



Investigation of Borreliosis and Leptospirosis Frequency in Patients with Chronic Renal Failure and Renal Transplantation

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Abstract

Background: In this study, it was aimed to research the prevalence of *B. burgdorferi* and *Leptospira* in the patients with chronic kidney failure and kidney transplants, and to determine the place of these agents in their etiologies.

Material and Method: In this study, totally 284 individuals were included. 193 of these were volunteered patients that were followed up in nephrology polyclinic and 91 of them were volunteered control individuals without any chronic disease who were coded for diagnosis as Z00. The patient group consisted of 174 patients who were followed up for chronic kidney failure (90.2%) and 19 patients who were followed up after kidney transplantation (9.8%). 79 (45.4%), 32 (18.3%), 7 (4%), 5 (2.9%), 3 (1.7%), 2 (1.2%) of 174 patients were with hypertension, diabetes, polycystic kidney, glomerulonephritis, kidney stone and amyloidosis, respectively. The reasons of 46 patients (26.5%) were unknown. 4 (26.3%), 3 (15.8%), 11 (57.9%) of the patients with renal transplantation were with polycystic kidney, hypertension and unknown etiology, respectively.

RPR test (Plasmatec, USA) which is a *Treponema pallidum* screening test was performed to 284 sera. Besides, ELISA method was used for the presence of *Leptospira* IgG antibody (AccuDiagTMLeptospira IgG USA) and *B. burgdorferi* (Viro-Immun Labor-Diagnostika IgG, Germany) IgG antibody according to manufacturer's instructions.

Results: All of the samples were found negative for *T. pallidum*. 6 of all cases (2.1%) were *B. burgdorferi* positive, whereas 4 patients (2.1%) in patient group and 2 individuals in control group (2.2%) were *B. burgdorferi* IgG positive. *Leptospira* IgG was positive in 27 patients (13.9%) in patient group and 3 individuals (3.3%) in control group. *Leptospira* IgG was negative in patients with renal transplantation. There was a statistically significant difference between patient and control groups according to *Leptospira* IgG positivity ($p=0.006$). There was no significant difference between the groups in terms of *Leptospira* and *Borrelia* IgG seropositivity according to etiological diagnosis groups ($p>0.05$).

Conclusion: During *B. burgdorferi* and *Leptospira* infections, the patients cannot be early diagnosed and treated. In this situation, it should be considered that especially *Leptospira* cases with kidney involvement may undergo chronic kidney failure. Thus, *B. burgdorferi* and *Leptospira* infections should be considered in the patients with kidney involvement especially with risk factors such as living in rural areas, husbandry and forestry, and at least screening test should be performed.

Keywords: Borreliosis, Leptospirosis, chronic renal failure, renal transplantation

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Introduction

Chronic renal failure (CRF) is a condition characterized by a chronic progressive deterioration in the capacity of the kidneys to regulate the fluid and solute balance and maintain the metabolic endocrine functions as a result of the decrease in the glomerular filtration rate. CRF is caused by systemic or infectious causes or occurs due to an unknown etiology. The most common one among these factors is diabetes mellitus, followed by hypertension, glomerulonephritis, amyloidosis, and polycystic kidney disease. Cases of an unknown etiology comprise 20% of the patients (1,2). Leptospirosis is an infection that occurs as a result of the transmission of *Leptospira* species from animals to humans. It may lead to a wide range of conditions from asymptomatic infection to diffuse vasculitis, severe renal impairment, aseptic meningitis, haemorrhagic diathesis and even serious complications and death when left untreated. Kidney involvement in leptospirosis is a complication manifest with various symptoms including pyuria observed in all the forms of the condition, hematuria, urinary sediment changes and renal failure (3). Lyme borreliosis is a disease transmitted by ticks from the genus *Ixoides* and observed more commonly among adults working in agriculture, animal husbandry and forestry during the summer. The initial symptom of the disease is a rash called erythema chronicum migrans, followed by cardiac, neurological and skeletal symptoms through hematogenous dissemination and finally an autoimmune phase (4). Lyme borreliosis can rarely be accompanied by glomerulonephritis. It is a cause of glomerulonephritis in animals and especially in dogs; and cases of glomerulonephritis in humans have been reported in the literature (5). Undiagnosed cases of borreliosis and leptospirosis, which may lead to glomerulonephritis and manifest itself with acute renal failure, may progress chronic kidney failure when left untreated. The gold standard in the diagnosis of *B. burgdorferi* and *Leptospira*, which are among the spirochetes, is the culture. However, it is time consuming and rather difficult to isolate the agent. Also, since bacterial growth takes a long time and requires a specific culture medium, the culture method is not practical as a routine laboratory test (3,4,6). Patients should be diagnosed and treated before end-stage renal failure develops. Late presentation of the CRF patients to the hospital and failure to diagnose the condition during the acute infectious phase; as well as overlooking microorganisms such as *Leptospira* or *Borrelia* - which are often missed during the diagnostic process - are among the causes why the disease group of unknown etiology has a high percentage¹. The aim of this study is to investigate the prevalence of *B. burgdorferi* and *Leptospira* among the patients who have undergone

kidney transplants due to chronic renal failure and to determine the role of these agents in their etiology.

Material and methods

Selection of the patient and control groups

A total of 284 subjects were enrolled in the study. The study group consisted of 193 patients who were followed up between 01 January 2014 –31 December 2014 in the nephrology outpatient clinic of the... Training and Research Hospital. The control group was formed of 91 individuals with no known chronic diseases who had applied to various outpatient clinics of the hospital for check up and received the diagnostic code Z00. Among the study group, 174 patients (90.2%) were treated for chronic renal failure and 19 (9.8%) patients were followed up subsequent to kidney transplants. Among the 174 patients with CRF, the etiology was associated with hypertension (HT) in 79 (45.4%) patients, diabetes in 32 (18.3%), polycystic kidney disease (PKD) in 7 (4%), glomerulonephritis (GN) in 5 (2.9%), kidney stones in 3 (1.7%), and amyloidosis in 2 (1.2%). In 46 patients (26.5%), the etiology was unknown. Among the 19 kidney transplant patients, 4 (26.3%) had polycystic kidney, 3 had HT (15.8%), and the cause was unknown in 11 (57.9%).

Investigation of the Patients' Sera Using the Serological Method

For this purpose, 5 ml of peripheral venous blood was collected from the patient- and control groups and centrifuged at 2000 x g for 10 minutes to separate the serum. A total of 284 serum samples collected from 193 patients and 91 control subjects have undergone the RPR test (Plasmatec, USA) to scan for *Treponema pallidum*, as well as ELISA tests for the presence of *Leptospira* IgG (AccuDiag™ *Leptospira* IgG USA) and *B. burgdorferi* IgG antibodies (Viro-Immun Labor-Diagnostika IgG, Germany). The tests were carried out in double wells using commercial kits according to the manufacturer's instructions.

Based on the reference cut-off values within the kit according to the manufacturer's instructions, the serum samples below 0.5 OD for *B. burgdorferi* IgG were accepted as negative, while those over 0.5 OD were considered to be positive. For *Leptospira* IgG, results below 1.2 were accepted as negative, while those over 1.2 were considered to be positive.

Statistical analysis

The statistical analysis of the data was performed using the SPSS 15.0 for Windows software package based on a 95% confidence interval. The inter-group comparison of IgG

positivity with ELISA was performed with the help of Fisher's exact test, while the mean age of the patients according to their gender was compared using the independent samples t-test. Statistical significance was based on a value of $p < 0.05$.

Results

Patient and control group populations

In the study group, 51.8% were male and 48.2% were female, while 74.7% of the control group were male and 25.3% were female. The mean age of the patients in the study group was 58.67 ± 17.26 years, while the mean age in the control group was 33.43 ± 18.6 years. When the study and control groups were investigated separately, no significant difference was observed between the ages of the male and female patients ($p > 0.05$).

B. burgdorferi and *Leptospira* positivity in the clinical samples using ELISA

According to the results of the RPR test carried out in the patient and control groups, which is the scanning method for *T. pallidum*, all the samples were negative. *B. burgdorferi* was positive in 6 (2.1%) among all the patients, of which four (2.1%) were in the study group and 2 (2.2%) were in the control group. In the study group, 3 (1.7%) of the 174 CRF patients and one (5.3%) out of the 19 organ transplant patients were positive for *B. burgdorferi*. No significant difference was observed in terms of *B. burgdorferi* IgG positivity between the study and control groups ($p > 0.05$). *Leptospira* IgG was positive in 30 (10.5%) among all the patients. *Leptospira* IgG was positive in 27 (13.9%) patients in the study group and in 3 (3.3%) subjects in the control group. None of the organ transplant patients was positive for *Leptospira* IgG. There was a significant difference between the study and control groups in terms of *Leptospira* IgG positivity ($p = 0.006$) (Table 1).

Table 1. According to the group of patients with RPR, *B. burgdorferi* and *Leptospira* positive rates

	GROUP				Total	p
	Patients		Control			
	n=193	%	n=91	%	n=284	
RPR	0	0	0	0	0	$p > 0.05$
Leptospira	27	14	3	3.3	30 (%10.5)	0.06
Borrelia	4	2,1	2	2,2	6 (%2.1)	$p > 0.05$

Based on the etiology of the disease, among the patients with CRF (n=174), 14 out of the 79 patients (17.7%) with hypertension-associated disease, 3 out of the 32 patients (9.4%), 7 out of the 46 patients (15.2%) with CRF of unknown etiology, 2 out of the 7 patients (28.5%) with polycystic kidney disease, and one out of the 5 patients (20%) with glomerulonephritis were positive for leptospira IgG; while one out of the 79 patients (1.2%) with hypertension-associated disease, one out of the 32 patients (3.1%) with diabetes mellitus, and one among the 46 patients (2.2%) with CRF of unknown etiology were positive for Borrelia IgG. Only one among the 19 organ transplant patients (5.3%) was found to be positive for Borrelia IgG. No significant difference was observed in terms of Leptospira and Borrelia IgG seropositivity based on the etiology of the disease ($p>0.05$) (Table 2).

Table 2. Borrelia and Leptospira IgG positivity rates of distribution in the etiologic diagnosis of CRF patients.

		Borrelia	Leptospira
CRF n=174	Etiology	n=4(%2.1)	n=27 (%13.9)
	HT n=79	1 (%1.3)	14 (%17.7)
	DM n=32	1 (%3.1)	3 (%9.3)
	Unknown n=46	1 (%2.2)	7 (%15.2)
	PKD n=7	0	2 (%28.5)
	GN n=5	0	1(%20)
RT n=19		1 (%5.3)	0

Discussion

While CRF may be associated with hypertension, diabetes mellitus, glomerulonephritis, polycystic kidney disease or infections, the etiology of 10-20% of the CRF cases remains unknown¹. In the study by Nadir et al. conducted in our country, CRF of unknown etiology was found as 12%, while CRF unknown etiology was 26.4% in this study (1,2). *B. burgdorferi* IgG seropositivity in all the subjects was 2.1%, while it was 10.5% for *Leptospira* IgG. In the study group, 3 out of the 174 CRF patients (1.7%), one out of the 19 (5.3%) organ transplant patients was positive for *B. burgdorferi*. No significant difference was observed regarding *B. burgdorferi* IgG positivity between the study and control groups. However, 27 patients (%13.9) in the study group were positive for *Leptospira* IgG, while 3 patients (3.3%) were found to be positive in the control group.

None of the organ transplant patients was positive for *Leptospira* IgG. The difference between the study and control groups in terms of *Leptospira* IgG positivity was statistically significant. In case of Weil's disease, which is the severest form of leptospirosis, the most common cause of mortality is renal involvement. Renal involvement may be observed in all forms of leptospirosis (7-9). The renal involvement of leptospirosis occurs through immune mechanisms and may be in the form of immune complex-mediated mesangioproliferative glomerulonephritis, interstitial nephritis and acute tubular necrosis; leading to cellular damage, increase in the inflammatory cells and accumulation of immune complexes, and tubulointerstitial nephritis (3,10,11). In the seroepidemiological studies in humans, its prevalence has been observed as 2-12%, while it was reported as 3.5-63% in animals (3). In an epidemiological study conducted in Samsun, leptospirosis was investigated in a study group of 279 agricultural workers, veterinarians and rice farmers, which are among the high risk groups for leptospirosis, and 200 healthy controls using the microscopic agglutination test (MAT). Seropositivity was found as 4.3% in the risk group, while it was 0.5% in the control group (6). In our country, case studies are more common than seroprevalence studies. In the study by Cetin et al. conducted on 13 patients, impairment in the kidney function was observed in all the patients (12). In one of the three leptospirosis cases from the southern Marmara region published by Gurcuoglu et al., the urea creatine levels were reported to be severely elevated. In the retrospective study by Covic et al. comprising a 5-year period, they have published 58 cases with acute renal failure due to leptospirosis (7,13). In a case study by Sav et al., leptospirosis was reported in a patient who presented with acute renal failure and jaundice (10). Also in this study, 28.5% among the patients with polycystic kidney disease, 20% of those with glomerulonephritis, and 15.2% of those with CRF of unknown etiology were positive for leptospira IgG. These results underline the necessity to investigate leptospira in the etiology of CRF. *B. burgdorferi*, the agent for Lyme disease, may also cause glomerulonephritis (14). In various studies from our country, *B. burgdorferi* antibody positivity in the risk groups was 6-35.9%, while it was 2-6.6% in the control groups (15,16). In a study conducted on healthy subjects in Isparta, seropositivity was 17% in those with a history of tick bites, while it was 2% in those without tick bites (17). Although glomerulonephritis associated with *B. burgdorferi* has been reported between 5-10% in dogs, it has very rarely been reported in humans. The infection has caused diffuse tubular necrosis with mesangial proliferation in dogs. Cases with membranous and membranoproliferative glomerulonephritis and minimal change disease associated with *B. burgdorferi* have been reported in the literature (18-20). In

Lyme disease, the cause of the nephrotic syndrome is the reduction of the T-cell activation due to the diminished expression of the CTLA-4 binding to CD80 (18). In this study, the prevalence of *B. burgdorferi* was 2.1% in the study group, while it was 2.2% in the control group. The prevalence of *B. burgdorferi* among renal transplant patients was found as 5.3%. In another study comparing *B. burgdorferi* IgG positivity in the risk group living in the villages of Erzurum and the control group, the prevalence was found as 2% in the risk group and 2.5% in the control group (14). In a study from Sweden, seropositivity for Lyme borreliosis among the group of guides participating in tours in the rural areas was found to be similar to the normal population (21). While culture is the gold standard in the diagnosis of *borrelia* and *leptospira*, since their isolation is difficult and time-consuming, other serological tests are also necessary for the diagnosis. The ELISA IgG and IgM tests are frequently preferred methods in routine use due to their practicality, rapidness and relatively lower cost. The *B. burgdorferi* and *leptospira* IgM antibodies start to rise within 2-4 weeks after the contraction of the infection. False positivity due to cross reactions is also common. Therefore, the positive ELISA results are recommended to be confirmed using the Western Blot method. The positive results from the serological tests are of value in combination with the clinical results (3,7,22). In this study, in order to overcome this disadvantage to some extent and to reveal cross reactions in the patient and control groups, the RPR test (Plasmatec, USA) was also performed and negative results were obtained from all the samples. In the patients positive for *B. burgdorferi* IgG, the *B. burgdorferi* IgG was assessed to be positive in the absence of a cross-reaction with *T. pallidum* antigens (4,9,22). In this study, *B. burgdorferi* and *Leptospira* IgG antibodies were studied through the ELISA method, and since their syphilis tests were negative, the patients with positive results were assessed to have a past infection. Late presentation to the healthcare center and thus missing the acute infection period before the diagnosis is made, or overlooking microorganisms such as *Leptospira* and *Borrelia*, which frequently go unnoticed, during the diagnosis may explain the high ratio of the group with unknown etiology (1). There is no study in the literature investigating the seroprevalence of *B. burgdorferi* and *Leptospira* in organ transplant patients. The present study is the first *B. burgdorferi* and *Leptospira* seroprevalence study conducted on a limited number of renal transplant patients from our country.

Conclusion

In conclusion, a possible association of chronic renal failure with infection due to spiral bacteria such as *B. burgdorferi* or *Leptospira* should be considered. In case of *B.*

burgdorferi and *Leptospira* infections, an early diagnosis and treatment may not be possible since the culture is difficult and time consuming and serological diagnostic methods are neglected during the acute phase. Renal involvement and progress to chronic renal failure especially in the patients with *Leptospira* should be kept in consideration. Therefore, in the patient group with renal involvement and particularly in those with risk factors such as rural origin and occupation in animal husbandry or forestry, *B. burgdorferi* and especially *Leptospira* infections should be considered in the diagnosis and at least a scanning test should be performed through serological methods.

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