A Case of Human Monocytic Ehrlichiosis in Serbia

Bogdan Arsić¹, Ana Gligić², Elizabeta Ristanović³, Branislav Lako⁴, Aleksandar Potkonjak⁴, Milan Peruničić⁵, Momčilo Pavlović⁶

¹Department of Infectious Diseases, General Hospital, Subotica, Serbia; ²Institute of Immunology and Virology “Torlak”, Belgrade, Serbia; ³Institute of Microbiology, Military Medical Academy, Belgrade, Serbia; ⁴Department of Veterinary Medicine, Faculty of Agriculture, Novi Sad, Serbia; ⁵Institute of Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade, Serbia; ⁶Pediatric Department, General Hospital, Subotica, Serbia

SUMMARY

Introduction Ehrlichiosis is a bacterial zoonosis transmitted by hematophagous arthropods – ticks. In humans, it occurs as monocytic, granulocytic, and ewingii ehrlichiosis. Pathological process is based on parasitic presence of Ehrlichia organisms within peripheral blood cells – monocytes and granulocytes. Case Outline Fifty-two year old patient was admitted to hospital due to high fever of over 40°C that lasted two days, accompanied with chills, muscle aches, malaise, loss of appetite, headache, confusion, breathing difficulties, and mild dry cough. The history suggested tick bite that occurred seven days before the onset of disease. Doxycycline was introduced and administered for 14 days, causing the disease to subside. Indirect immunofluorescence assay was used to analyze three serum samples obtained from this patient for Ehrlichia chaffeensis antibodies, and peripheral blood smear was evaluated for the presence of Ehrlichia and Ehrlichia aggregation into morulae. Conclusion Ehrlichiosis should be considered in each case where there is a history of tick bite together with the clinical picture (high fever, chills, muscle aches, headache, generalized weakness and malaise, and possible maculopapular rash). The presence of Ehrlichia chaffeensis antibodies was confirmed in a patient with the history of tick bite, appropriate clinical picture and indirect immunofluorescence assay. This confirmed the presence of human monocytotropic ehrlichiosis, a disease that is uncommonly identified in our country.

Keywords: Ehrlichia chaffeensis; human ehrlichiosis; fluorescent antibody technique; indirect; doxycycline

INTRODUCTION

Ehrlichiosis, as human infectious disease, was described in 1986, although Ehrlichia has been studied as a cause of disease in animals until 1968, when Ehrlichia canis was identified as the cause of disease of a large number of military dogs in Vietnam. The similarity between E. canis and E. sennetsu (previously known as Rickettsia sennetsu), causing fever in humans in Japan, was then observed. Recent findings have suggested that Ehrlichiae, together with Rickettsiae, form a subgroup of Proteobacteria [1]. Bacteria from the Ehrlichia genus were classified to the Anaplasmataceae family [2]. Based on their genetic characteristics, Ehrlichia organisms were divided into 3 genogroups. Genogroup I includes E. chaffeensis (cause of human monocytotropic ehrlichiosis – HME), E. canis, and E. ewingii. Genogroup II (Anaplasma group) includes Anaplasma phagocytophilum – human granulocytic ehrlichiosis – HGE, and E. equi. Genogroup III (called Neorickettsiae) includes E. sennetsu and E. risticii [1].

The first case of human ehrlichiosis, similar to E. canis, was described in the USA in late 1980s [3]. Patients with specific antibodies to E. chaffeensis were later described in other continents and countries: Israel [4], Thailand [5], and Argentina [6]. E. chaffeensis was isolated from the tick Ixodes ricinus in Germany [7] and Bulgaria [8]. The human infections caused by E. chaffeensis were confirmed by numerous seroepidemiological studies in Italy [9], Czech Republic [10], Portugal [11], Slovenia [12], and Croatia [13], while there has been one case reported in Serbia [14]. Retrospective studies showed that Ehrlichiae, as causes of infectious diseases, had been present in human population even before 1980s [15].

The aim of this paper was to present clinical characteristics of the serologically confirmed case of ehrlichiosis in a patient who had previously been bitten by a tick.

CASE REPORT

Fifty-two year old patient was admitted to Department for Infectious Diseases of General Hospital in Subotica due to high fever of over 40°C that lasted for two days and was accompanied by chills, muscle aches, malaise, loss of appetite, headache, confusion, breathing difficulties, and mild dry cough. Seven days before the onset of disease, the patient noticed a tick in his scrotal region and removed it himself. Before admission to hospital, the patient took antipyretics only. On admission, he had high fever, was adynamic to prostration, and confused. The remaining physical findings appeared normal, as well as local findings at the site of tick bite.

Correspondence to:
Bogdan ARSIĆ
Department of Infectious Diseases
General Hospital
Izvorska 3, 24000 Subotica
Serbia
momodec@tippnet.rs
Laboratory findings immediately upon admission were the following: WBC 2.9×10^9/L, platelets 72×10^9/L, RBC 4.44×10^12/L, hemoglobin 129 g/L, AST 82 U/L (normal level: up to 37), ALT 55 U/L (normal level: up to 42), CK 1890 U/L (normal level: up to 195), and CRP 29.2 mg/L. Urine examination showed normal results. Blood and urine cultures were negative to *Mycobacterium tuberculosis*. Serologic reactions to *Rickettsia prowazekii*, *Coxiella burnetii*, *Brucella abortus*, *Borrelia burgdorferi*, as well as to TPHA were negative. Furthermore, seroreactive reactions to EBV, CMV and HSV were negative. RF and ANA were also negative. Lung X-ray, and the abdominal and urinary tract ultrasound suggested normal findings. Peripheral blood smear showed morulae in small number of monocytes (less than 10%) staining darker than the rest of the monocytes.

On the second day of hospital stay, doxycycline was introduced at a dose of 2×100 mg orally, and on the following day, the patient’s fever subsided. Treatment lasted 14 days, after which the patient had no subjective complaints and the physical findings were normal.

Laboratory findings at discharge: WBC 7.5×10^9/L, platelets 220×10^9/L, RBC 4.1×10^12/L, hemoglobin 120 g/L, AST 32 U/L, ALT 34 U/L, CK 155 U/L, CRP 15.2 mg/L. During the following six months, the patient used to come for regular controls and he had no subjective complaints, while his physical and laboratory findings remained normal.

At the Department for Immunology of the Military Medical Academy, indirect immunofluorescence assay was used to analyze three serum samples from this patient for *E. chaffeensis* antibodies, and peripheral blood smear was evaluated for the presence of Ehrlichia and Ehrlichia aggregation into morulae. Serum samples were taken on days 7 (July 24, 2006), 14 (July 31) and 50 (September 5) of the illness, and the titers of IF antibodies were < 1:32, 1:64, and 1:128, respectively. The peripheral blood smear was dated July 19, 2006.

**Indirect Immunofluorescence Assay (IFA)**

The antigen for identification of *E. chaffeensis* antibodies (Lot no. 02-0266N) was obtained as a suspension of *E. chaffeensis* replicated in canine monocytes. The suspension was spread over 8-field microscopy slides, dried at room temperature for at least three hours, and fixed with cold acetone (Merck pro analysi) for 10 minutes at -18°C. The slides were then dried 10 minutes at room temperature, and immediately used as an antigen, or stored for later use at -20°C.

The immune control serum was human serum obtained from convalescents infected with *E. chaffeensis* with titers of 128 or higher. The control negative serum was human serum negative to *E. chaffeensis* antibodies at a dilution of 1:16.

The immune control serum was human serum obtained from convalescents infected with *E. chaffeensis* with titers of 128 or higher. The control negative serum was human serum negative to *E. chaffeensis* antibodies at a dilution of 1:16. After 30 minutes of humidity chamber incubation at 37°C and PBS wash-out, in order to remove antibodies not bound to antigen, a mixture of fluorescein labeled goat antihuman IgG (INEP, Belgrade) was added, as well as Evans blue for better contrast when reading the reaction. After 30 minutes of humidity chamber incubation at 37°C, slides were washed out, dried, and after adding glycerin, the IFA reaction was studied using Leitz fluorescent microscope (at 400× magnification). The last serum dilution showing observable immunofluorescence in monocyte cytoplasm with good positive and negative control was considered to be the titer of *E. chaffeensis* antibodies.

**DISCUSSION**

The disease of the humans known as humane monocytic or monocytotropic ehrlichiosis is caused by *Ehrlichia chaffeensis*. It dwells and replicates in the cytoplasm of certain host cell types, mainly neutrophil leukocytes, leukocytes and monocytes, within vacuoles that are bound to cellular membrane. The name of the disease HME suggests the tropism of the organism toward peripheral blood monocytes [13]. Until now, only one report on HME in Serbia has been published. Dokić et al. [16] presented the case of HME in a patient admitted to Military Medical Academy.

The HME is a complex zoonosis. The causing organism persists in the nature through the infection of various mammals. Dogs, wolves and deer are considered to be the primary reservoirs of *Ehrlichia chaffeensis*. Fox and goats have been experimentally infected as well. The primary vector is a tick *Amblyomma americanum*. *Ehrlichia chaffeensis* may also be transmitted by a tick *Dermacentor variabilis* [17]. Most ehrlichiosis cases have history of tick bites (as in our patient), although in 32% of cases this information may be missing [18]. Therefore, the absence of definite tick attachment should never dissuade physician from considering the diagnosis of HME.

There is no specific clinical sign that would help in distinguishing this condition. In both HME and HGE, the symptoms may vary in their intensity, ranging from mild disease to life- threatening conditions. The most common symptoms are elevated body temperature (>90%), headache (>85%), fever, muscle aches (>80%), nausea (40%), vomiting (40%), loss of appetite (40%), overall weakness and malaise (>80%), and confusion (20%). The diffuse skin changes are uncommon (10%), and if present, they are manifested as maculopapular rash without any predilection. The central nervous system infection is present in 20% of patients with HME and may manifest as aseptic meningitis with lymphocytic pleocytosis and elevated protein level in cerebrospinal fluid, cranial nerve neuropathy or encephalopathy [19, 20]. Because the signs and symptoms are nonspecific, the clinicians must frequently incorporate clues from the clinical and epidemiologic history and consider other features. Most of these symptoms and signs were present in our patient, and the history of tick bite made our diagnosis of HME easier.
The etiological diagnosis of human ehrlichiosis is based on serological diagnostics or identification of causative microorganism in the elements of peripheral blood. In the peripheral blood smear, morulae are found in granulocytes (25-80%) and in monocytes (less than 10%). Morulae are cytoplasmic vacuoles in which Ehrlichia replicates. The most commonly used staining method is Wright's stain. The serological reaction that is used for diagnosis is indirect immunofluorescence (IF) assay [21]. According to the Center of Disease Control (CDC), the first sample should be taken as early as possible, preferably in the first week of symptoms, and the second sample should be taken 2 to 4 weeks later [22]. The IF assay is considered positive if there is an increase or decrease in titer of antibodies 4 to 4 weeks later [22]. The IF assay is considered positive of symptoms, and the second sample should be taken 2 days [22]. After treatment for 14 days with doxycycline, our patient had no subjective complaints, while his physical and laboratory findings were normal.

The human ehrlichiosis is a newly identified disease that is still being rarely diagnosed. Ehrlichiosis should be considered in each case where there is a history of tick bite together with clinical picture (high fever, chills, muscle aches, headache, generalized weakness and malaise, and possible maculopapular rash). The diagnosis is made with high certainty using serological assays, and the disease is effectively treated with doxycycline.

**ACKNOWLEDGMENT**

The *E. chaffeensis* antigen and the positive control serum were donated by Dr. Gregory Dasch from Center for Disease Control, Atlanta, USA. This research was a part of the Project: Research of Lyme Disease and Other Vector-borne Zoonoses in Vojvodina, number: 114-451-1892/2011, Provincial Secretariat for Science and Technological Development of AP of Vojvodina.
Случај хумане моноцитне ерлихиозе у Србији

Богдан Арсић1, Ана Глигић2, Елизабета Ристановић3, Бранислав Лако4, Александар Поткоњак4, Милан Перуничић5, Момчило Павловић6
1Одељење за инфективне болести, Општа болница, Суботица, Србија;
2Институт за имунологију и вирусологију „Торлак“, Београд, Србија;
3Институт за микробиологију, Војнодржавна академија, Београд, Србија;
4Департман за ветеринарску медицину, Пионирски факултет, Универзитет у Новом Саду, Нови Сад, Србија;
5Институт за инфективне и тропске болести, Клинички центар Србије, Београд, Србија;
6Дечје одељење, Општа болница, Суботица, Србија

КРАТАК САДРЖАЈ
Увод Ерлихиоза је бактеријска зооноза која се преноси хематофагним артролопадама – крпељема. Код људи се јавља као моноцитна, гранулоцитна и ewingji ерлихиоза. Патоложки процес је последица унутрашњег паразитирања ерлихије у моноцитима и гранулоцитима периферне крви.

Приказ болесника Болесник стар 52 године примљен је на одељење због високе двомечне фебрилности (преко 40°C) која је праћена дртациом, боловима у мишићима, маласалошћу, губитком апетита, главоболом, конфузношћу, отежаним дисањем и оскудним сувим кашљем. У анамнези је добијен податак о уједу крпеља седам дана пре пријема. Лабораторијски налази су указали на тромбоцитопенију, леукопенију, анемију и повећање активности трансами- наза у серуму. Болесник је лечен доксициклином 14 дана, након чега су се тегобе повукле. Методом индирактне имунофлуоресценције анализирани су три узорка серума овог болесника на присуство антитела на бактерију Ehrlichia chafeeensis и прегледан је узорак размаза периферне крви на присуство ерлихија и конглетерата ерлихије у морулу, које представљају цитоплазматске вакуоле.

Закључак Код болесника с податком о убуду крпеља, одговарајућом клиничком сливу и серолошким тестом индирактне имунофлуоресценције доказана су антитела за бактерију Ehrlichia chafeeensis, што указује на хуману моноцит-торопну ерлихиозу, болест која се код нас ретко доказује. На ерлихиозу треба мислити када уз клиничку сливу (висока фебрилност, грозница, болови у мишићима, главобола, општа слабост и маласалост, евентуално макулопапулозна осна) постоји податак о убуду крпеља.

Кључне речи: Ehrlichia chaffeensis; хумана ерлихиоза; индирактна имунофлуоресценција; доксициклини