory infection and GBS; on the other hand, in gastrointestinal infection such relationship was not significant.

Despite the lack of significant relationship between the recent infection with CMV, and GBS (P=0.259), 17.1% of the cases had positive serological evidence in favor of recent infection with this pathogen in comparison to 5.7% of the controls. These results match the results of the previous surveys. For instance, in the case-control study of Jacobs *et al.* CMV was the most common viral factor related to GBS and serological evidence of recent infection with CMV was present in 13% of GBS patients (12).

Visser *et al.* studied the clinical and the electrophysiological characteristics of 20 patients with GBS related to CMV and compared it with the previous results of studying 43 patients with GBS related to CBJ, and 71 other patients who were not related to any of these two infections. They had come to this conclusion that the clinical pattern of GBS related to CMV is different from the two other groups, in the way that these patients are younger than the other two groups, they need more assisted ventilation, the sensory nerve involvement is more severe, and the cranial nerve involvement is more common (5). The results of our study are, to some extent, different. 5 out of 6 patients with the history of CMV infection did not have cranial nerve involvement. The average of sensory nerve involvement in these 6 patients had no difference with other patients. None of these patients required assisted ventilation. On the other hand considering that 2 of these patients had AIDP pattern, 2 had AMSAN pattern, and 1 of them had pure sensory pattern. We can conclude that in the patients with positive history of CMV infection, electrophysiologic evidence of sensory nerve involvement is common. A few of these studies took the relationship between GBS and history of infection with EBV into consideration; although the results of these studies support the possible role of EBV in the development of GBS. In 10% of the patients of Jacobs *et al.* there was serologic evidence of the recent infection with EBV (12). In this survey the positive EBV IgM was seen in 2.5% of the patients with GBS, which was 1 of 35 patients. It is evident that there is the need for doing more studies with more samples in order to proof this theory. The only patient in this study, who had the positive history of recent infection with EBV, had bilateral facial palsy and bladder dysfunction. The clinical type was classic GBS and the electrophysiological type was AIDP.

Concerning *H. influenzae* infection, various studies have different antigens in their serologic assays. *H. influenzae* is a gram negative bacterium which is defined as two types of strains: encapsulated and non-encapsulated (non-typable). Gervaix *et al.* showed that there is a relationship between vaccination with HIB vaccine and GBS (13). The results from the study of Kago *et al.* who used outer membrane protein of type B H influenzae revealed that history of HIB infection can be seen in 7% of Miller-Fisher syndrome patients, and 2% of GBS patients. Comparing history of this infection in MFS patients with its history in the control group was significant (P<0.0001) (6).

Mori *et al.* used non-encapsulated *H. influenzae* in their study. The result revealed that 13% of patients with GBS have positive non-encapsulated *H. influenzae* antibody titer. This percentage had significant difference with the control group (P=0.03) (14). In the study of Jacobs *et al*. 1% of 154 patients with GBS had history of *H. influenzae* infection, but these researchers did not mention the type of antigen they used (12).

In our study, the patients of case and control group were studied only in terms of the existence and HIB IgG titer. Measuring only IgG, may have lead to the overestimation of HIB positive patients in our study. In this survey 97.1% of patients in the case group and 88.6% of patients in the control group had a history of previous infection (positive IgG titer) with *H. influenzae*, and the relationship between this history and

GBS was not significant. But the average of HIB IgG titer in the patients with GBS was significantly more than the control group (5.21 *vs.* 2.97, *P*=0.002 respectively). In addition, a difference in the populations of GBS patients studied may have affected the frequency. Finally, geographical differences may produce a difference in frequency. Different immunogenetic background, for example different human leukocyte antigen haplotype, may produce different susceptibilities to *H. influenzae*.

Our results provide further evidence that *H. influenzae* and probably CMV, can be associated with GBS.

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