Association of *Campylobacter jejuni* infection and Guillain-Barré syndrome: a cohort study in the northwest of Iran

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Recent studies have suggested that *Campylobacter jejuni* is a common pathogen causing Guillain–Barré syndrome (GBS). This study aimed to determine the frequency and clinical and electrophysiological features of *C. jejuni* infection in children with GBS. We carried out a prospective study on a cohort of 48 children with GBS admitted to Tabriz Children’s Hospital in the northwest of Iran from January 2003 to March 2005. Serologic investigations were used to diagnose preceding *C. jejuni* infection. Evidence of a recent *C. jejuni* infection was found in 23 (47.9%) of the patients. *C. jejuni*-associated GBS patients were younger than others (p=0.010), and they had a rapid progression to reach peak disability (p=0.018). Neither the peak disability nor the residual one-year disability was different between the *C. jejuni*- positive and *C. jejuni*-negative patients. The patients with preceding *C. jejuni* infection were more likely to have axonal neuropathy (p=0.021).

Key words: Campylobacter jejuni, clinical, electrophysiological, Guillain-Barré syndrome.

Since the marked decline in poliomyelitis incidence, the Guillain-Barré syndrome (GBS) has become the most common cause of acute flaccid paralysis. The GBS is an autoimmune disorder of the peripheral nervous system resulting in progressive weakness and areflexia. The average annual incidence of clinically defined GBS in the developed world is between 1 and 4 cases per 100000 population. Epidemiologic studies in all parts of the world have confirmed the association between GBS and previous acute infection, especially of the respiratory or gastrointestinal tract. Many studies have reported that 50%-75% of patients had an infectious illness 1-3 weeks before onset of neurologic symptoms. Previous diarrheal illness occurred in 10-30% of patients. Bacteria of the *Campylobacter jejuni* (*C. jejuni*), a gram-negative rod, are now a common cause of bacterial gastroenteritis in both developed and developing countries. It has been recently identified as a major cause of antecedent infections in GBS patients. *Campylobacter* is thought to stimulate this autoimmune disease through a mechanism called molecular mimicry, whereby campylobacter contains ganglioside-like epitopes in the lipopolysaccharide moiety that elicit autoantibodies reacting with peripheral nervous system antigens.

Serological studies have shown that the frequency of preceding *C. jejuni* infection in GBS patients ranges from 15% in Italy to 62% in China. Differences in various frequencies of *C. jejuni*-associated GBS could be related to different assay systems and criteria used in the diagnosis of *C. jejuni*. Since the mean duration of fecal excretion of *C. jejuni* in an infected person is only 16 days, and because of the 1- to 3-week lag between infection and the onset of GBS, many GBS patients with preceding *C. jejuni* infection might have falsely negative stool cultures. Thus, due to the limitations of culture techniques, serologic studies in combination with clinical histories are useful in identifying patients. A variety of antibody assays for detecting isotype specific antibodies
have previously been reported. Enzyme-linked immunosorbent assay (ELISA) appears to be the most commonly used method to measure antibodies in serum.\textsuperscript{7,12,13}

The immune response to \textit{C. jejuni} infection is similar to that in other infectious diseases: IgG and IgM levels in serum rise in response to infection and remain high for 3-4 weeks before declining to the baseline; IgA serum levels, however, rise during the first few weeks of infection and then fall rapidly.\textsuperscript{14,15}

This study was designed to determine the frequency and clinical and electro-physiological features of \textit{C. jejuni} infection in children with GBS.

**Material and Methods**

Between January 2003 and March 2005, 60 children with the diagnosis of GBS were admitted to Tabriz Children’s Hospital (a 200 -bed acute-care teaching hospital) and were included in this study. This hospital is the largest children’s medical center in the northwest of Iran, providing tertiary referral care for critically ill patients.

The diagnosis of GBS was defined clinically according to the criteria of Asbury and Cornblath.\textsuperscript{1}

All the patients were asked about recent infection, vaccinations or surgical procedures (in the last month) using a structured questionnaire. The clinical details including cranial nerve abnormality, autonomic abnormalities, cerebrospinal fluid (CSF) protein levels, status at the time of admission, duration of acute hospital stay, days of mechanical ventilation, status at discharge and outcome at 3, 6 and 12 months were recorded. The functional grade of motor deficit was calculated according to the standard GBS score as follows: 0: healthy, 1: minor signs and symptoms and capable of running; 2: able to walk 5 meters without assistance, but unable to run; 3: able to walk with assistance, 4: confined to bed or chair bound, 5: requires assisted ventilation, and 6: exitus.\textsuperscript{16}

All patients underwent at least one electro-physiologic examination in the acute phase of disease. Nerve conduction studies included motor nerve conduction (MNC), sensory nerve conduction (SNC) and F-wave response studies performed using the standard techniques of supramaximal percutaneous nerve stimulation and surface electrode recording. MNC studies were performed on posterior tibialis, ulnar and peroneal nerves and SNC on median and sural nerves. Each value of MNC, SNC and F-wave latency was compared with age-matched normal values. Needle electromyography (EMG) was performed in all patients in at least two proximal and two distal limb muscles. The electrodiagnostic classification of the patients was based on electrophysiologic criteria for subtypes of GBS as defined by Hadden et al.\textsuperscript{17}

Poliovirus infection was excluded by cultures that are routinely performed for patients with acute flaccid paralysis as a requirement of the national program of poliomyelitis eradication. Serum samples were obtained 1-7 days after the onset of GBS. Sera were stored at -80°C until tested. Serum samples were screened for IgG and IgM antibodies against \textit{C. jejuni} by ELISA (ELISA Kit, Diapro, Italy) at dilutions of 1:200 and 1:2000. Patients were considered \textit{C. jejuni}-positive if they had high optical densities for both IgM and IgG classes at serum dilutions of 1:200 and 1:2000.

Microbiologic investigations for \textit{C. jejuni} were not performed in this study.

All subjects received, in addition to supportive care, 400 mg/kg of intravenous immunoglobulin for five days.

**Statistical Methods**

Differences in proportions were statistically tested by chi-square or Fisher’s exact test. All other comparisons between groups were made with Student’s unpaired t-test or Mann-Whitney U test.

**Results**

We recruited 60 children with GBS. Twelve patients were subsequently excluded from final analysis due to insufficient serum sample for serological studies. Twenty-three (47.9%) patients were found to be positive for \textit{C. jejuni}.

The most common manifestation was limb weakness, with various degree of motor weakness, in all subjects. On admission, all patients had areflexia or hyporeflexia. Sensory symptoms, mostly as pain (limbs), were noted in 18 of 48 patients (37.5%). Explanation
of sensory symptoms such as numbness or paresthesia was not sufficiently reliable due to the young age of our patients. Autonomic dysfunction (urinary and arrhythmia) was observed in 5 patients only.

Demographic and clinical features of C. jejuni-positive and C. jejuni-negative patients are presented in Table I.

The mean (SD) age of patients was 5.1 (±3.3) years. Subjects were grouped as aged ≤5 years and >5 years: 78.3% of C. jejuni-positive subjects vs 48% of C. jejuni-negative patients were under 5 years (Odds ratio [OR]: 3.9, 95% confidence interval [CI]: 1.10-13.80; p=0.031).

The mean (SD) time to reach peak disability was 5.8 (±6.6) days: 3.5 (±3.0) days for C. jejuni-positive and 7.9 (±8.2) days for C. jejuni-negative patients (p=0.018).

Only 3 of 48 patients (6.3%) were able to walk independently at the peak of illness (grade 0-2). Overall, 81.2% of our patients were in grade ≥4: 73.9% of C. jejuni-positive compared with 68% of C. jejuni-negative subjects (p=0.63). Three (6%) patients received assisted ventilation (grade 5), and all of them were C. jejuni-positive (p=0.09).

There were complications in 9 patients (18%), mostly pulmonary symptoms. There was, however, no significant difference between the two groups (p=0.50). No mortality was observed.

All cases were followed up for one year. At the third month, 15 (7 C. jejuni-positive and 8 C. jejuni-negative) patients were not able to walk independently (p=0.63). At the sixth month, 6 patients (4 C. jejuni-positive and 2 C. jejuni-negative) were not able to walk independently (p=0.71). At the twelfth month, 3 patients (1 C. jejuni-positive and 2 C. jejuni-negative) were not able to walk independently (p=0.55).

Albuminocytologic dissociation on CSF examination (defined as CSF protein >40 mg/dl and CSF cell count <15/mm$^3$) was detected in 35 (73%) of all patients. In 13 (27%) patients, protein levels were normal; CSF was obtained only in the first week of illness. There was no significant difference in CSF findings between the two groups.

Neurophysiological examination was performed by a physiatrist, and four patterns emerged: Demyelinating type in 21 (43.8%) patients, axonal type in 18 (37.5%) patients, demyelinating type of GBS with secondary axonal loss (mixed type) in 5 (10.4%) patients, and unclassified in 4 (8.3%) patients (Table II).

When unclassified patients were excluded, the patients with preceding C. jejuni infection were more likely to have acute axonal neuropathy or axonal degeneration in association with acute inflammatory demyelinating polyradiculoneuropathy (OR: 3.98, 95% CI: 1.2 -13.24; p=0.021).

### Table I. Demographic and Clinical Features of Patients with GBS

<table>
<thead>
<tr>
<th></th>
<th>C. jejuni-positive</th>
<th>C. jejuni-negative</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Age ≤5 years</strong></td>
<td>18 (78.3%)</td>
<td>12 (48%)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Age &gt;5 years</strong></td>
<td>5 (21.7%)</td>
<td>13 (52%)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (male/female)</strong></td>
<td>11/12</td>
<td>16/9</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Preceding events</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Upper respiratory infection</td>
<td>12 (52.2%)</td>
<td>13 (52%)</td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>8 (34.8%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td>1 (4.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Mumps</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (8.7%)</td>
<td>9 (36%)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Seasonal incidence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>4 (17.4%)</td>
<td>7 (28%)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>7 (30.4%)</td>
<td>5 (20%)</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>8 (34.8%)</td>
<td>9 (36%)</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>4 (17.4%)</td>
<td>4 (16%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Cranial nerve involve (n=13)</td>
<td>8 (34.8%)</td>
<td>5 (20%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Autonomic change (n=5)</td>
<td>3 (13%)</td>
<td>2 (8%)</td>
<td>0.60</td>
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</tbody>
</table>
Table II. Electrophysiologic Pattern of Patients with GBS

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>C. jejuni-positive n=23</th>
<th>C. jejuni-negative n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDP</td>
<td>5 (21.8%)</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>Acute motor or motor and sensory axonal neuropathy</td>
<td>12 (52.2%)</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>AIDP + AD (mixed type)</td>
<td>3 (13%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>3 (13%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

AIDP: Acute inflammatory demyelinating polyradiculoneuropathy. AD: Axonal degeneration.

Discussion

Campylobacter jejuni infection was first reported as a potential cause of GBS in 1982. Shortly thereafter, several reports described patients who developed GBS soon after infection with C. jejuni. We found that 23 of 48 (47.9%) children with GBS had evidence of C. jejuni infection, which is higher than most reports from western countries. Rees et al. reported a prevalence of 26% from the United Kingdom and Jacobs et al. reported 32% from the Netherlands. Studies from the United States and Italy found rates of 36% and 15%, respectively, of C. jejuni infection in patients with GBS. However, our findings are relatively in accordance with research reports from Japan and China. Serologic evidence of recent C. jejuni infection was found in 92 (45%) of 205 Japanese patients with GBS. In Beijing, 62% of GBS patients had evidence of C. jejuni infection in patients with GBS.

The reasons for the higher frequency of C. jejuni-associated GBS in our study are not clear, but it might be related to the higher incidence of enteritis in developing countries, including Iran. The annual incidence of campylobacter enteritis in England and the United States is about 50/100,000 of the general population and about 300/100,000 of children 1-4 years old. In Iran, the incidence of campylobacter enteritis has not yet been investigated. Another explanation may be related to the younger age of our patients (mean 5 years), since, as mentioned earlier, campylobacter enteritis is also more common in children aged 1-4 years.

Differences in frequencies of C. jejuni-associated GBS might be related to different systems and criteria used for diagnosis of C. jejuni positivity. A comparative study carried out in Japan and the Netherlands on the presence of anti-C. jejuni antibody in GBS showed that serological assay systems vary considerably between laboratories.

Our criteria for diagnosis of C. jejuni were made particularly stringent to exclude any false-positive serologic results. It has been reported that isolated IgM response to C. jejuni may also occur after salmonella enteritis. In addition, we excluded patients with elevated levels of a single class of IgG antibody since it has been shown that some people, such as those who regularly drink raw milk, have elevated levels of IgG antibodies.

Only 8 of 23 C. jejuni-positive patients in our study gave definite history of diarrheal illness in the last month, so asymptomatic infection occurred in 15 patients, in whom diagnosis was based on serologic analysis. The incidence of antecedent gastrointestinal symptoms does not necessarily reflect the frequency of preceding C. jejuni infection. In GBS, asymptomatic C. jejuni infection is frequent. Rees et al. reported asymptomatic infection in 8 of 27 C. jejuni-positive patients, but they also included patients with elevated levels of isolated IgG antibody and history of diarrheal illness within 12 weeks of neurologic symptoms.

Patients with C. jejuni-associated GBS were younger than the C. jejuni-negative group. A possible explanation for this distinct pattern of age-specific incidence of GBS might relate to the more frequent infection with C. jejuni and poor personal hygiene in children aged 1-4 years.

The mean time to reach peak disability in C. jejuni-positive patients was shorter than in C. jejuni-negative groups. Some studies reported that there is a subgroup of patients presenting with a hyper-acute onset commonly after having gastroenteritis that is characterized by rapid progression, slow recovery and substantial residual disability. The findings of our study suggest that many of these patients have had C. jejuni infection, similar to other studies.
Some reports suggest that GBS patients with C. jejuni infection suffer from a more severe form of GBS with poor recovery. Our case material is slanted toward severe cases (about 82% were grade ≥4) mostly because of referral bias due to the availability of a pediatric intensive care unit at our hospital. However, there was no significant difference in the degree of overall disability at the peak of the illness in our study, although all patients who needed mechanical ventilator were C. jejuni-positive. Our follow-up showed that complete recovery was achieved in 22 (95%) C. jejuni-positive and 23 (92%) C. jejuni-negative patients at 12 months, and this difference was not significant.

We found that in C. jejuni-associated GBS, clinical features (limb weakness, cranial nerve involvement, autonomic changes, complications, overall disability and recovery) did not differ from C. jejuni-negative patients. However, time to reach nadir was shorter in the C. jejuni-positive group.

Until recently, GBS was defined as a single homogeneous clinical entity. New studies, however, show that GBS can be divided into electrophysiological and pathologic patterns. The most frequently encountered pattern of GBS in Europe and North America is acute inflammatory demyelinating polyneuropathy. In severe cases, axonal degeneration may accompany the demyelination. In the early 1990s, pure motor axonal form of GBS, termed acute motor axonal neuropathy (AMAN), was recognized in northern China, and later found to occur in other countries. This subgroup of GBS is characterized by electrophysiological and pathological evidence of axonal degeneration of the motor neuron and possible association with anti-ganglioside GM1 antibody, C. jejuni infection, or both. Unfortunately, it was not possible for us to investigate anti-ganglioside GM1 antibody in this study. In our study, C. jejuni-positive patients showed a significantly higher percentage of axonal type of GBS compared to C. jejuni-negative patients. Some similar findings were reported in other studies. Barzegar et al. reported a prevalence of 27.5% axonal type in 40 Iranian children with GBS in the northwest of Iran, but they did not perform any serological investigation of C. jejuni infection.

Clinical and electrophysiological features of our subjects were relatively similar to other studies reported from Asian countries, especially China. The majority of Chinese patients presenting with acute areflexic paralysis in the summer months met electrodiagnostic criteria for a purely motor axonopathy, but nevertheless made a good recovery. Furthermore, a high percentage of affected children had IgG and IgM antibodies against C. jejuni as compared to hospital controls. This suggests that C. jejuni infection may be responsible for a large proportion of patients presenting with predominantly motor axonal neuropathy.

In conclusion, C. jejuni is a common preceding infection in children with GBS in Iran. This agent results in axonal type of GBS, with good prognosis. However, further studies are needed to investigate the association of anti-ganglioside GM1 antibody and C. jejuni infection.

REFERENCES


