Evaluation of the Association Among Cerebrospinal Fluid Protein, Inflammatory Markers, and Electromyography in Pediatric Guillain-Barre Syndrome

Dilek Agircan*, Ozlem Ethemoglu*, Mustafa Calik**, Tulin Gesoglu Demir*

*Harran University, Harran Faculty of Medicine, Department of Neurology, Sanliurfa, Turkey
**Harran University, Harran Faculty of Medicine, Department of Child Neurology, Sanliurfa, Turkey

Abstract

Aim: Previous studies have shown that the cerebrospinal fluid (CSF) protein level correlates with the number of demyelination criteria in electromyography in adult patients with Guillain-Barré syndrome (GBS), which is a potentially life-threatening postinfectious disease. We aimed to assess the association between CSF protein level, inflammatory markers, and electrophysiological values in the diagnosis of pediatric patients to act quickly in treating GBS.

Methods: In this cross-sectional study, thirty-nine children with GBS were retrospectively analyzed from the medical records of patients who were treated as inpatients between 2013 and 2021. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, C-reactive protein, uric acid, CSF protein levels, and electrophysiological parameters of the patients on admission were recorded. Hughes disability scores (HDS) were evaluated to determine the severity of disability on admission and at the 3rd month.

Results: Cerebrospinal fluid protein was positively correlated with tibial and peroneal motor nerve distal latency (DL) and negatively correlated with tibial and peroneal sensorial nerve conduction velocities (NCV). In the acute inflammatory demyelinating polyneuropathy group, 3rd-month HDS was significantly lower than in the acute motor axonal neuropathy group. A positive correlation was found between first-admission HDS and 3rd-month HDS. There was no significant difference between the electrophysiological subgroups and inflammatory markers.

Conclusion: In pediatric GBS patients, well-standardized ranges of the tibial and peroneal motor nerves DL, as well as medial plantar and peroneal superficial NCV, may be sensitive markers. Early rehabilitation programs could prevent disability in immobile patients.

Keywords: Child, Guillain-Barre syndrome, uric acid, neural conduction, medical records

Introduction

Guillain-Barré syndrome (GBS) is a potentially life-threatening postinfectious disease characterized by rapidly evolving symptoms, which usually include ascending symmetrical weakness or paralysis, as well as hyporeflexia or areflexia. While some neurologists restrict treatment to patients with severe disease, others treat even patients with mild disease whose nerve conduction studies (NCS) are normal on electromyography (EMG) to avoid deterioration (1). Therefore, it is important to identify patients with a poor prognosis.

Because of increased blood-nerve barrier permeability, active myelin damage and increased antibody deposition result in increased cerebrospinal fluid (CSF) protein levels. Furthermore, high CSF protein levels are a marker of injury that correlates with progression and disability in adult patients with GBS (2). The literature found a correlation between the increase in CSF protein levels and the number of NCS demyelination criteria in adult GBS patients (3).
Numerous studies have assessed the relationship between the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), C-reactive protein (CRP), uric acid (UA), albumin, and prognosis in adult GBS patients (2-6). It has been suggested that NLR could be a prognostic factor for disability and respiratory failure in GBS patients (7). It has also been shown that high levels of UA and albumin are protective factors for patients with GBS (8). Chang et al. (6) demonstrated that UA levels in CSF were significantly increased in adult patients with acute inflammatory demyelinating polyneuropathy (AIDP), suggesting that CSF UA may be related to the pathogenesis of demyelination in patients with GBS.

The publications focused on adult patients, and we did not find any studies evaluating the effects of UA level or the correlation of specific EMG parameters and CSF protein on prognosis in pediatric GBS patients. In this study, we aimed to evaluate the association between CSF protein, inflammatory markers, and EMG in pediatric patients with GBS.

Methods

Compliance with Ethical Standards

The Clinical Research Ethics Committee of Harran University approved the study on August 16, 2021, under the number HRU/21.14.15, and it complies with the Declaration of Helsinki.

Study Design

In this cross-sectional study, electronic medical records of 39 patients with GBS who were under 18 years old and treated as inpatients at the Harran University Faculty of Medicine, Neurology, and Pediatric Neurology Departments between January 2013 and June 2021 were used. The patients diagnosed with GBS according to the Brighton criteria (9), who underwent CSF analysis and electrophysiological findings within the first 24 hours, were included in the study. The exclusion criteria were as follows: Steroid use, local or systemic infections, malignancy, and chronic diseases such as hematological, autoimmune, cardiovascular, renal, and hepatic disorders (Figure 1). Standard motor and antidromic sensory NCS were performed on at least four motor nerves (median, ulnar, tibial, and peroneal) and four sensory nerves (median, ulnar, medial plantar, and superficial peroneal). In motor nerves, distal latency (DL), amplitude, and duration of compound muscle action potential (CMAP), nerve conduction velocity (NCV), conduction block (CB), and temporal dispersion were evaluated. The F-wave minimum latency was also measured. The amplitude of the sensory nerve action potential (SNAP), peak latency, and NCV were measured in sensory nerves. The patients were electrophysiologically diagnosed with GBS according to the European Standardized Telematic Tool to Evaluate Electrodagnostic Methods criteria (10) and distributed to the demyelinating group (AIDP), axonal group [acute motor axonal neuropathy (AMAN) and acute motor and sensorial axonal neuropathy (AMSAN)], and NCS normal group. All electrophysiological studies were conducted and evaluated by the same person.

White blood cell (WBC), neutrophil, lymphocyte, thrombocyte, CRP, UA, and albumin values, which were drawn from all patients within the first 24 hours after admission, were retrospectively recorded. Neutrophil-lymphocyte ratio was calculated as the ratio of neutrophil cell count to lymphocyte cell count, and PLR was calculated as the ratio of thrombocyte cell count to lymphocyte cell count. The disability of the patients was evaluated using the Hughes disability scale (HDS) at admission and during the control examination after 3 months. It is an accepted disability scale ranging from 0 to 6 for GBS patients and is as follows: 0/healthy, 1/minor symptoms and capable of running, 2/able to walk 5 m or more without assistance but unable to run, 3/able to walk 5 m across an open space with help, 4/bedridden or chair-bound, 5/requiring assisted mechanical ventilation for at least part of the day, and 6/death (11).

Figure 1. Flowchart of patient selection
Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 22.0 (IBM Corp., 2013). IBM-SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp.) software package. To test whether the data showed a normal distribution, the Kolmogorov-Smirnov test and the Shapiro-Wilk test were used. Student’s t-test, one-way ANOVA, and Tukey’s post hoc tests were used to compare normally distributed continuous variables. Categorical variables are expressed as numbers and percentages compared with the chi-square test. Differences in HDS between admission and after 3 months were examined using the Wilcoxon test. Pearson’s test was used to identify correlations. A p-value of 0.05 was considered statistically significant for all comparisons and correlations.

Results

After administering the inclusion and exclusion criteria, 39 pediatric GBS patients were included in the study. Of these patients, 22 were boys (56.4%) and 17 were girls (43.6%). The mean age was 5.74±5.13 years. 13 (33.3%) patients had AMAN; 10 (25.7%) patients had AIDP; 16 (41%) had normal NCS. There were no AMSAN patients. In eight patients, we found that sensory nerves could not be obtained with decreased SNAP, prolonged DL, or reduction in NCV in the lower extremities; these patients were included in the AIDP group because they had demyelination findings in their motor nerves. EMG was performed on day 3.5 in the NCS normal group and on day 6.5 in the NCS abnormal group after complaints had started.

Fifteen patients were diagnosed with GBS with level 1 diagnostic certainty according to the Brighton Criteria. While the number of patients diagnosed with level 2 diagnostic certainty was also 15, 9 patients were diagnosed with level 3. No patients had level 4 diagnostic certainty (Table 1).

All AIDP patients had a reduction in NCV, prolonged DL, absent F-waves, or prolonged minimum F-wave latencies, 9 (90%) patients had CB. Twelve of 13 AMAN patients had absent F-waves or prolonged minimum F-wave latencies. One of the AMAN patients had a conduction block, and another had a reduction in NCV (Figure 2).

CSF protein was significantly higher in the AIDP group than in the AMAN and NCS normal groups (p<0.001). It was also higher in the AMAN group than in the NCS normal group, but this difference was not statistically significant (p=0.891).

There was no statistically significant difference between the WBC, neutrophil, leukocyte, NLR, PLR, CRP, albumin, UA, and CSF protein values and the electrophysiological subgroups of the patients. A comparison of the laboratory and clinical characteristics between the subgroups is presented in Table 2. 3rd-month HDS were significantly lower in the AIDP group compared to the AMAN group (p=0.006) (Figure 2).

Although the 3rd-month HDS in the AMAN group was statistically higher than that in the other groups, the recovery rates at 3rd month compared with the initial HDS were significant in all three groups (p-AMAN=0.002, p-AIDP=0.007, p-NCS normal=0.001). A positive correlation was found between first-admission HDS and 3rd-month HDS (p=0.036, r=0.338). There was no correlation between the inflammatory markers and disability scores on admission or at 3rd-month (Table 3).

CSF protein was positively correlated with NCS parameters of tibial and peroneal motor nerve DL (respectively p=0.003, r=0.672 - p=0.033, r=0.564) and was also negatively correlated with NCS parameters of medial plantar and superficial peroneal sensorial NCVs (respectively p=0.041, r=0.897 - p=0.012, r=0.610).

Table 1. Diagnostic levels of patients according to the Brighton criteria between the GBS subgroups

<table>
<thead>
<tr>
<th>Brighton criteria level</th>
<th>AIDP, n (%)</th>
<th>AMAN, n (%)</th>
<th>Normal NCS, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 (40)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>6 (40)</td>
<td>7 (46.7)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Chi-square test

AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, NCS: Nerve conduction studies
In the peak phase of GBS among pediatric patients, 75% are unable to walk unaided, 30% have tetraparesis, 35-50% suffer from cranial nerve involvement, and 15-20% develop respiratory and/or autonomic failure. The severity of the infiltrative period in inflammatory neuropathies determines the clinical course of GBS. Hence, many studies have investigated the underlying pathogenesis and prognostic factors (2,3,12-14). CSF total protein is highest in the demyelinating GBS subtype and correlates with disease severity (15). The CSF protein level was higher in the AIDP subtype than in the AMAN subtype in our study. The frequency of GBS in different clinical subtypes varies according to geographical area. AIDP is the most common subtype in European countries, whereas AMAN is predominant in Asia (16). Tekgul et al. (17) demonstrated that the frequency of children with AMAN in Turkey was approximately 35%. In our study, the frequency of AMAN was found to be 33%, similar to the previous study, and it was more common than AIDP. In addition to nine AIDP patients, one AMAN patient had a CB. Conduction block is considered an electrophysiological hallmark of AIDP; however, the low proximal amplitude seen in the acute period of AMAN might be interpreted as a pseudo-CB. Moreover, AIDP associated with severe axonal damage could be indistinguishable from the pure axonal form of GBS. Therefore, patients with prolonged distal motor latency and pseudo-CB should be followed up with EMG before GBS subtypes are determined (18).

Some studies in the literature have revealed that:

### Table 2. Comparison of the laboratory and clinical characteristics between the GBS subgroups

<table>
<thead>
<tr>
<th></th>
<th>AIDP</th>
<th>AMAN</th>
<th>NCS Normal</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF protein</td>
<td>155.99±93.94</td>
<td>54.41±34.71</td>
<td>44.73±37.95</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.891</td>
</tr>
<tr>
<td>WBC</td>
<td>10.14±2.22</td>
<td>10.26±3.41</td>
<td>9.78±2.60</td>
<td>0.994</td>
<td>0.946</td>
<td>0.891</td>
</tr>
<tr>
<td>NEU</td>
<td>1.50±0.90</td>
<td>1.38±1.11</td>
<td>1.87±1.40</td>
<td>0.966</td>
<td>0.725</td>
<td>0.515</td>
</tr>
<tr>
<td>PLT</td>
<td>430.98±232.25</td>
<td>372.71±92.57</td>
<td>372.92±123.96</td>
<td>0.632</td>
<td>0.998</td>
<td>0.61</td>
</tr>
<tr>
<td>LYM</td>
<td>3.96±1.54</td>
<td>5.17±2.27</td>
<td>3.71±1.94</td>
<td>0.326</td>
<td>0.134</td>
<td>0.948</td>
</tr>
<tr>
<td>NLR</td>
<td>1.50±0.90</td>
<td>1.38±1.11</td>
<td>1.87±1.40</td>
<td>0.966</td>
<td>0.725</td>
<td>0.515</td>
</tr>
<tr>
<td>PLR</td>
<td>110.75±37.68</td>
<td>82.50±32.51</td>
<td>126.64±72.81</td>
<td>0.436</td>
<td>0.747</td>
<td>0.087</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.07±0.06</td>
<td>0.21±0.33</td>
<td>2.69±9.77</td>
<td>0.998</td>
<td>0.564</td>
<td>0.55</td>
</tr>
<tr>
<td>UA (mg/dL)</td>
<td>3.71±0.61</td>
<td>3.77±0.82</td>
<td>3.71±1.86</td>
<td>0.495</td>
<td>0.99</td>
<td>0.412</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.38±0.38</td>
<td>4.25±0.49</td>
<td>4.06±0.44</td>
<td>0.766</td>
<td>0.188</td>
<td>0.494</td>
</tr>
<tr>
<td>HDS-A</td>
<td>3.40±0.84</td>
<td>3.84±0.37</td>
<td>3.56±0.62</td>
<td>0.219</td>
<td>0.796</td>
<td>0.451</td>
</tr>
<tr>
<td>HDS: 3rd</td>
<td>1.60±0.51</td>
<td>2.38±0.65</td>
<td>1.56±0.51</td>
<td><strong>0.006</strong></td>
<td>0.985</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>

*One-Way ANOVA
Mean ± standard deviation, p1, significance between AIDP and AMAN; p2, significance between AIDP and NCS normal group; p3, significance between AMAN and NCS normal group.


### Table 3. Correlation between inflammatory markers and disability scores

<table>
<thead>
<tr>
<th></th>
<th>HDS-A</th>
<th>HDS-3rd</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>NLR</td>
<td>-0.177</td>
<td>0.281</td>
<td>0.157</td>
<td>0.339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLR</td>
<td>-0.042</td>
<td>0.8</td>
<td>0.37</td>
<td>0.822</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.117</td>
<td>0.477</td>
<td>-0.111</td>
<td>0.501</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>0.028</td>
<td>0.867</td>
<td>0.23</td>
<td>0.158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.149</td>
<td>0.367</td>
<td>-0.146</td>
<td>0.374</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation test
HDS-A: Hughes disability scale on admission, HDS-3rd: Hughes disability scale at 3rd-month control, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, CRP: C-reactive protein, UA: Uric acid

**Discussion**

In the peak phase of GBS among pediatric patients, 75% are unable to walk unaided, 30% have tetraparesis, 35-50% suffer from cranial nerve involvement, and 15-20% develop respiratory and/or autonomic failure. The severity of the infiltrative period in inflammatory neuropathies determines the clinical course of GBS. Hence, many studies have investigated the underlying pathogenesis and prognostic factors (2,3,12-14). CSF total protein is highest in the demyelinating GBS subtype and correlates with disease severity (15). The CSF protein level was higher in the AIDP subtype than in the AMAN subtype in our study.

The frequency of GBS in different clinical subtypes varies according to geographical area. AIDP is the most common subtype in European countries, whereas AMAN is predominant in Asia (16). Tekgul et al. (17) demonstrated that the frequency of children with AMAN in Turkey was approximately 35%. In our study, the frequency of AMAN was found to be 33%, similar to the previous study, and it was more common than AIDP.

In addition to nine AIDP patients, one AMAN patient had a CB. Conduction block is considered an electrophysiological hallmark of AIDP; however, the low proximal amplitude seen in the acute period of AMAN might be interpreted as a pseudo-CB. Moreover, AIDP associated with severe axonal damage could be indistinguishable from the pure axonal form of GBS. Therefore, patients with prolonged distal motor latency and pseudo-CB should be followed up with EMG before GBS subtypes are determined (18). To the best of our knowledge, CSF protein elevation has not been compared with a specific NCS in pediatric GBS patients. DiCapua et al. (3) showed that the level of CSF protein correlated with the number of electrophysiological abnormalities related to demyelination on NCS but not with any single specified criterion in adult patients with GBS. Other studies have demonstrated that CSF protein levels do not correlate with the number of electrophysiological abnormalities or any specific criterion (2,19). In our study, CSF protein levels showed a positive correlation with peroneal and tibial motor nerve DLs, but a negative correlation with medial plantar and peroneal superficial sensory NCVs in pediatric patients. According to this correlation, we suggest that the DLs of the peroneal and tibial motor nerves, as well as the medial plantar and peroneal superficial sensory NCSs in the lower extremity, may be more sensitive than other nerves to demonstrate early demyelination. However, further studies are required. Some studies in the literature have revealed that...
Axonal damage is associated with a poor prognosis (20). It has been suggested that low CMAP is associated with slow recovery or poor outcomes in adults, whereas it is associated with a good prognosis in children (21). Tekgul et al. (17) demonstrated that the recovery process was slower in the AMAN group than in the AIDP group during the first 12 months, and after this duration, the recovery grades evened. In contrast, it was also shown that GBS disability scores at 6 months were higher in AIDP than in AMAN (22). Despite the contradictory data in the literature, our study revealed significantly higher HDS levels at 3 months in AMAN compared to other groups. Our patients’ recovery rate was slower in the AMAN group during the first 3 months, although the rate of improvement in all groups was statistically significant.

Verma et al. (12) showed that a poor functional outcome at 6 months was associated with the axonal pattern on electrophysiological assessment and muscle weakness on admission. It has been suggested that the risk of sequelae correlates with the initial severity, especially the loss of walking ability during the acute phase of GBS (13). Barzegar et al. (23) recommended an early rehabilitation program to prevent further side effects and deconditioning secondary to immobility in pediatric patients who had some predictors such as a disability score >3, autonomic and cranial nerve involvement, and absence of CMAP. In accordance with the literature, a positive correlation was found between first-admission HDS and 3rd-month HDS in our patients. In this context, early rehabilitation programs could prevent disability in immobile pediatric patients.

The hallmarks of GBS are rapidly progressive bilateral and rather symmetric limb weakness. EMG and CSF can be used to support the diagnosis in clinically difficult cases. EMG can also be useful for differentiating the electrophysiological subtypes of GBS. However, these tests were found to be normal in the first 1-2 weeks of illness (20). The Brighton criteria from level 1 to level 4 describe the level of diagnostic certainty based on clinical presentations and additional test results. Level 1 represents the strongest level of diagnostic certainty, whereas level 4 is the weakest diagnostic level for diagnosing patients with insufficient GBS data. Level 3 represents patients who have clinical signs of GBS without CSF or EMG results in the absence of an alternative diagnosis for weakness, whereas at level 2, one of these test results supports the diagnosis (9). In our cohort, there were no patients with Level 4 diagnostic certainty because of our inclusion criteria. Among patients with normal NCS, 9 had level 3 and 7 had level 2. The European Academy of Neurology/Peripheral Nerve Society Guidelines recommend performing a second electrodiagnostic study later during the disease course, which can be helpful because abnormalities may take several weeks to develop (24). In our study, the timing of electrophysiological assessment was earlier in patients with normal NCS. The comparison of the 3rd month HDS between the NCS normal and AIDP groups revealed no significant difference, drawing attention to the importance of electrophysiological follow-up, although it is not practical.

In the literature, many studies have investigated the relationship between hematological and biochemical parameters and prognosis in adult GBS patients (2,4,5). Researchers suggested that NLR levels could be a prognostic factor in adult patients (2). Ethemoglu et al. (4) revealed that NLR was higher at the 3rd-month control in pediatric GBS patients and might be useful in estimating the course of the disease. Although decreased NLR and PLR are not associated with disease severity, they may indicate AIDP (25). It is indicated that autoimmune conditions such as GBS may stimulate the inflammatory response and thus lead to an increase in CRP production, which is significantly associated with a poor prognosis in patients with GBS. C-reactive protein can be used as a risk assessment and prognostic marker, according to Altaweel et al. (5). It has also been shown that GBS significantly reduces serum albumin levels, and this decrease is correlated with disease severity (4,8,25,26). In the current study, no correlation was found between NLR, PLR, CRP, and albumin levels in patients with electrophysiological subgroups, and there was no correlation between these parameters and prognosis.

Reduced serum UA levels have been associated with neurological diseases such as multiple sclerosis, neuromyelitis optica, Alzheimer’s disease, and Parkinson’s disease. Patients with GBS showed reduced serum UA levels compared with healthy controls. It has been demonstrated that CSF UA levels increased in adult patients with AIDP and suggested that it could be related to demyelination. To the best of our knowledge, although it has been shown that serum UA level is a protective factor in adult GBS patients, there are no studies evaluating serum UA in the pediatric group (6,8). Even serum UA level was not found to be associated with electrophysiological subgroups in our study, which is important because it is the first study to evaluate UA in children with GBS.

**Study Limitations**

Nevertheless, our study had some limitations, such as a retrospective design and a small sample. The other limitations included a short clinical follow-up and a lack of electrophysiological follow-up. Despite all these limitations, our study is valuable because it comprehensively addresses pediatric patients with GBS by considering their electrophysiological, clinical, blood, and CSF parameters. Our study contributes to the importance of EMG in the
early diagnosis of GBS in pediatric populations because it highlights well-standardized ranges of tibial and peroneal motor nerve DL, as well as medial plantar and superficial peroneal sensory NCVs.

Conclusion

Our study represents a pioneering endeavor, being the first to investigate UA levels in pediatric patients with GBS, even though our findings did not reveal a significant association between inflammatory markers such as NLR, PLR, and CRP, as well as serum UA levels and electrophysiological subgroups. In our opinion, well-standardized ranges of tibial and peroneal motor nerves DL and medial plantar and superficial peroneal sensorial NCVs, which are added to the routine study program, might be a sensitive marker in the diagnosis of pediatric GBS patients. Disability at presentation is more important than subtypes in determining prognosis, indicating the importance of early rehabilitation. Further studies with larger patient populations and serial NCSs are required.

Acknowledgements

We are grateful to our brilliant electrophysiological technician, Mehmet Fatih Ozmodanli, for his endless determination to work and support in obtaining data.

Ethics

Ethics Committee Approval: The Clinical Research Ethics Committee of Harran University approved the study on August 16, 2021, under the number HRU/21.14.15, and it complies with the Declaration of Helsinki.

Informed Consent: Retrospective study.

Authorship Contributions


Conflict of Interest: No conflicts of interest were declared by the authors.

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References


