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CAMPYLOBACTER JEJUNI AND POSTINFECTIVE CHRONIC SEQUELS: JOINTS MANIFESTATIONS

SUMMARY

The aim of the paper was to investigate the occurrence of musculoskeletal disorders in patients following enterocolitis caused by *C.jejuni* and the role of *C.jejuni* in triggering the seronegative spondyloarthropathies detecting specific antibodies in patient sera. We investigated 146 patients with *C.jejuni* diarrhea and 30 patients with seronegative spondyloarthropathies. Stool samples were submitted to the Center for Microbiology at the Public Health Institute for *C.jejuni* detection. In each patient with seronegative spondyloarthropathies, one serum sample was examined for antibodies against *C.jejuni* using complement fixation test. Rheumatic manifestations seen in 146 (11%) patients with *C.jejuni* enterocolitis were arthralgia, myalgia, enthesitis and reactive arthritis. The cases of reactive arthritis caused by *C.jejuni* are the first reported cases in Serbia and Montenegro. In the group of patients (30 pts) with seronegative spondyloarthropathies, (Reiter's syndrome, Morbus Bechterew, enteropathic arthritis, psoriatic arthritis, reactive arthritis, oligoarthritis) antibodies against *Campylobacter jejuni* were noticed in 26,7%. There were statistically significant differences between the presence of antibodies in the group of patients and the control group ($p < 0.05$). No *C.jejuni* were discovered in the stools of these patients. In conclusion, based on the data obtained, *C.jejuni* may be an important factor in triggering the reactive arthritis, but for the detection of its role in other seronegative spondyloarthropathies, long-term follow-up studies of patients with *C.jejuni* enterocolitis are necessary. The prevention of infective disease can forestall chronic sequels, too.

Key words: *Campylobacter jejuni*, postinfective sequels, musculoskeletal system

INTRODUCTION

Campylobacter jejuni (*C.jejuni*), and *Campylobacter coli* (*C.coli*), thermophilic campylobacters, represent the main cause of bacterial diarrhea in developed countries (1) and one of the most frequent causes of enterocolitis in developing countries (2) especially in early childhood (3). Clinical manifestations of enteroco-

litis caused by *Campylobacter species* are diarrhea, fever, abdominal pain, and in some patients, fecal blood (4). Symptom severity differs in developing and developed countries. *C.jejuni* is the most often isolated agent among thermophilic *Campylobacters* (2). After *C.jejuni* infection, severe chronic sequelae may occur, such as reactive arthritis (5), post-infective neuropathy, Guillain-Barré syndrome (GBS) and Miller-Fisher Syndrome (MFS).

Seronegative spondyloarthropathies comprise ankylosing spondylitis (AS), reactive arthritis/Reiter syndrome, spondyloarthritis associated with psoriasis and chronic inflammatory bowel diseases, and a form of juvenile chronic arthritis (pauciarticular, late onset type). All forms of spondyloarthropathies are associated with the histocompatibility antigen HLA B-27, although the strength of this association varies markedly not only among the various disease forms but also among the various ethnic and racial groups worldwide (6). These diseases tend to occur more often among males who are in their late teens and early twenties, and may start with features such as enthesitis (inflammatory lesions of the entheses, i.e. sites of ligamentous or tendinous attachment to bone), dactylitis or oligoarthritis, and in some cases may progress to sacroiliitis and spondylitis, with or without extra-articular features such as acute anterior uveitis or muco-cutaneous lesions. On the other hand, it is considered that among other bacteria (*Chlamydia trachomatis*, *Salmonella spp*, *Shigella spp*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*) *C.jejuni* plays a role in the pathogenesis of seronegative spondyloarthropathies (7)

Reactive arthritis still remains not quite explained, but determined with certain HLA types: HLA-B27 (5).

We have evaluated the prevalence musculo-skeletal manifestations (ReA) of *C.jejuni* triggered by postinfective sequels in patients with *C.jejuni* diarrhea, and patients with seronegative spondyloarthropathies.

MATERIAL AND METHODS

Two groups of patients were investigated: 146 patients with *C.jejuni* diarrhea, 30 patients with seronegative spondyloarthropathies. In addition, one control group for serological investigation was included: 30 sex- and age-matched healthy subjects for the group of patients with seronegative spondyloarthropathies. Control groups have been selected during a systematic check-up.

Stool samples obtained from either outpatient or hospitalized patients with diarrhea were submitted to the Center for Microbiology at the Public Health Institute for *C.jejuni* detection. Data about clinical characteristics of disease were obtained by questionnaire and patients were also interviewed personally or by telephone. Patients who complained about joint symptoms were directed to rheumatologist.

Patients with seronegative spondyloarthropathies were selected and rheumatologic investigations were conducted at the Institute of Rheumatology and Cardiology, "Niska Banja".

In patients with seronegative spondyloarthropathies, single stool sample was investigated for the presence of *C.jejuni* and one serum sample for antibodies against *C.jejuni*.

For the detection of thermophilic *Campylobacters* the Columbia agar base was supplemented with 5% sheep blood and antibiotics (cefoperazon, 1.5g/l, colistin 10⁶ U, vancomycin 1g/L, amphotericin B 0.2g/L), (bioMérieux, Marcy l'Etoile, France). Inoculated plates were incubated at 42°C for 48 hours in a microaerophilic atmosphere (gas generating system "Torlak", Belgrade, RS).

Colonies of *Campylobacter* were presumptively identified by staining (carbolfuchsin 1%). S- and spiral shaped bacteria having a "gull wing" morphology were observed. Colonies also tested positive for oxidase and catalase production. Strains presumptively identified as *Campylobacters* were differentiated to the species level by the API Campy (bioMérieux, Marcy l'Etoile, France).

Data about clinical characteristics of disease were obtained by questionnaire and patients were also interviewed personally or by telephone.

CFT was performed in 96 microtitration wells plate using the *C.jejuni* antigen, hemolytic system and complement (Serion Diagnostica, Wuertzburg, Germany) and serum dilution from 1:8 to 1:128. Positive results were considered to be 1:16 and higher dilutions.

Statistical analysis was performed using the Fisher's exact test and program for statistical analysis, EpiInfo 6.1.

RESULTS

In patients with diarrhea, *C.jejuni* infection were proven in 211 out of 14264 stool cultures (1.48%). Out of 211 patients, 146 gave data related to the illness. Postinfection sequels included joint affections in 16 (11%) patients: 9 females and 7 male patients, with mean age of 29.55 ±SD24.06 years, and 18.14 ±SD14.96 years, respectively. The mean age for that group was 24.56± SD 25.56 years.

Musculoskeletal disorders were represented as arthralgia, myalgia, enthesopathy and arthritis reactiva (Table 1).

Table 1. Musculoskeletal manifestations in patients with diarrhea caused by *C.jejuni*

Musculoskeletal manifestations	Number of patients (%)
Arthralgia	13 (8.9)
Arthritis reactiva	2 (1.4)
Myalgia	2 (1.4)
Enthesopathy	1 (0.7)
No manifestations	130 (89)
Total	146 (100)

In three patients, arthralgia was seen in a radiocarpal joint; in three patients carpometacarpal joints were affected, one of which was associated with myalgia; in two patients arthralgia occurred in spinal joints, in one patient arthralgia was associated with sternocostal joints and with myalgia and body weight loss; in one patient arthralgia was associated with elbow joint, in one patient with the hip joint; in one patient, arthralgia was associated with sternocostal joints and with myalgia and weight loss; one patient had a knee joint affected. In one patient, arthralgia was seen in the ankle joint and carpometacarpal joints. Arthritis reactiva associated with restricted movements in involved proximal interphalangeal joints was seen in a patient with underlying connective tissue disease, scleroderma, and in one patient with suspected connective tissue disease. To our knowledge, the cases of reactive arthritis caused by *C.jejuni* are the first reported in Serbia. The patient suffering from enthesopathy had involvement with the Achilles' tendons.

Of 30 patients with seronegative spondyloarthropathies, there were 19 males (mean age 38.47±SD 16.65) and 11 females (mean age 29.82 ±SD10.22) with the mean age value for the group of 35.3±SD15.04. The most frequent clinical diagnosis was arthritis reactiva (Table 1).

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C.jejuni was not discovered in any stool sample of these patients. Antibodies against *C.jejuni* were proven in eight patients with arthritis reactiva, oligoarthritis, Morbus Bechterew and spondyloarthropathies (Table 2).

Table 2. Presence of antibodies against *C.jejuni* in patients with seronegative spondyloarthropathies

Clinical diagnosis	Number of patients (%)	Number of positive samples
Arthritis reactiva	8 (26.7)	4 (13.3)
Reiter's syndrome	5 (16.7)	0
Spondyloarthropathies	6 (20)	1 (3.3)
Morbus Bechterew	2 (6.7)	1 (3.3)
Arthritis psoriatica	4 (13.3)	0
Oligoarthritis	5 (16.7)	2 (6.7)
Total	30 (100)	8 (26.7)

In the control group, antibodies against *C.jejuni* were detected in one serum sample. Fisher's exact test revealed significant statistical difference between patients and control group (p=0.026; p<0.05).

DISCUSSION

C.jejuni may trigger two types of post-infective sequels: neurological, whose manifestations are GBS, and musculoskeletal, which can be classified as arthritis reactiva (ReA) or tendonitis, enthesopathy and bursitis.

Musculoskeletal disorders associated with *C.jejuni* diarrhea have been described in early studies (8). Despite that fact, their pathogenesis still remains insufficiently understood. In the pathogenesis of a similar disease, caused by other enteric bacteria (*Yersinia enterocolitica*), the host and bacterial factors are important. In this study, musculoskeletal disorders were recorded in 11% of patients with diarrhea caused by *C.jejuni*: arthralgia in 8,9%, ReA and myalgia in 1,4%, enthesopathy in 0,7% of patients. Hannu et al. (9) have proven musculoskeletal disorders in 9% of patients with *C.jejuni/coli* diarrhea: in 8% ReA, and in 1% tendonitis, enthesopathy or bursitis. According to Loch and Krogfelt, 16% of patients with *C.jejuni/C.coli* diarrhea developed musculoskeletal disorders (10). In the outbreak caused by thermophilic campylobacters, joint affection occurred in 21% of patients (11). According to results of Skirrow and Blaser (1), 1% of patients with *C.jejuni* diarrhea suffer from ReA.

In a long-term follow-up study of patients with muscle skeletal disorders after an outbreak of *C.jejuni* diarrhea, in 4/9 patients rheumatic disorder persisted after five and a half years, and in one patient, the relapse of reactive arthritis occurred after seven years (12).

In this study, *C.jejuni* is possibly related to seronegative spondyloarthropathies in 26,7% patients. Most of them (4 pts) had the diagnosis of reactive arthritis. Loch et al. (13) detected *C.jejuni* as a triggering bacterium in 6/22 patients with ReA. In the study involving patients from Papua New Guinea, of 23 patients with oligoarthritis, antibodies against *C.jejuni/C.coli* were found in the sera of two patients as the proof of recent preceding infection (14). One research revealed *C.jejuni* DNA in the synovial fluid in two of 12 patients with oligoarthritis (15). Investigation on early arthritis in southern Sweden revealed the *Campylobacter* as preceding bacteria in the majority of cases of arthritis reactiva (16). In addition, our study revealed the presence of antibodies against *C.jejuni* in the patients with

arthritis reactiva, whereas these antibodies were rarely found in patients with other diagnoses. Despite the presented data, there are studies which have not proved the role of *C.jejuni* in etiology of seronegative spondyloarthropathies (17, 18).

CONCLUSION

In conclusion, based on the data obtained, *C.jejuni* may be an important factor in triggering the reactive arthritis, but for the detection of its role in other seronegative spondyloarthropathies, long-term follow-up studies of patients with *C.jejuni* enterocolitis are necessary. Considering the fact that different studies represented diversity in prevalence

of some musculoskeletal manifestations, further researches that would involve population-based studies are necessary to obtain more precise results of chronic postinfection sequels after *C.jejuni* diarrhea.

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CAMPYLOBACTER JEJUNI I POSTINFEKTIVNE HRONIČNE SEKVELE: MANIFESTACIJE NA ZGLOBOVIMA

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SAŽETAK

Ovim istraživanjem ispitivana je pojava mišićnoskeletnih poremećaja kod bolesnika nakon enterokolitisa izazvanog *Campylobacterom jejuni* (*C.jejuni*), a uloga *C.jejuni* u započinjanju seronegativnih spondiloartropatija praćena je detekcijom specifičnih antitela u serumu obolelih. Ispitivano je 146 bolesnika sa dijarejom izazvanom *C.jejuni* i 30 bolesnika sa seronegativnim spondiloartropatijama. Bakteriološki pregled stolice na prisustvo *C.jejuni* vršen je u Centru za mikrobiologiju Instituta za javno zdravlje u Nišu. Pored toga, kod bolesnika sa seronegativnim spondiloartropatijama ispitivan je i jedan uzorak seruma na antitela prema *C.jejuni* reakcijom vezivanja komplemenata. Od 146 obolelih od enterokolitisa izazvanog *C.jejuni*, reumatske manifestacije, u vidu artralgijs, mijalgija, entesitisa i reaktivnog artritisa, javile su se kod 11% bolesnika. Prema našim saznanjima, ovo su, do sada, prvi opisani slučajevi reaktivnog artritisa izazvanog *C.jejuni* u Srbiji. U grupi od 30 bolesnika sa seronegativnim spondiloartropatijama, kod 26, 7% obolelih su dokazana antitela prema *C.jejuni*. Utvrđeno je da postoji statistički značajna razlika između prisustva antitela u grupi obolelih i u kontrolnoj grupi ($p < 0.05$). U grupi obolelih od seronegativnih spondiloartropatija nije otkriveno prisustvo *C.jejuni* u stolici. Na osnovu dobijenih rezultata, zaključuje se da *C.jejuni* može biti značajan faktor u započinjanju reaktivnog artritisa, ali su neophodne dugotrajne studije koje bi pratile bolesnike sa enterokolitisom izazvanim *C.jejuni* da bi se rasvetlila njegova uloga u nastanku drugih seronegativnih spondiloartropatija. Pritom, prevencija ovog infektivnog procesa može da spreči prevenciju hroničnih postinfektivnih sekvela.

Ključne reči: *Campylobacter jejuni*, postinfektivne sekvele, mišićnoskeletni sistem