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ORIGINAL PAPER

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Analysis of Clinical and Laboratory Characteristics of Patients with Leptospirosis in Five-year Period

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ABSTRACT

Introduction: Leptospirosis is an infection caused by spiral bacteria from the family Leptospiraceae, and is considered to be the most widespread zoonosis in the world. **Aim:** To analyze the clinical and laboratory characteristics of patients with Leptospirosis over five years. **Methods:** The study included 160 patients aged 17-79 years, who in the period 01.01.2014. to 21.12.2018. were hospitalized at the Clinic for Infectious Diseases of the University Clinical Center Tuzla. They were diagnosed based on medical history, clinical examination, laboratory and microbiological results. The definitive diagnosis was confirmed by serological testing from the patients' blood. **Results:** In the observed period, the highest number of patients were in 2014 80/160, and the lowest in 2015 15/160. Male patients were more likely to suffer from 118 (73.8%) than female 42 (26.3%). The mean age was ± 56.5 years. The most common symptoms in patients were: fever (95.6%), headache (93.8%), malaise (87.5%) and myalgia (85.6%). In all patients, 160/160 (100%) accelerated erythrocyte sedimentation and elevated C-reactive protein was observed. The following findings were reported from white blood cell findings: leukocytosis in 81/160 (50.6%), neutrophilia in 103/160 (64.4%) and lymphopenia in 128/160 (80%) patients. Liver findings had the following values: elevated AST in 142/160 (88%) and ALT in 130/160 (81.3%) patients. Total bilirubin was elevated in 105/160 (65.6%) and direct in 107/160 (66.9%). Thrombocytopenia was in 142/160 (88%) patients. Urea was elevated in 103/160 (64.4%) and creatinine in 101/160 (61.3%) patients. **Conclusion:** it is very important that physicians in their day to day practice, especially in ambiguous febrile conditions, differentially diagnose leptospirosis and establish a timely diagnosis, this is key to adequate

and timely therapy, and therefore to reducing to development of complications and mortality.

Keywords: leptospira infection, Weil Disease, diagnosis of leptospirosis.

1. INTRODUCTION

Leptospirosis is a bacterial infection from a group of zoonoses caused by pathogenic *Leptospira* from the family Leptospiraceae. The entrance door to this cause in the microlesion of the skin and mucous membranes. The infection can be caused by direct contact with an infected animal or indirect contact via soil or water contaminated with the urine of the infected animal (1). Human leptospirosis is present on all continents except Antarctica and is considered the most widespread zoonosis in the world (2). Leptospirosis is a global public health problem and is estimated to have an incidence ranging from 0.1-1/100.000 inhabitants in temperate climates to $\geq 10/100.000$ inhabitants in humid tropical areas annually (3). Due to the often non-specific clinical symptoms of infections, the failure to make a differential diagnosis and the low availability of specific laboratory tests, especially in developing countries, it is assumed that there are always a large number of undetected cases. Leptospirosis is manifested by a different clinical picture of the disease. About 90% of leptospirosis is manifested by signs and symptoms of nonspecific anicteric febrile illness that mimics the symptoms of other diseases (4). The mortality rate for these forms of leptospirosis is less than 1%, but it is increasing in elderly patients with comorbidities (1, 5). Weil's disease is a serious, potentially lethal form of leptospirosis accompanied by hepato-renal insufficiency

and pneumonitis, hemorrhagic diathesis and impaired consciousness. Mortality in developed Weils syndrome is high and is >10% (5). In a microbiology laboratory, leptospirosis is usually diagnosed with a microscopic agglutination test (MAT), detection of antibodies by ELISA test, culturing bacteria from blood, CSF, urine and tissue, or polymerase chain reaction (PCR). Due to the field availability and ease of use, the diagnosis of leptospirosis is most commonly confirmed, with clinical and biochemical parameters, by an increase in IgM antibody titers in the acute phase of the disease (6).

2. AIM

To analyze the clinical and laboratory characteristics of patients with Leptospirosis for over five years.

3. METHODS

In this retrospective study the clinical, biochemical and serological characteristics of 160 patients with leptospirosis aged 17-79 years treated at the Infection Disease Clinic of the University Clinical Center Tuzla in period January 1, 2014, to December 31, 2018, were analyzed. Patient data were obtained through insight into their medical histories. The diagnosis of leptospirosis was determined by anamnestic data, clinical examination, laboratory and microbiological results. The definitive diagnosis was confirmed by serological testing from the patients' blood using a factory Leptospira IgM and IgG Elisa test (Virion/Serion GmbH, Würzburg, Germany). Interpretation of anti-leptospiral IgG and IgM antibody results was performed according to the manufacturer's specification (<15 U/ml negative, 15-20 U/ml borderline and >20 U/ml positive for IgM and <10 U/ml negative, 10-15 U/ml borderline and >15 U/ml IgG positive).

Laboratory analyses were interpreted from the blood and included baseline parameters of the inflammatory response, urea, creatinine, transaminases, complete blood count, and prothrombin time.

The study was approved by the Ethics Committee of the Public Health Institution University Clinical Center Tuzla No. 02-09/2-46/19.

Statistical data processing included basic tests of descriptive statistics and was performed using INM SPSS 23.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

4. RESULTS

Distribution of patients by months and years of disease

In the period from January 1, 2014, to December 31, 2018, 160 patients were hospitalized with the diagnosis of leptospirosis at the JZU UKC Tuzla, Infectious Diseases Clinic. Most patients were admitted in 2014 80/160, and 15/160 in 2015 (Figure 1).

Distribution of patients by gender

Of the 160 patients with leptospirosis enrolled in this study, 118 (73.8%) were male and 42 were female (26.3%) (Figure 2).

Distribution of patients by age

The age range of patients ranged from 17 to 79 and the mean age was ± 56.5 years. The highest prevalence of patients with leptospirosis was in the age group of 51-60 years

Figure 1. Distribution of patients by months and years

Figure 2. Distribution of patients by gender

Figure 3. Distribution of patients by age

with 50 cases (31.3%). The lowest prevalence was found in the age group of 15-20 years (1.3%) (Figure 3).

Results of serological testing

An ELISA test showed that 143 (89.4%) patients had IgM positive antibodies and 17 (10.6%) patients had borderline IgM antibodies. IgG antibodies were positive in 41 (25.6%) and negative in 119 (74.4%).

Clinical manifestations of the disease

The most common symptoms in patients with leptospirosis were: fever (95.6%), headache (93.8%), malaise (87.5%), and myalgia (85.6%). Other symptoms are listed in Table 1.

In all 160 patients (100% of patients), the accelerated erythrocyte sedimentation (ESR) and elevated C-reactive protein (CRP) were observed. The following findings were reported from white blood cell findings: leukocytosis in 81/160 (50.6%), neutrophilia in 103/160 (64.4%) and lymphopenia in 128/160 (80%) patients. Analysis of liver enzymes revealed elevated aspartate amino transferase (AST)

Symptoms and signs	No(%) of patients
Fever	153 (95,6)
Headache	150 (93,8)
Weakness	140 (87,5)
Myalgia	137 (85,6)
Dark color of urine	118 (73,8)
Weight loss	103 (64,4)
Abdominal pain	77 (48,1)
Icterus	70 (43,8)
Vomiting	88 (55,0)
Conjunctival suffusion	68(42,5)
Oliguria	68 (42,5)
Petechiae	37 (23,1)
Diarrhea	34 (21,3)
Positive meningeal signs	8 (5,0)
Dialysis treatment	6 (3,8)
Exitus letalis	4 (2,5)

Table 1. Symptoms and signs of patients with leptospirosis

in 142/169 (80%) and alanine amino transferase (ALT) in 130/160 (81,3%) patients. Total bilirubin was elevated in 105/160 (65,5) and direct in 107/160 (66,9%). Most patients 142/160 (88,0%) had thrombocytopenia. Urea was elevated in 103/160 (64,4%) and creatinine in 101/160 (61,3%) patients. The most common comorbidities were diabetes in 39/160 (24,4%) and high blood pressure in 16/160 (10%) patients. Four patients or 2,4% died during hospitalization. Dialysis was performed in 6 (3,6%). Extended prothrombin time (PT) was recorded in 116/160 (72,5%) patients (Table 2.)

A therapeutic approach in the treatment of leptospirosis

Most patients were treated with penicillin monotherapy, 94/160 (58.8%), third-generation cephalosporins 46/160 (28.4%) and doxycycline 13/160 (8.1%).

The average length of hospitalization was $\pm$ 12.7 days; the maximum hospital stay was 51 days and the minimum 2 days.

5. DISCUSSION

Leptospirosis is often manifested by uncharacteristic symptoms and signs. As a disease, it belongs to a group of medical conditions described as undifferentiated febrile diseases. Due to the non-specific onset of the disease characterized by sudden onset with fever, chills, and headache, the disease is often unrecognized by experienced clinicians. A wide range of possible differential diagnoses includes malaria, typhoid fever, rickettsioses, and ehrlichiosis, and some viral infection such as dengue, chikungunya fever, hantavirus fever or influenza.

The most common symptoms in the cases studied by Chawla et al. were fever (96,6%), followed by jaundice (63,3%) and conjunctival suffusion (40%) (4). Similar symptoms in their study are described by Prabhua et al. (7). One of the most common symptoms in our study was

Pathological ranges		
Laboratory findings	Reference ranges	No (%) of patients
↑ ESR	≤20 mm/1st hours	160 (100)
Leukocytosis	3.4-9.7x10 ⁹ /L	81 (50,6)
Neutrophilia	44.0-72.0 %	103 (64,4)
Lymphopenia	20.0-46.0 %	128 (80,0)
Thrombocytopenia	158-424x10 ⁹ /L	142 (88,0)
↑CRP	0.0-3.3 mg/L	160 (100)
↑AST	15-37 U/L	142 (88,0)
↑ALT	30-65 U/L	130 (81,3)
↑Total bilirubin	5-21 mmol/L	105 (65,5)
↑Direct bilirubin	0-5 mmol/L	107 (66,9)
↑Urea	3,2-7,4 mmol/L	103 (64,4)
↑Creatinine	80-115 mmol/L	101 (61,3)

*CRP- C-reactive protein, ESR- erythrocyte sedimentation rate, AST- aspartate aminotransferase, ALT- alanine aminotransferase

Table 2. Laboratory findings in patients with leptospirosis *CRP- C-reactive protein, ESR- erythrocyte sedimentation rate, AST- aspartate aminotransferase, ALT- alanine aminotransferase

headache (89,9%). It was described as pulsating bitemporal pain. Other symptoms were dominated by: malaise (83,85), myalgia (82%), dark urine (70,7%), loss of appetite (61,7%), jaundice (41,9%), vomiting (52,7%), abdominal pain (46,1%), decreased urination (40,7%), rash (23,1%), diarrhea (22,2%) and positive meningeal signs (4,8%). Suffusion (dilatation of conjunctival vessels without purulent exudates), as characteristic recognition symptom of leptospirosis that rarely occurs in other infectious diseases in our study, was found 68/160 (42,2%) patients. Conjunctival suffusion was accompanied by jaundice, which we found in 41,9% of our patients. In the study by Prabhua et al. (7) jaundice was observed in 44% of patients.

An important laboratory parameter of leptospirosis is thrombocytopenia, and it occurs in 40-86,6% of infections (2). In our study, 88% of patients had thrombocytopenia, which did not result in hemorrhagic syndrome. In their study, Rahul et al. describe 71% of thrombocytopenia cases (8). In a study conducted by Lina et al. in the Philippines, 15 of 59 patients with leptospirosis had an incidence of thrombocytopenia of 61% and was present in most patients suffering from severe leptospirosis (9).

The results of the study by Linda et al. suggest that thrombocytopenia is associated with various complications and worse prognosis of leptospirosis. Mortality rates are higher in patients with thrombocytopenia compared with patients who did not have laboratory-confirmed thrombocytopenia (10).

Therefore, early recognition of thrombocytopenia, prevention of further complications and mortality in leptospirosis are important. The mortality rate in our study was 2,5% and all patients belonged to the age group >70 years. The fatal outcome was due to the development of Weil's disease with severe kidney and liver damage. Lopez et al.

(11) found in their research that with increasing age, the risk of death increased. Age and gender are very important risk factors for leptospirosis as it has been established that male sex and older age are conducive to the onset of this disease. In our study, 73,8% of patients were male and 26,3% female. A similar ratio has been observed in most studies conducted in different fields. In a study conducted between 2011 and 2016 76% of all patients with reported leptospirosis in Germany were male (12). In a study involving 60 patients serologically positive to leptospirosis, Linda et al. stated that there were 41 men and 19 women (10).

In Northern India, the average age of patients was 32.6 years. There was a higher percentage of male patients (57%), while women patients accounted for 43% of patients (13). Our study showed that the average age of patients was 56.5 years, and the patients were most often in the age group of 51-60 years. As in other similar studies, there were 73,8% more sick men in our country, compared to 26,3% women.

Individuals over the age of 60 have been described as having a 7,3 times higher risk of death than those aged 19 to 20 (13). In a study in Northern Iran, the death rate was 6,1% (14). The WHO states that timely dialysis, as well as fluid and electrolyte balances, have reduced mortality from leptospirosis in recent years (3). Dialysis treatment in our study was needed for 3,8% of patients. Lecour et al. in Brasil, as many as 22% of patients describe the need for dialysis treatment (15).

Elevated bilirubin values are always a consequence of hepatocellular damage and interruption of intercellular connections between hepatocytes, leading to leakage of bilirubin from the bile ducts (16). Sethi et al. (13) also describe cases with high ALT and AST. Most patients had increased ALT (81,3%), AST (88%), total bilirubin (65,5%) and direct bilirubin (66,9%).

Our five-year study proved that the highest number of patients with leptospirosis was present in the second half of 2014. This was undoubtedly contributed by the unprecedented rainfall and devastating floods that engulfed our country.

All patients after serological evidence of the presence of specific IgM antibodies underwent antibiotic treatment. Most patients, 94 of them, were successfully treated with penicillin monotherapy (58,8%). In the second-largest successful treatment, this group of patients was treated with third-generation cephalosporins 46 (28,4%). The rest of the successfully-treated patients, 13 of them, were treated with doxycycline (8,1%). There is strong evidence in the literature that administering antibiotics to individuals with risk factors and clinical signs of leptospirosis prevents the development of more severe forms of the disease. A placebo-controlled trial in the Philippines has shown that administration of intravenous penicillin shortens the duration of leptospirosis, prevents impaired renal function, days of hospitalization, and spreads *Leptospira* with urine (17).

According to the Centers for Disease Control and Prevention, milder forms of leptospirosis are treated with doxycycline, while more severe forms are treated with third generations intravenous penicillin or cephalosporins (18). Mild asymptomatic cases generally do not require specific antibiotic treatment. An analysis of the research papers,

conducted by the Cochrane Library, concluded that there was still insufficient evidence for or against the preventive antibiotic treatment of persons who are, for example, occupationally exposed to the possibility of leptospira infection (19). Research from India shows that antibiotic prophylaxis does not prevent infection with *Leptospira* in endemic areas, but has a significant protective effect in reducing morbidity and mortality (20).

6. CONCLUSION

It is very important that physicians in their daily practice, especially in obscure febrile conditions, differentially diagnose leptospirosis and establish a timely diagnosis. Particular attention should be paid to the age group, which this work has shown to be risky as well, which includes respondents from 51-60 years. This is key to adequate and timely therapy, which will reduce the development of complications and mortality. Public health education is one of the most important methods for the prevention of leptospirosis.

- **Author's contribution:** Each author gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. Each author had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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