

HUMORAL IMMUNE RESPONSE TO *CAMPYLOBACTER JEJUNI* IN PATIENTS WITH ENTEROCOLITIS AND GUILLAIN-BARRÉ SYNDROME

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Abstract - *Campylobacter jejuni* is one of the most important causes of diarrheal disease worldwide. In addition, it can cause neurological post-infectious sequels, such as Guillain-Barré syndrome (GBS). Humoral immune response to *C. jejuni* was monitored in patients with *C. jejuni* enterocolitis, GBS patients and healthy persons, by ELISA. Statistical significance between patients with enterocolitis and healthy persons, as well as among GBS patients and healthy controls, was proven. Statistical significance in IgA among the examined groups was also noticed. The highest values of IgM were found in the patients with GBS, while the highest values of IgG were found in those with enterocolitis. *C. jejuni* is a significant cause of antecedent infection in GBS. ELISA techniques can be considered a reliable method in determining the presence of serum antibodies in patients with enterocolitis caused by *C. jejuni*, as well as in patients with GBS.

Key words: *C. jejuni*, enterocolitis, GBS

INTRODUCTION

Campylobacter jejuni (*C. jejuni*) has been detected as one of the most important causes of diarrheal disease in the world (Cawthraw et al., 2002). *Campylobacteriosis* is primarily a zoonosis (Chartzipanagiotu et al., 2003), usually manifested as mild enteritis. Clinically, the infection is characterized by the appearance of prodromal symptoms: about 30% of patients suffer from influenza-like infection such as fever, headache, myalgia, dizziness. Symptoms and signs of abdominal infection can occur after two to three days in the form of diarrhea and abdominal pain (Al-Shamahy et al., 2007). Inflammatory diarrhea is commonly present in developed countries, while noninflammatory diarrhea predominates in developing countries

(Al-Shamahy et al., 2007). In addition, as a result of infection transient bacteremia, septic arthritis, meningitis, peritonitis, cholecystitis, hepatitis and fulminant sepsis may occur. Several cases of myocarditis as a complication of *C. jejuni* infection have been reported (Chartzipanagiotu et al., 2003). Post infectious complications are the results of immune mechanism activation. They include manifestations on the peripheral nervous tissue, usually presented as an acute polyneuropathy, Guillain-Barré syndrome (GBS), or symptoms on the muscular-skeletal system, such as Reiter's syndrome and reactive arthritis (Nachamkin, 2002).

For the first time, in 1982, Tathersfield and Rhodes described GBS following *C. jejuni* infection (Rhodes

et Tattersfield, 1982). The estimated incidence of GBS after *C. jejuni* infection is 1:1058, and after *C. jejuni* O:19 infection, 1:158 (Nachamkin et al., 1998). Other serotypes also associated with GBS are: O:41, O:1, O:2, O:4, O:4 complex (4, 13, 16, 43, 50), O:5, O:10, O:16, O:23, O:37, O:44, O:64 O:35 and O:13/65 (Endtz et al., 2000). The cross-reactive antibodies against *C. jejuni*, lipooligosaccharides (LOS) and gangliosides of the human nervous tissues play a paramount role in one of the main mechanisms in GBS pathogenesis-molecular mimicry (Yuki et al., 1999; Schmiedt-Ott et al., 2006). Although stool culture is the gold standard for infection diagnosis, it is often negative due to the postponed stool sampling, antibiotic treatment, or even asymptomatic *C. jejuni* infection.

Given the importance of *C. jejuni* in acute infectious and post-infectious sequelae, especially in GBS, serum antibody monitoring should be the method of choice for the diagnosis of a recent infection with *Campylobacter*, since stool culture for *C. jejuni* is often negative in these patients. There are several methods in serology for the detection of a recent *C. jejuni* infection, such as complement fixation (Jones et al., 1980), western blots (Nachamkin et Hart, 1985) and ELISA techniques (Kaldor et al., 1983). Nevertheless, ELISA is considered one of the most sensitive and specific methods for standardization in patients with enterocolitis (Strid et al., 2001) and GBS (Ang et al., 2007). However, humoral immune response detected by ELISA has not been investigated in Serbia, neither in enterocolitis nor in GBS patients.

MATERIALS AND METHODS

Patients and sera

Sera were obtained from 30 patients suffering from uncomplicated *C. jejuni* enterocolitis with positive stool culture confirmed at the Institute for Public Health in Niš, Serbia. The time at which sera were sampled ranged from 10 to 21 days after a positive culture. In addition, 30 sera were collected from Serbian GBS patients admitted to the Institute of Neurology, School of Medicine, University of Belgrade and Institute of Neurology, School of Medicine, University of

Niš, where the disease was neurologically confirmed. The time at which sera were sampled ranged from 5 to 30 days after the onset of symptoms. Sera from healthy individuals were collected during their regular health check at the Institute of Occupational Medicine, Niš.

ELISA

For antibody detection, the ELISA technique was performed using a commercial kit (Serion Immundiagnostica GmbH, Würzburg, Germany) and positive sample notification was completed by the computer program Virion test evaluation ver. 4.0.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software package for Windows. The statistical significance of the absolute frequency of the samples was tested with the chi-square test; p values <0.05 were considered to be significant. To compare values between multiple characteristics of the tested samples, Kruskal-Wallis test was used, while the post hoc analysis for analyzing quantitative differences among the groups was tested with the Mann-Whitney U -test.

RESULTS

In the patients with enterocolitis, serum antibodies to *C. jejuni* were monitored in 15 male and 15 female patients, ages 36.2 ± 16.36 ($x \pm SD$). The group of patients with neurologically proved GBS was also comprised of 15 male and 15 female patients: two children at the age of 5, and 28 adults, older than 18 years. The control group of healthy individuals involved 17 males and 13 females with an average age of 37.6 ± 6.1 ($x \pm SD$).

In the group of patients with enterocolitis, elevated serum antibodies to *C. jejuni* for any of the three classes of immunoglobulins (Ig) were found in 18 (60%) patients.

Positive values for IgA, IgM and IgG were found in 11 (36.7%), five (16.7%) and 14 (46.7%) patients,

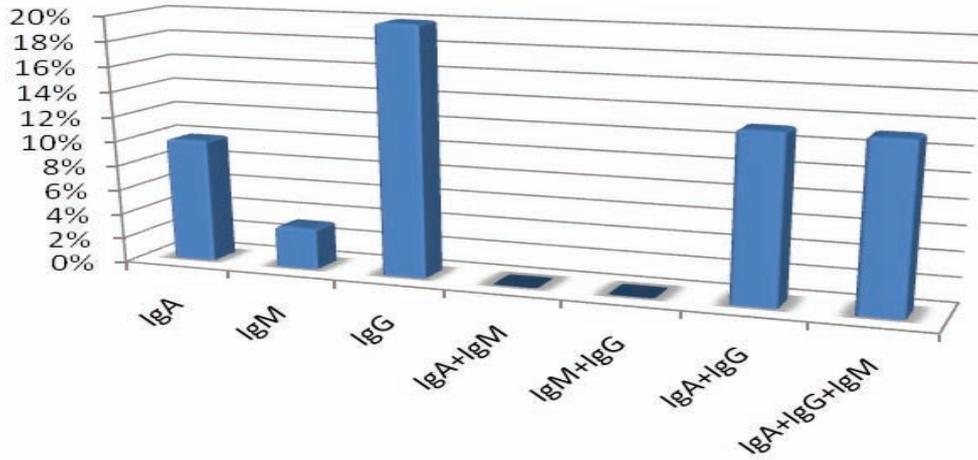


Fig. 1. Classes of specific immunoglobulins in patients with enterocolitis.

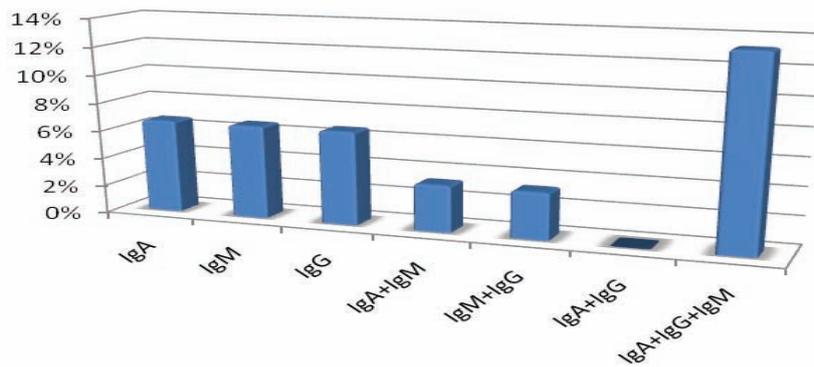


Fig. 2. Classes of specific immunoglobulins in patients with GBS.

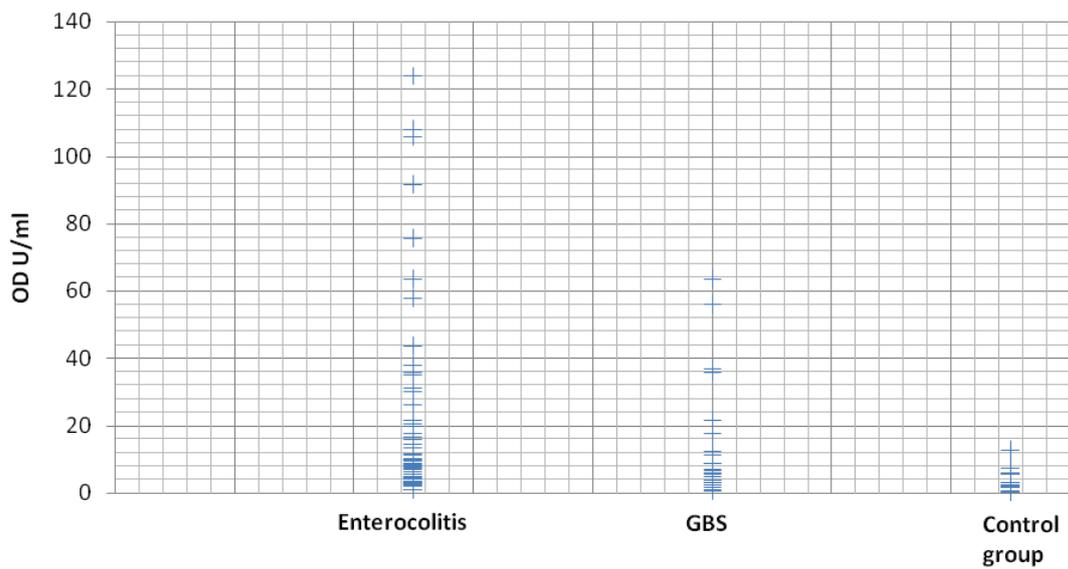


Fig. 3. Scatterplots of IgA ODs in *C. jejuni* for various patient groups.

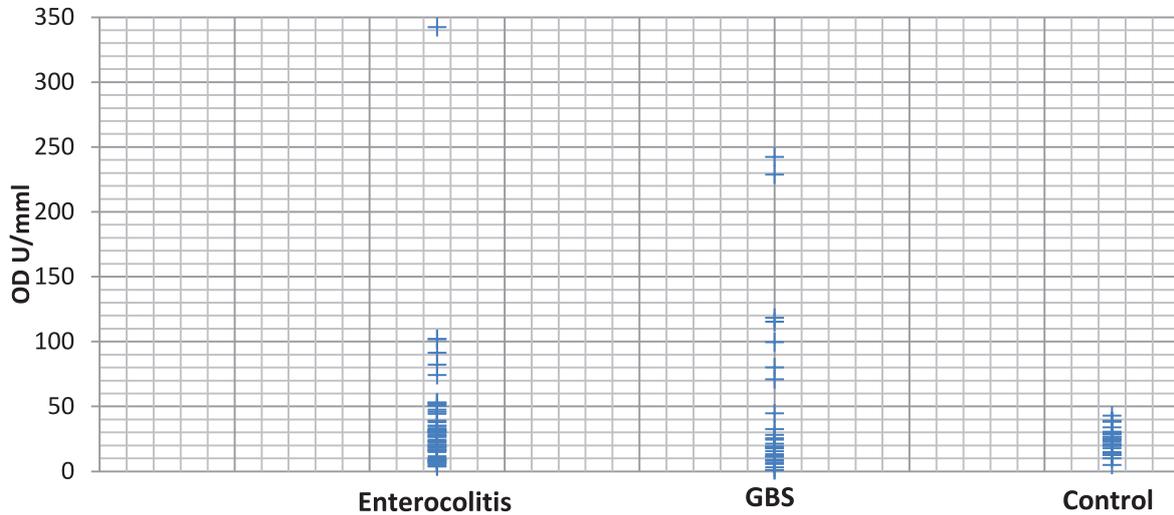


Fig. 4. Scatterplots of IgM ODs in *C. jejuni* for various patient groups .

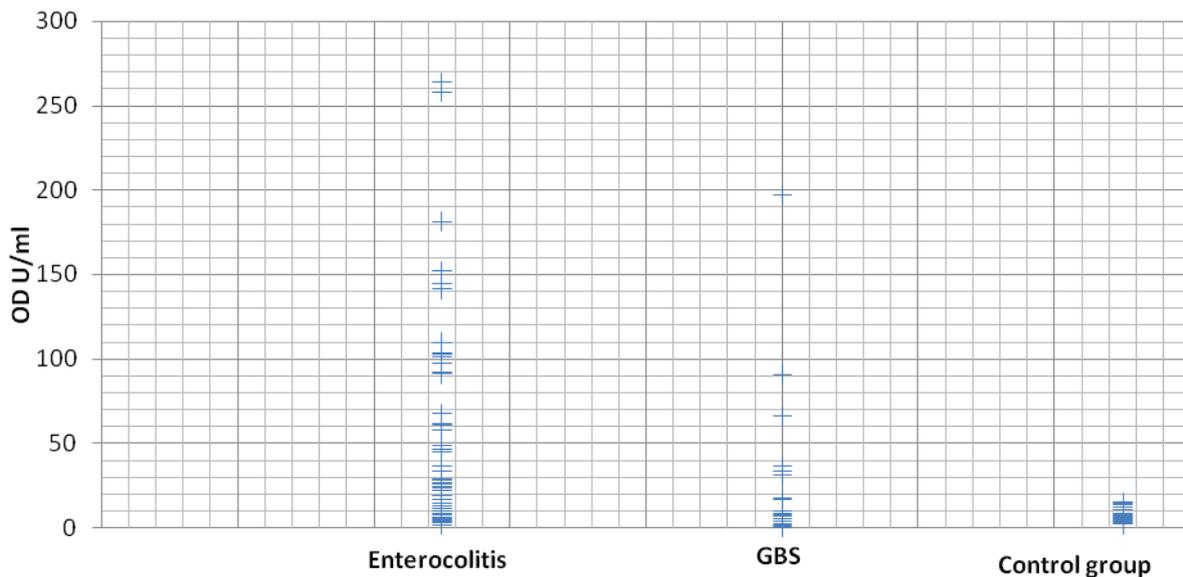


Fig. 5. Scatterplots of IgG ODs in *C. jejuni* for various patient groups

respectively. Only one class of Ig was detected in 10 patients: three were IgA, one IgM and six IgG positive. Both, IgA and IgG responses were observed in four patients (13.3%), and the presence of all three classes was recorded in 13.3% individuals (Fig. 1).

One or more classes of Ig within the group of patients with GBS were verified in 40% of the patients. Elevated values of IgA, IgM and IgG were detected

in 7 (23.33%), 8 (26.67%) and 7 (23.33%) patients, respectively. Only one Ig was detected in six patients: each class of IgA, IgM and IgG was demonstrated in two patients. All three classes of immunoglobulins were detected in four patients (13.33%) (Fig. 2).

The presence of any of the three classes of anti-*C. jejuni* antibodies was not proven in the control group.

A statistically significant difference among the positive serum samples of the patients with enterocolitis and the healthy individuals, as well as between the GBS patients and healthy controls, was proven, ($p = 0.0001075$), while a difference was not detected between patients with enterocolitis and GBS ($p = 0.1213353$).

IgA optical density (OD) values in patients with enterocolitis, GBS patients and in the healthy controls ranged from 0.9-124.0 U/ml, 0.6-226.2 U/ml, and 0.1-12.6 U/ml, respectively (Fig. 3).

Monitoring of IgA OD within the examined groups of patients revealed a significant statistical difference (Kruskal-Wallis test, $p < 0.001$). In addition, the Mann-Whitney U test verified a significant statistical difference between the group of patients with enterocolitis and the control group of healthy persons ($p < 0.001$); then between the group with GBS and the control group ($p < 0.002$). The same test verified statistically significant difference between the group with enteritis and the group with GBS ($p = 0.046$).

IgM OD values in patients with enterocolitis, GBS patients and in the healthy controls ranged from 6.6-342.3 U/ml, 0.9-448.1 U/ml, and 4.9-8.9 U/ml, respectively (Fig. 4).

The highest IgM antibody values were found within the group of GBS patients, followed by the group with enteritis, while the lowest values were detected within the control group of healthy persons. However, a statistical significance between the IgM OD values was not proved (Kruskal-Wallis test, $p = 0.369$).

IgG OD values in patients with enterocolitis, GBS patients and in healthy controls ranged from 5.4-535.1 U/ml, 0.4-197.1 U/ml and 0.4-197.1 U/ml, respectively (Fig. 5).

The highest values of IgG OD were verified in the group of patients with enterocolitis, then in the group of patients with GBS, while the lowest levels

were found in the control group of healthy persons. Statistical significance ($p < 0.001$) existed among patients with enteritis and GBS ($p = 0.012$), between patients with enteritis and the control group ($p < 0.001$), while significance was not recorded between the GBS patients and the group of healthy individuals ($p < 0.553$).

DISCUSSION

C. jejuni is one of the most common causes of enterocolitis in both developed and developing countries, but it is also responsible for provoking post-infectious sequelae, which, although relatively rare, can cause severe, life-threatening conditions or permanent disability. Post-infectious sequelae may affect the peripheral nervous system leading to GBS, Miller Fisher syndrome (MFS) and related polyneuropathy (Takahashi et al., 2005).

In our patients with *C. jejuni* enterocolitis, antibodies were detected in 60%, positive values for IgA, IgM and IgG were found in 11 (36.7%), 5 (16.7%) and 14 (46.7%) patients, respectively.

Kaldor and colleagues (1983) followed the levels of serum antibodies in 137 patients with *C. jejuni* enteritis in Australia in the period 1975-1982. Detectable levels were present in 59% of patients' sera 6 or 7 days after the onset of illness, and in 79% of patients sera 10 or more days after the onset (Kaldor et al., 1983). Herbrink et al. (1988) found that serological diagnosis of *C. jejuni* enteritis could be made in 34 (74%) out of 46 cases.

Since polio has been successfully eradicated in most parts of the world, GBS has become the most common cause of acute flaccid paralysis (Nachamkin et al., 1998). Although different infectious agents are involved in GBS pathogenesis, *C. jejuni* infection has been proved to be the most common preceding event. After *C. jejuni* infection, three forms of GBS can occur: acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN) and acute motor-sensory axonal neuropathy (AMSAN) (Nachamkin et al., 1998). In

Serbia, the first fully documented case of AMAN associated with *Campylobacter* infection was described in 2010. Isolated *C. jejuni* was characterized as subsp. *jejuni* biotype II, serotype O: 19 (Miljković-Selimović et al., 2010).

The association of GBS and campylobacter is diverse in distinctive geographical areas. In patients with GBS, the frequency of previous *C. jejuni* infection ranges from 14% (Winer et al., 1988) to 88% (Kuroki et al., 2001). The lack of accurate data on the frequency of GBS-related infection caused by *Campylobacter* originates from the lack of serological testing. In small serological studies, in one-third to one-half of patients with GBS high levels of antibodies to *C. jejuni* were registered at the time of the appearance of neurological symptoms (Allos et al., 1993). Several studies have shown that patients with GBS with preceding *C. jejuni* infection have severe clinical disease and poor outcome (Huges and Ress, 1997).

In the present study, 40% of the GBS patients had a positive finding and demonstrated the presence of one or more Ig classes to *C. jejuni*, pointing to its etiologic role. The percentage of anti-*C. jejuni* antibodies in our patients was higher than the results in the study in the UK from 1991 to 2001. That study revealed that between 9% and 14% of GBS-serologically proven cases could be linked to symptomatic *Campylobacter* infection (Tam et al., 2003), which also confirms the importance of *C. jejuni* as the cause of enterocolitis in that geographic region.

In the present study, IgA OD values in patients with enterocolitis were in the range of 0.9-124.0 U/ml, while in GBS patients they were in the range of 0.6-226.2 U/ml. Although the levels were elevated in both groups, their presence in GBS patients was more significant. IgM OD values were elevated in both patients with enterocolitis and patients with GBS, showing no statistical differences between these two groups. IgG OD values in patients with enterocolitis were in the range of 5.4-535.1 U/ml. In GBS patients they were in the range of 0.4-197.1 U/ml, showing the absence of statistical differences be-

tween these two groups. Antibody response in GBS patients revealed the acute nature of the disease, as was observed in the study conducted by Ang et al. (2003). They detected an elevation in all three classes of immunoglobulins in GBS patients, with statistical significance in IgA OD, and increased levels of IgM and IgG, which were not statistically proved (Ang et al., 2007). In the present study, elevated values for IgA and IgM OD in GBS patients were detected, with statistical significance for IgA in GBS sera.

The stronger humoral immune response in GBS patients than in patients with uncomplicated enterocolitis needs further clarification. Streed et al. (2001) investigated the effect of *C. jejuni* serotype on antibody response in patients with enterocolitis. They did not find a correlation between the serotypes and Ig levels except for serotype O:19, one of the serotypes most often associated with GBS. All three classes of Ig were significantly elevated in the patients. In addition, enhanced reactivity to *C. jejuni* antigens in GBS patients was noticed (Ang et al., 2002), which may be explained by a generally hyper-reactive immune response when challenged with *C. jejuni* (Ang et al., 2007). Both, microbial and host factors may lead to autoimmunity, neural damage and dysfunction.

C. jejuni is a significant cause of antecedent infection in GBS. Based on the obtained results, the ELISA technique can be considered a reliable method for determining the presence of serum antibodies in patients with *C. jejuni* enterocolitis as well as in patients with GBS. It can also be a valuable tool in the etiological diagnosis of preceding *C. jejuni* infection in GBS. Thus, besides neurological examination, specific microbiological and immunological tests are necessary for the etiological diagnosis of GBS.

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