Turk J Public Health 2016;14(3)
Available online at: http://tjph.org/ojs/index.php/

Research Article

Seroprevalance of rickettsioses among healthy individuals in Mersin province, Turkey

İlker Alakuş[a], Elif Şahin Horasan[b], Ahmet Öner Kurt[c], Güliz Evik[d], Gülden Ersöz[e], Gönül Aslan[f], Ali Kaya[g]

[a] MD. Mersin University, School of Medicine, Dept. of Infectious Diseases and Clinical Microbiology, Yenişehir, Mersin

[b] Assoc. Prof.. Mersin University, School of Medicine, Dept. of Infectious Diseases and Clinical Microbiology, Yenişehir, Mersin

[c] Assoc. Prof. MD. Mersin University, School of Medicine, Dept. of Public Health, Yenişehir, Mersin

[d] MD. Mersin University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Yenişehir, Mersin

[e] Prof. Dr. MD. Mersin University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Yenişehir, Mersin

[f f] Prof. Dr. MD. Mersin University, School of Medicine, Department of Medical Microbiology, Yenişehir, Mersin

[g] Prof. Dr. MD.Mersin University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Yenişehir, Mersin

Received date 17.05.2016, Accepted date: 19.12.2016

Abstract

Objective: Studies relevant to rikettsial biology, its pathogenesis and diagnosis have increase due to the increase of rickettsioses incidents in the world, particularly over the the past two decades. We investigated the seroprevalence of the ricketsia disease in the Mersin province of Turkey. **Methods:** This study included 450 healthy individuals aged 5 years and over, who had attended family health centers in Mersin city center. Serum samples collected during November 2011 were evaluated. Rickettsia antibody assays were used indirect immunofluorescenc techniques.

Corresponding Author: Güliz Evik, Mersin University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Çiftlikköy Kampüsü, Yenişehir, Mersin. Phone: 234-8037595182, 234-7045639114; E-mail: gulizevik@gmail.com

Data were analyzed using a descriptive analysis, the Chi-Square Test and the Student T Test for comparison of variables. **Results:** The prevelance of ricketsia positivity was determined as 9.8%. The seropositivities were higher in males, in the unemployed, in the occupational risk groups, in people who live in rural regions, in people who work in gardens/farms, in picnickers, in countryside walkers, in hunters, in owners of livestock, in people with no history of rash diseases. When the relationship of spotted fever group (SFG) seropositivity the risk factors was investigated, there was no statistical significance. **Conclusion:** According to our study rickettsial disease is detected in Mersin and it was seen that the rickettsiosis disease need for more comprehensive studies to show the prevalence in all of Turkey.

Keywords: Rickettsioses, seroprevelance, antibodies.

Mersin İlinde sağlıklı bireylerde riketsiyozis seroprevalansı

Özet

Amaç: Riketsiyozların özellikle son yirmi yıldır dünyada artan insidansı nedeniyle, riketsiyaların biyolojisi, hastalığın patogenezi ve tanı yöntemlerinin geliştirilmesi ile ilgili çalışmaların artış gösterdiği bilinmektedir. Çalışmamızda Türkiye'de Mersin ilinde riketsiya hastalığının seroprevelansını belirlemeyi amaçladık. **Yöntem:** Çalışmaya Mersin il merkezindeki Aile Sağlığı Merkezlerine başvuran 5 yaş üstü 450 birey dahil edildi. Serum örnekleri Eylül - Aralık 2011 tarihlerinde toplandı. Bu çalışmada indirekt immünfloresan antikor testi uygulandı. Verilerin özetlenmesinde tanımlayıcı istatistikler ve değişkenlerin karşılaştırılmasında Ki-Kare Testi ve Student T Testi kullanıldı. **Bulgular:** Çalışmamızda seropozitiflik oranı %9.8 olarak tespit edilmiştir. Seropozitiflik, erkeklerde, işsizlerde, riskli meslek grubunda, kırsal bölgede yaşayanlarda, bahçe ve çiftçilikle uğraşanlarda, piknik yapanlarda, doğa yürüyüşü yapanlarda, avcılık yapmayanlarda, hayvan beslemeyenlerde, öyküsünde döküntülü hastalık bulunmayanlarda daha yüksektir. Benekli ateş grubu (BAG) seropozitifliği ile risk faktörleri arasındaki ilişki araştırıldığında, istatistiksel olarak anlamlı bir fark bulunmadığı saptandı. **Sonuç:** Mersin ilinde riketsiyal hastalıklar saptanmış olup, riketsiyanın tüm Türkiye'deki prevelansının gösterilmesi için daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Riketsiya, seroprevalans, antikor

Introduction

Rickettsioses are worldwide zoonoses caused by bacteria (Genera *Rickettsia* and *Orientia*) and transmitted by arthropods (lice, fleas, ticks and mites). These zoonoses are among the oldest known vector borne diseases. In Europe, only *Rickettsia* spp. are etiological agents of rickettsioses. The gold standard in diagnosis is immunofluorescent antibody testing.[1] Where other tests are not available, the Weil Felix test can be used.[1] There are several classifications of *Rickettsia* spp. The most widely used is one that divides this genus into the spotted fever group (SFG) and the typhus group (TG).[2]

It has been shown studies on the biology, pathogenesis and methods for diagnosing rickettsioses are increasing due to high incidence of rickettsioses in the past two decades.[1,3] In Turkey, the number cases of the Mediterranean SFG have been increasing since 1990, especially in Thrace region and in Istanbul.[4] Today, exposures to tick bites or to tickborne pathogens have increased due to risky behaviours relating to rickettsioses, such as picnicking, jogging,

camping, hunting, or safaris. Other relevant activities include frequent exposure to domestic or wild animals that are reservoir in endemic areas, working or living in wooded areas, vineyards or countrysides in which ticks are ubiquitous. Mediterranean spotted fever is considered endemic in Mediterranean countries. Rickettsiosis has been increasing in all Mediterranean countries. Ticks that are vectors are present in almost every region of Turkey, varying in numbers and characteritics, but there are limited studies on rickettsioses in Turkey despite increasing numbers of Mediterranean spotted fever cases and exposures to tickborne pathogens. Most previous studies have been of patients with clinical symptoms and sample sizes were quite small. Therefore, in our population-based study, a questionnaire was used in order to identify risky behaviour models and the presence of rickettsial infections, and also to test for seropositivity in blood samples.

In the present study, rickettsial diseases were investigated in healthy individuals who had been living in Mersin province.

Methods

The target population consisted of 777,164 people aged 5 years and over, living in the center of Mersin province. The sample size was estimated by using EPI Info Version 3.5.1 software with a 95% confidence interval and 3% error. When the disease prevalence was considered to be 11.7%, the minimum sample size was estimated as 441. We planned to include 450 individuals aged 5 years and over, who had attended family health centers in Mersin city center. First, the total population aged 5 years and over in the Mersin city center was stratified on the basis of sex and age groups in decades. Afterwards the weighted numbers of the study population were calculated according to sex and each age group. For a better representation of our study sample, we collected data from several family health centers. For this reason, 25 family physicians (FPs) (totally there are 183 FPs in the city center) have been chosen by systematic sampling and 18 persons from the registered population of each FP were included in the study sample.

The study was approved by Mersin University Faculty of Medicine Ethics Committee. Patients above 5 years of age who had been admitted to a Family Health Center for any reason were included. Before enrolling in the study, each patient gave written informed consent.

Data collection

The sociodemographic data of participants were recorded on a data form on which there were 13 questions, three of them open-ended. The sociodemographic data form included age, gender, address (rural region-village-street name), occupation and educational level. This form also recorded field working, gardening, picnic frequency, countryside walks, camping, hunting, safari, history of animal husbandry, and history of spotted diseases with fever. Filling the data form took about five minutes. No one refused to participate in the research.

Blood samples taken from participants were centrifuged at 4000 rpm for 5 minutes. Then, 1 cc serum samples were put into Eppendorf tubes and stored at -30^{o}C until the assay. Before assaying, serum samples were taken to room temperature for 1 hour. Similarly, a Rickettsia IFA IgG test kit was taken from +4^{o}C to room temperature for 30 minutes before starting the assay. In this study, indirect immunofluorescence antibody (IFA) test was used as it is the most generally preferred among the serologic tests, and has been considered as the “gold standard”.[1] It has been used by many laboratories with high sensitivity and specificity. So far as the validity rates are concerned, our study was of the seroepidemiologic type, and used the Rickettsia IFA IgG (Focus Diagnostics, Cypress, California, USA, Product Code: IF0100G) commercial kit. The procedure was performed according to recommended protocol by manufacturer.[5]

Table 1. Demographic characteristics of the study group and comparison of the factors involved in seropositivity

	Seropositive		Seronegative		Total		χ^2
	n	%	n	%	n	%*	p
Gender							
Male	24	10.8	199	89.2	223	49.6	0.486
Female	20	8.8	207	91.2	227	50.4	0.486
Employment status							
Employed	12	8.5	130	91.5	142	31.6	0.414
Unemployed	32	10.4	276	89.6	308	68.4	0.520
Risky occupation							
Yes	4	21.1	15	78.9	19	4.2	2.859
No	40	9.3	391	90.7	431	95.8	0.091
Living region							
Rural	6	11.3	47	88.7	53	11.8	0.162
Urban	38	9.6	359	90.4	397	88.2	0.687
Gardening, farming							
Yes	15	12.8	102	87.2	117	26.0	1.659
No	29	8.7	304	91.3	333	74.0	0.198
Picnicking							
Yes	22	11.3	173	88.7	195	43.3	0.883
No	22	8.6	233	91.4	255	56.7	0.347
Countryside walking							
Yes	14	13.0	94	87.0	108	24.0	1.634
No	30	8.8	312	91.2	342	76.0	0.201
Hunting							
Yes	2	9.5	19	90.5	21	4.7	0.002
No	42	9.8	387	90.2	429	95.3	0.968
Livestock farming							
Yes	9	9.2	89	90.8	98	21.8	0.050
No	35	9.9	317	90.1	352	78.2	0.823
History of rash diseases							
Yes	1	5.3	18	94.7	19	4.2	0.458
No	43	10.0	388	90.0	431	95.8	0.498
Total	**44**	**9.8**	**406**	**90.2**	**450**	**100.0**	

*Column percent

The study participants who were from sectors of agriculture, hunting, and forestry were defined as in the "occupational risk" group. When all the study groups were classified according to the type of living places, province centers and districts were accepted as urban regions, and villages were accepted as rural region. Data were analyzed using a descriptive analysis, the Chi-Square Test and the Student T Test for comparison of variables. The level of statistical significance is accepted as $p \leq 0.05$.

Results

Of the participants, 227 (50.4%) were female; the mean age of the participants was 32.7±18.3 (range, 5-79). The proportion of active employed people was 31.6% (n=142). Sociodemographic characteristics of the participants and associations with suggested risk factors were presented in Table 1.

Rickettsia IgG antibody was positive in 44 (9.8%) of the subjects. None of the serum samples was positive for TG rickettsia IgG antibody. The seropositivities were higher in males (10.8%), in unemployed subjects (10.4%), in the occupational risk groups (21.1%), in the people who live in rural region (11.3%), in the people who work in gardens and farms (12.8%), in the picnickers (11.3%), in the countryside walkers (13.0%), in the hunters (9.8%), in those handling livestock (9.9%), in those who had no history of rash diseases (10.0%) than any the other groups. When the relationship of the SFG seropositivity to the risk factors was investigated, none were statistically significant (Table 1). There was no statistically significant difference between seropositivity and age (Table 2).

The distribution of educational level is presented in Table 3. When the seropositivity status of the participants was evaluated according to the educational level (university graduates) were found to be higher than the other groups but this difference was not statistically significant (Table 3).

Table 2. The distribution of age groups of participants

Age groups	Seropositive		Seronegative		Total	
	n	%	n	%	n	%*
5-14	9	10.5	77	89.5	86	19.1
15-24	9	10.0	81	90.0	90	20.1
25-34	3	3.7	79	96.3	82	18.2
35-44	4	5.8	65	94.2	69	15.3
45-54	9	15.5	49	84.5	58	12.9
55-64	4	10.8	33	89.2	37	8.2
65 and above	6	21.4	22	78.6	28	6.2
Total	**44**	**9.8**	**406**	**90.2**	**450**	**100.0**

*Column percent, χ^2= 11.290, p= 0.080

Table 3. The distribution of education level of participants

Education level	Seropositive		Seronegative		Total	
	n	%	n	%	n	%*
Primary school and under	22	10.0	198	90.0	220	51.1
Secondary school	6	8.0	69	92.0	75	17.4
High school	9	9.8	83	90.2	92	21.3
University graduates	6	13.6	38	86.4	44	10.2
Total	**43**	**10.0**	**388**	**90.0**	**431**	**100.0**

*Column percent, χ^2= 0.986, p= 0.805

Discussion

There have been many studies about rickettsiosis in Turkey.[6-10] Vural et al.[10], studied ricketsiosis seroprevalence in rural Antalya in 1995. They found 13.2% seropositivity by IFA in healthy people who were in continuous contact with animals and they reported that the disease was endemic in that region. Tekin et al.[11], conducted a study in Northern Anatolia and aimed to determine the high-risk behaviour models for ricketsioses together with seropositivity. They looked for the presence of anti-rickettsia IgG antibody by using the IFA technique. In total, 11.7% of the subjects were seropositive for the spotted fever group rickettsia IgG. But, there was no seropositivity for the typhus group. We found the rate for this seropositivity was 9.8% in our region.

In the studies from other countries, Marshal et al.[12], found SFG seropositivity higher in rural people when compared to urban people in Northern Greece. Tekin et al.[11] reported that seropositivity was 1.48 fold higher in rural people than in urban people. Vural et al.[10] reported that dwelling in a rural region was a significant risk factor. In our study, the seropositivity was 11.3% for people who live in a rural region. And it is higher than for the other groups.

Some results of studies about Rickettsia seropositivity have reported these to be higher in males and other studies have reported them to be higher in females.[13-16] In our study, the seropositivity was higher in males 10.8% compared to females females but there was no statistically significant difference between these in our study.

Tekin et al.[11] collected 580 blood samples to search the presence of anti-rickettsia IgG antibody, using the IFA technique. They reported that the mean age of seropositive and seronegative individuals were significantly different as 46.7±18.6 and 32.9±20.2, respectively. Kuloğlu et al.[13], examined 30 patients diagnosed with spotted fever ricketsiosis between 1997 and 2003 at Trakya University. The mean age of the study group was 53.6±15 years. Ozgunes et al. reported 20 rickettsiosis cases with a mean age of 38.0[14]; which was similar to the mean age of our study group. In our study, the seropositivity in the occupational risk groups is 21.1% (n=4); whereas it is 9.3% (n=40) in non-risky occupations. Although this difference is not statistically significant, the ratio is high and remarkable. Despite the fact that the rate is more than two times, this is due to the small number of those with occupational risks.

In previous studies, picnicking was the reported risk factor which was followed by country walking and gardening. However, in our study, none were statistically significant. Tekin at al. reported that doing gardening was a risk factor with a seropositivity rate of 18.8%.[11] In our study, similar to other studies, we found no positivity for the typhus group rickettsia.

Conclusion

Case series from our country have revealed that rickettsioses and seropositivity are more frequent than expected. In this study, seropositivity was 9.8% in our region. Besides the epidemiologic rate, risky behaviours and populations at risk were also determined. In our country, especially during the summer, ricketsial diseases must be considered in differential diagnoses when a patient presents with fever and muscle and joint pain. In addition, risky behaviour models should be evaluated in detail and people should be informed about rickettsioses in order to improve the level of awareness. We believe that this study is important as the first step of further studies with larger samples and should be supported.

Potential conflicts of interest

None of the authors any potential financial or nonfinancial conflicts of interest related to this manuscript.

References

1. Mandell GL, Bennett JE, Dolin R. In: Principles and Practise of Infestious disease 7th ed. USA: Introduction of rickettsioses, 2010;186:2495-2498.
2. Portillo A, Santibáñez S, García-Álvarez L, Palomar AM, Oteo JA. Rickettsioses in Europe Microbes and Infection 2015;17: 834-838.
3. Parola P, Paddock CD, Raoult D. Tick-Borne rickettsioses around the world: Emerging diseases challenging old concepts. Clin Microbiol Rev 2005;18: 719-756.
4. Topçu AW, Söyletir D. Riketsiya ve Erlihliya Enfeksiyonları In: Enfeksiyon Hastalıkları ve Mikrobiyolojisi: 3. baskı, İstanbul: 2008;150:1979-1990.
5. Scola La B, Raoult D. Laboratory diagnosis of rickettsioses: Current approaches to diagnosis of old and new rickettsial diseases J Clin Microbiol 1997; 35:2715-2727.
6. Eren N, Hamzaoğlu O. Türkiye'de Bulaşıcı Hastalıklar (1925-1993). Ankara: Türk Tabipleri Birliği, 1996:80-81.
7. Mert A, Tabak F, Dumankar A, Eroğlu C, Öztürk R, Aktuğu Y. Dört Marsilya humması olgusu. Klimik Derg 1997;10: 146-148.
8. Kuloğlu F, Rolain JM, Akata F. First Isolation of Rickettsia Conorii From Humans in the Trakya Region of Turkey. Eur J Clin microbiology Infect disease 2004;23:609-614.
9. Aydin L, Bakirci S. Geographical Distribution of Ticks in Turkey. Parasitol Res 2007;101 (Suppl 2):163-166.
10. Vural T, Ergin C, Sayin F. Investigation of Rickettsia conorii antibodies in the Antalya area. Infection 1998;26:170-172.
11. Tekin A, Gözalan A, Çöplü N, Yılmaz G, Köksal İ. Türkiye'nin Karadeniz Bölgesinden seçilmiş merkezlerde Riketsiya seropozitivitesi ve risk faktörleri; Dicle Tıp Derg 2015;37(3): 204-210.
12. Marshall GS, Stout GG, Jacobs RF, et al. Antibodies Reactive to Rickettsia Rickettsii Among Children Living in the Southeast and South Central Regions of the United States. Arch Pediatr Adolesc Med 2003;157: 443-448.
13. Kuloğlu F, Filiz A,Tansel Ö, Gürcan Ş. Son Altı Yılda Trakya Bölgesindeki Benekli Ateş Grubu Riketsiyoz Olgularının Özellikleri; Klimik Dergisi 2004; (17)2: 87-90.
14. Özgüneş N, Ergen P, Yazıcı S, Aksoy Y, Bekler G, Sargın F.; Yirmi Riketsiyoz Vakası. Klimik Derg 200114: 91-92.
15. Gençer S, Özer S, Ak Ö. Riketsiyozlu 19 hastanın değerlendirilmesi. XII. Türk Klinik Mikrobiyoloji ve Enfeksiyon Hastalıkları Kongresi Bildiri Özet Kitabı 2005, ss: 253.
16. Vural T, Ergin Ç, Kurşun AE. Antalya Yöresinde Rickettsia Conorii Antikorlarının Araştırılması. Mikrobiyol Bült 1995; 29:370-374.