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Patients with suspected visceral leishmaniasis in Istanbul

Huseyin Cakan[1], Suat Saribas[2]*, Vecdet Oz[1], Erdal Polat[2], Mustafa Aslan[2] and Bekir Kocazeybek[2]

[1]Institute of Forensic Sciences, Istanbul University, Turkey.

[2]Microbiology and Clinical Microbiology Department, Cerrahpasa Faculty of Medicine, Istanbul University, Turkey.

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We performed a four year study to investigate the Visceral Leishmaniasis (VL) cases in, Turkey. Fifty-nine patients with suspected VL from Istanbul were included in this work. Bone marrow and blood samples of these patients were tested for possible VL infection using several methods including serological tests, microscopy, PCR. Nineteen (32.2%) patients had positive results for VL after one or more of the tests performed, while only 7 patients (11.8%) had positive results with all the tests including Giemsa stain. Four (6.8%) patients had negative results based on all the serological tests performed except for positive results with Giemsa stain, culture and PCR. The other 4 (6.8%) patients had positive results with Formol-gel, ELISA IgG (>1.1 ISR) and IFAT IgG, (>1/256) but negative results were obtained with direct microscopic examination, culture and PCR. Using PCR *Leishmania infantum* DNA was detected in 11(18.6%) of the (*Leishmania*) cultures originated from the bone marrow samples. *Plasmodium vivax* was found in 2 (3.4%) patients and leptospira was detected in 1 (1.7%) patient. One (1.7%) patient was diagnosed with *Pneumonia* (*Streptococcus pneumoniae*) . Forty (67.8%) patients had negative results after direct microscopic examination, culture, serological tests and PCR. The kappa coefficients = 0.80 = 1.00, = 0.51, = 0.55 and = 0.45 were evaluated for PCR and direct microscopic examination, PCR and culture, PCR and ELISA, PCR and IFAT and PCR and Formol-Gel, as perfect agreement, perfect agreement, moderate agreement and moderate agreement fair moderate, respectively. The probability values (p) for comparisons of all the above tests with PCR showed a significant correlation (p < 0.000) In conclusion, we found that no single method alone was sufficient enough to diagnose VL accurately; however, combined with PCR, all these methods can reveal better and sensitive results ultimately leading to a correct diagnosis. We also suggest that PCR has to be applied with other laboratory diagnostic tests in order to increase the sensitivity in diagnosis and decrease the possible defects in diagnosis.

Key words: Visceral leishmaniasis, *Leishmania infantum*, Turkey.

INTRODUCTION

Visceral Leishmaniasis (VL) is a parasitic disease caused by *Leishmania infantum*. It is transmitted through bites of infected sand flies (female Phlebotomus). The reservoirs of VL vary from region to region; dogs, Canidae, wild rodents and house carnivorous are the most common reservoirs (Kilic. 2002). VL is usually seen in children and occasionally in adults (Unat et al., 1991; Pearson et al., 2000; Kilic. 2002). This disease is present in different regions of the world but widely seen in the coasts of the Mediterranean countries including Turkey. VL cases were common the coastal Turkish cities such as Izmir, Aydın, Denizli, Manisa, Mugla, Kastamonu, Istanbul, Bilecik, Karabuk as well as other cities located inlands like Kars, Erzurum, Tokat, Konya, Nigde and Aksaray suggested that the disease can be seen in any part of Turkey (Ok et al., 2002; Ozensoy et al., 2002; Ertabaklar et al., 2003). VL particularly affects individuals who have poor social-economical status and live under poor hygiene conditions. According to a Turkish Health Ministry report,

*Corresponding author. E-mail: suatsaribas@yahoo.com. Tel.: 0090212 4143000-22417, 00905326168150

Table 1. Travel history of patients with definite visceral leishmaniasis and the comparison of some demographic characteristics.

Patient No	Age	Gender	The first location	Current location	Hospital	Travel history
1	7	M[1]	U ak	Kagıthane/IST[3]	Sisli Etfal Hospital	+
2	12	F[2]	Zonguldak	Bagcılar/IST	Bakırkoy SIH[4]	+
3	4	M	Istanbul	Mecidiyeköy/IST	i li Etfal Hastanesi	-
12	6	F	Kastamonu	Kastamonu	Sisli Etfal Hospital	-
29	2	F	Kastamonu	Inebolu/Kastamonu	Sisli Etfal Hospital	-
30	12	M	Hatay	Hatay	Sisli Etfal Hospital	+
31	2	M	Istanbul	Kavacık/IST	Kartal Government Hospital	-
36	3	F	Istanbul	Kavacık/IST	Kartal Government Hospital	-
43	1	F	Istanbul	Halkalı/IST	Bakırköy SIH	-
46	6	M	Kütahya	Kütahya	Afyon Kocatepe Medical Faculty	+
54	16	M	Kars	Izmit	Cerrahpasa Medical Faculty	+

M[1]: Male, F[2]: Female, IST[3]: Istanbul, SIH[4]: Social insurance hospital

the annual rate of VL was limited to 40 cases (Ok et al., 2002). There are 12 millions leishmaniases cases world-wide and 200 000 deaths as a result of VL every year according to WHO report (One World Health Organization: Treatment in Leishmaniasis (Access date: www.oneworldhealth.org/diseases/leishmaniasis.php.:12. 05.2006).

Pre-diagnosis of VL can be made based on clinical findings; however, definite diagnosis requires detection of amastigotes in smears prepared from the clinical samples. ELISA, IFAT and Formol-Gel are generally used to diagnose VL and recent reports have shown that PCR method generated more sensitive and specific results. (Schallig et al., 2001; Lachaud et al., 2001; Ozensoy et al., 2002; Reithenger et al., 2002).

In this study, we aimed to investigate bone marrow and blood samples obtained from the patients with suspected VL. We used PCR, Giemsa staining, culture and other serological methods to determine VL. We then compared PCR results with those obtained from the laboratory diagnostic tests.

MATERIAL AND METHODS

We investigated 59 patients with suspected VL between 2002 and 2006 in Istanbul. Bone marrow and blood samples were collected from the hospitalized patients with suspected VL. Three N.N.N (Novy-MacNeal Nicolle) media preparations were used for culture per patient and smears were prepared from these cultures. Blood samples were tested using Formol- gel, ELISA IgG (Leishmania Ab R- Biopharm, Germany) and IFAT (It was tested with *Leishmania infantum* which we prepared) tests. Most of the laboratory work was performed in the Forensic Science Institute's Forensic Microbiology and Parasitology Laboratory of Istanbul University. All PCR studies were conducted at the Charite Institute of Microbiology and Hygiene, (Molecular Parasitology Department) of the Humboldt University of Berlin using the portions of the bone marrow samples.

Statistical analysis

The data were processed with SPSS for Windows version 11.5 (SPSS, Inc., Chicago, IL). Statistical analysis of test results was performed in terms of sensitivity, the degree of agreement between tests and Kappa ( ) values.

The degree of agreement between PCR, direct microscopic examination, culture, serological tests (ELISA, IFAT, Formol-Gel) was determined by calculating Kappa ( ) values with 95% confidence intervals. Kappa values express the agreement beyond change, and a value of 0.21-0.60 represents a fair to moderate agreement. 0.60-0.80 a substantial agreement >0.80 almost perfect agreement beyond change (Altman, 2001)

The calculation of the degree of agreement between the tests and the values for PCR-Direct Microscopic Examination, PCR-Culture, PCR-ELISA, PCR-IFAT, PCR- Formol-Gel were based on the results obtained with the suspected VL serum samples.

RESULTS

The travel histories and demographic characteristics of the patients diagnosed with VL were shown in Table 1. Five of the 11 patients were living in another place different from Istanbul and 6 of the 11 patients were living in Istanbul. Only five patients have a travel history in this patient group. In 19 (32.2%) patients, positivity was detected for VL after using one or more tests (Table 2). Seven (11.8%) patients had positive results after all the tests were performed including Giemsa stain. Otherwise 4 (6.8%) patients were negative with all the serological tests, but these 4 patients were positive after Giemsa stain, culture and PCR analyses. The other 4 (6.8%) patients were positive using Formol-gel, ELISA IgG >1.1 ISR, IFAT IgG >1/256, but direct microscopic examination, culture and PCR showed negative results. *Leishmania infantum* was identified by PCR [R.A RFLP] in 11(18.6%) of *Leishmania* cultures (Figure 1) from bone marrow samples inoculated onto N.N.N media (Picture 1, 2). *Plasmodium vivax* was detected in 2 (3.4%) patient and leptospira was in 1 (1.7%) patient. *Pneumoniae* (*Streptococcus pneumonia*) was found in 1 (1.7%) patient and 40 (67.8%) patients were negative with direct microscopic examination, culture, serological tests and PCR.

The kappa coefficients = 0.80 = 1.00, = 0.51, =

Table 2. The Distribution of patients with suspected Visceral Leishmaniasis who has one or more positivity in visceral leishmaniasis after the diagnostic tests.

No	Patient no	Direct	Culture	ELISA	IFAT	Formol-Gel	PCR
1	1	+	+	+	+	+	+
2	2	+	+	+	+	+	+
3	3	+	+	+	+	+	+
4	6	(-)	(-)	+	+	+	(-)
5	8	(-)Pneu	(-)	(-)	(-)	+	(-)
6	12	+	+	+	+	+	+
7	18	(-)	(-)	+	+	+	(-)
8	29	+	+	(-)	(-)	(-)	+
9	30	+	+	+	+	+	+
10	31	+	+	+	+	+	+
11	36	+	+	(-)	(-)	(-)	+
12	42	(-)	(-)	+	+	+	(-)
13	43	+	+	(-)	(-)	(-)	+
14	46	+	+	(-)	(-)	(-)	+
15	47	(-)Leptospira	(-)	(-)	(-)	+	(-)
16	49	(-)Malaria	(-)	+	(-)	(-)	(-)
17	51	(-)	(-)	+	+	+	(-)
18	54	+	+	+	+	+	+
19	56	(-) Malaria	(-)	(-)	(-)	+	(-)

PCR: Polymerase Chain Reaction IFAT: Immun fluorescense antibody test.
Pneu = Pneumoniae.

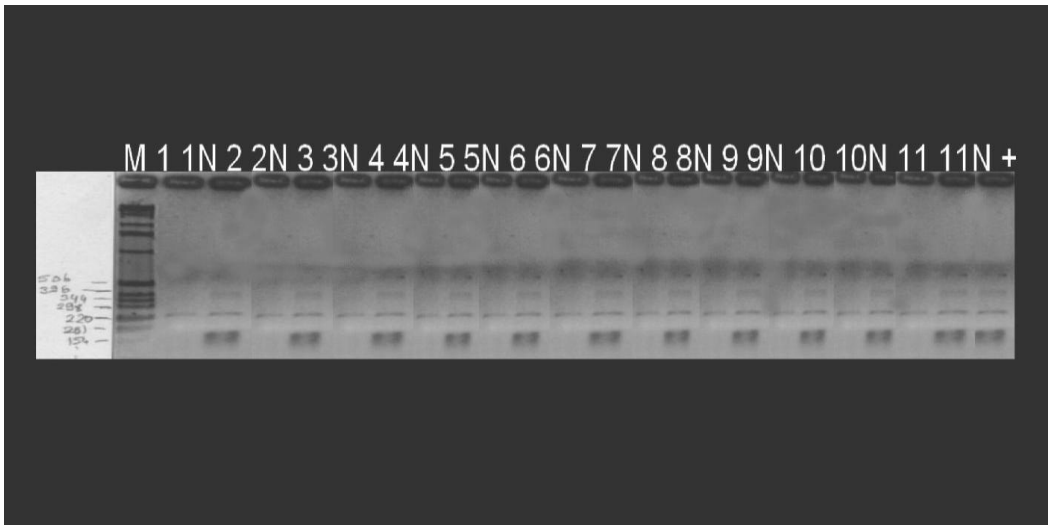
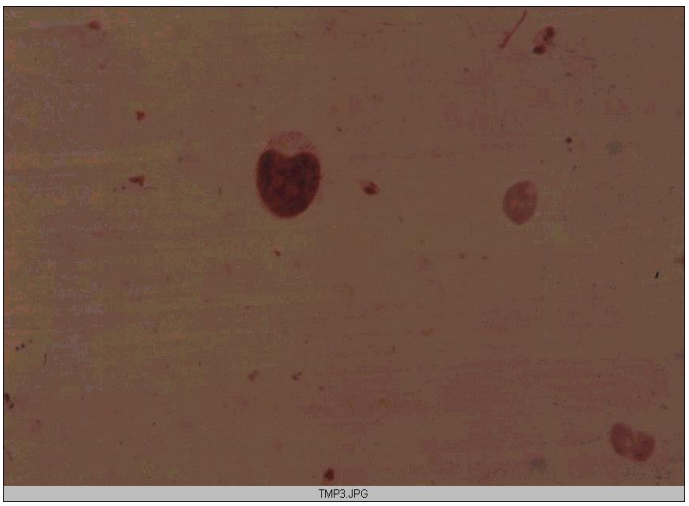


Figure 1. The bands of PCR [R.A (RFLP)] products of bone marrow samples in the agorose-gel (M = marker N = nested products).

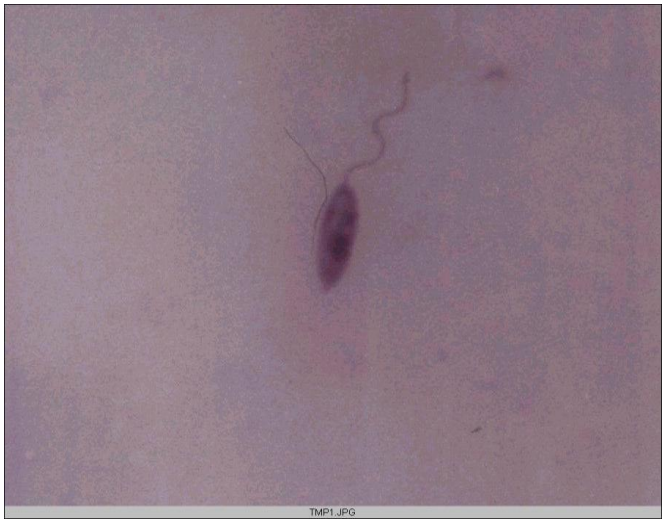
0.55 and = 0.45 were evaluated for PCR and direct microscopic examination, PCR and culture, PCR and ELISA, PCR and IFAT and PCR and Formol-Gel, as perfect agreement, perfect agreement, moderate agreement and moderate agreement fair moderate, respectively. The probability values (p) for comparisons of all the above tests with PCR showed a significant correlation ($p < 0.000$) (Table 3).

DISCUSSION

Leishmaniasis is an important parasitic disease which



Picture 1. Amastigote form of Leishmania in bone marrow (Giemsa 10X100).



Picture 2. Promastigote form of leishmania in N.N.N. media (Giemsa 10 x 100).

affects large populations. Although Leishmaniasis has been known for years, proper diagnosis, control, treatment of this disease are always difficult. Basic diagnosis for VL is often problematic due to negligence and physicians often do not consider the possibility of VL when a patient comes with the symptoms of the disease. In order to control Leishmaniasis, quick diagnosis is imperative so that proper treatment for patients can be arranged (Ozdener, 2005). The use of fast and reliable methods such as PCR has gained big importance in VL diagnosis. Among the eleven patients diagnosed with definitive VL, we found some patients were original residents of Istanbul, while the others were from the other cities of Turkey (Table 1) confirming early studies indicated the sporadic of the disease (Ok et al., 2002). It is conceivable that factors such as migrations due to socio- economical problems and easy travel have made the spread of diseases from one place to another easy. The VL cases have to be reported to the authorities of the cities which have sporadical VL cases like Istanbul in order to take some precautions to control the reservoir dogs and vector *Phlebotomus.* VL cases were seen sporadically almost in every regions of Turkey. VL cases are seen annually as average 40 in Turkey according to the official reports (Ok et al., 2002; Ozbel et al., 2002).

The each result from laboratory diagnostic tests for patients with suspected VL and patients with one or more positive results with diagnostic tests (Table 2) was compared with PCR and statistically cappa test was used for comparison. After the evaluation of cappa test results, culture results were found statistically very significant and in concordance with PCR results. Direct microscopy results were also found statistically significant. ELISA and IFAT showed statistically moderate significance but Formol-Gel showed a weak association.

Laboratory methods are also useful in making diagnosis for parasitic infections in addition to clinical symptoms, clinical history, and travel history, geographical location. Molecular methods based on the amplification of nucleic acids are commonly used actually for the diagnosis of parasitic infections. Diagnosis of the parasitic infections is not a trivial task. For instance scarce parasites in samples or parasite's atypical morphology make proper diagnosis difficult. There is always a contamination risk in the culture methods. The direct methods for detecting parasites are reliable; however they have a low sensitivity. More sensitive PCR method can be used in readily available blood samples from patients (Osman et al., 1997; Aviles et al., 1999; Fissore et al., 2004; Silva et al., 2004).

Non-invasive serological tests which have high sensitivity and specificity are also very helpful in the specific discriminative diagnosis of patients who are thinking as VL. The gold standard is to show parasites in a smear of bone marrow. However bleeding, insufficient sampling can be a problem during bone marrow aspiration. In addition amastigote forms cannot be found in aspiration samples.

A few DNA of parasite can be used in PCR and the sensitivity and specificity increase with the use of PCR. PCR is a promising and alternative method and also candidate for being gold standard in future. The number of parasites decreases after treatment and a few parasites can be obtained with the aspiration of bone marrow. These cause difficulties in direct microscopy because of the scarce of parasites. Piarroux et al. (1999) reported that no method has 100% sensitivity but the most sensitive method is PCR in the diagnosis of VL. They also reported that serological methods also have high sensitivity but they fail to diagnose the relapses. We had successful results with the use of PCR. PCR has some

Table 3. The statistical evaluation of test results after the comparison of PCR with the other laboratory tests of VL suspected patients

		PCR (0)	PCR (1)	kappa*	
		N	N		p
Direct Microscopy	(0)	44 (91.7%)	0 (0%)		
	(1)	4 (8.3%)	11 (100%)	0.80	0.000
Culture	(0)	48 (100%)	0 (0%)		
	(1)	0 (0%)	11 (100%)	1.00	0.000
ELISA	(0)	43 (89.6%)	4 (36.4%)		
	(1)	5 (10.4%)	7 (63.6%)	0.51	0.000
IFAT	(0)	44 (91.7%)	4 (36.4%)		
	(1)	4 (8.3%)	7 (63.6%)	0.55	0.000
Formol-Gel	(0)	41 (85.4%)	4 (36.4%)		
	(1)	7 (14.6%)	7 (63.6%)	0.45	0.000

N = Patient number, (0) = Negative test result, (1) = Positive test result, = kappa coefficient, p = probability value (SPSS 11.5 Programme), * kappa correlation and probability value (p).

disadvantages like to be expensive, difficulties in performing test, contamination risk, and expensive equipment. Twenty-three (38.6%) out of 70 patients were diagnosed VL by PCR in a German study of two years (Harms et al., 2003). Mathis and Deplazes (1995) applied ssrRNA gene sequence for clinical samples collected from human and dogs by PCR and compared with culture results. They only detected a false negative result by PCR in one sample and they suggested that it was depend on collecting sample improperly.

Schönian et al. (2003) applied different PCR methods in 113 (69.7%) out of 162 cases with suspected leishmaniasis various different clinical samples for the diagnosis of leishmaniasis in Israel, Palestinian and Germany and they showed the importance of PCR in the diagnosis of leishmaniasis. Moreover, they stated that RFLP (RA) is a very useful method for the identification of species. Sundar and Rai (2002) reported that PCR has 100% sensitivity when compared with ELISA and IFAT in 48 patients with VL in India. Cascio et al. (2002) investigated 10 (71.4%) out of 14 patients with suspected VL by direct examination, culture, PCR and serological methods and they compared all of the methods and they identified the causative agent in the species level. Silvia et al. (2004) detected VL in 19(22.3%) patient out of 85 patient and compared PCR with ELISA and IFAT methods. They emphasized the sensitivity and specificity of PCR method and they stated that PCR could not sufficiently discriminate past and new infections and also cross reactions from other infections. Brustoloni et al. (2007) evaluated the sensitivity and specificity of PCR in VL diagnosis in archived Giemsa-stained bone marrow slides of 91 patients with VL and 79 controls with other diseases and they compared PCR with conventional diagnostic techniques, like direct microscopy and parasite culture. While PCR showing high sensitivity (92.3%) and good specificity (97.5%), direct examination detected 79.1% and culture 59% of positive samples. In addition they observed that PCR was able to detect VL in 16 of 19 patients (84.2%) with negative microscopy. They concluded that PCR in Giemsa-stained bone marrow slides is a suitable tool for VL confirmation. There are difficulties in culture and culture needs long time to recover parasites. The diagnostic value of direct microscopy which was one of traditional diagnostic methods is not sufficient and the reliability of ELISA is very low. For all above reasons, recently most of the researchers agreed on PCR which was faster and more sensitive method compared with other methods for the diagnosis of VL. (Osman et al., 1997; Aviles et al., 1999; Fissore et al., 2004; Silva et al., 2004; Piarroux et al., 1999; Harms et al., 2003 ; Mathis and Deplazes 1995; Schonian et al., 2003; Sundar and Rai, 2002; Cascio et al., 2002; Brustoloni et al., 2007).

Similar to other studies, we had more sensitive results by PCR compared with serological tests and direct microscopy but we noticed that sample taking procedure is very important in PCR.

The direct microscopy is still gold standard in either our study or in other studies. However, the sensitivity can be low according to the mistakes in sample taking procedure. Sensitivity in diagnosis increases with PCR. Serological methods are also show high sensitivity and specificity, but parasites can be detected in cases below cut-off value by direct microscopic examination or the DNA of parasites can be detected by PCR. PCR is an important noninvasive tool which can be used for the diagnosis of VL with great accuracy. PCR can also be used to predict cure of the disease (Maurya et al., 2005). On the contrary, false negative results can be obtained occasionally according to the improperly sample taking or PCR inhibitors (Reithenger et al., 2002; Mathis and

Deplazes, 1995).

We suggest that PCR can be used collectively with other methods in stead of being alternative against other methods in the diagnosis and follow-up the leishmaniasis.

Serological methods with low reliability are commonly used for the detection of leishmaniasis incidence and the relationship between them in humans and dogs of endemic areas. The results of any serological tests must be evaluated and judged with other tests and clinical and epidemiological data (Ozbel et al., 2002). Kilic et al. compared the performances of agglutination tests [DAT (Direct Agglutination Test) and FAST (Fast Agglutination Screening Test)] with IFAT and ELISA on serum samples of 59 patients with the clinical suspicion of VL. The agreements (and between, the groups were higher than 90.0% (0.80). They considered DAT; the most suitable and low cost test (Kilic et al. 2008). Brustoloni et al. (2007) compared the conventional methods for diagnosis of 116 children with the suspected VL. They showed that the association of microscopy and serology can provide diagnosis in 98.5% of cases. The highest sensitivity in laboratory diagnosis among the cases was obtained with a combination of BMA (bone marrow aspirates) direct examination and IFAT. They concluded that conventional methods for diagnosis of VL are still indispensable in regions with poor life standart.

Ertabaklar et al. (2003) reported that IFAT and DAT had high specificity and sensitivity and they also stated that a patient must be diagnosed serologically at first and after, this diagnosis must be confirmed parasitologically (4). Ak (1989), tested sera of 50 patients for antibodies by IFAT and ELISA. Positive results were obtained in 6(12%) patients by IFAT and leishmania amastigotes were detected in bone marrow smears at the same time. Positive results were obtained in 1/16, 1/64, 1/256 and 1/512 dilutions of 2, 20, 3 and 3 sera of patients, respectively and negative results were obtained in sera of 22 patients.

Ozbilgin et al. (1997) made cultures of bone marrows of 59 patients to the NNN media and also they dyed smears by Giemsa stain. They also detected antibodies in serum samples with IFAT, ELISA and DAT methods. They detected Leishmania amastigotes in 15(25.4%) of 59 patients.

Ozbel et al. (2002) reported a positive Leishmania serology in one child who were treated before for kala-azar out of 54 children by ELISA, IFAT and DAT methods in West Black Sea Region. 4 kala- azar cases were reported previously in the same village of Nebioglu (West Black-Sea region).

Ozensoy et al. (2002) reported that PCR has a high sensitivity compared with direct smear and IFAT in the diagnosis of VL from the bone marrow aspiration samples collected from human and dogs.

VL was not detected as epidemic or endemic disease in our study; it was seen as sporadic cases like other regions of Turkey. However, the reservoir dogs can be found in everywhere, especially in rural areas and Phelebotomus which were vectors of VL have always the ability to get parasites from the dogs and transmits to the humans.

As a result, VL is an endemic disease for the most of the regions of Turkey, but it is a sporadic disease for stanbul and neighborhood. We suggest that PCR is a useful method for the diagnosis of VL. There are difficulties in PCR test procedure and needs expensive equipment. Sometimes the samples are not enough to test. We also suggest that PCR has to be applied with other laboratory diagnostic tests in order to increase the sensitivity in diagnosis and decrease the possible defects in diagnosis. Migrations caused by social-economical difficulties and travel easiness, made the transportation disease possible from one place to another places easily and VL must always in the mind in suspected cases.

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REFERENCES

AKsaray M (1989). Serological studies with different antigens in Kala-Azar diagnosis. Turk. Parasitol. J. 13: 39-42

Altman DG (1991). Practical statistics for medical research. Aufl. Boca Raton, London, New York, Washington D.C.: Chapman & Hall; pp. 1–499.

Aviles H, Belli A, Armijos A, Monroy FP, Harris E (1999). PCR detection and identification of Leishmania parasites in clinical specimens in Ecuador: a comparison with classical diagnostic methods. J. Parasitol. 85: 181-7.

Brustoloni YM, Lima RB, Cunha RV Dorval ME, Oshiro ET, De Oliveira ALL, Pirmez C (2007). Sensitivity and specificity of polymerase chain reaction in Giemsa-stained slides for diagnosis of visceral leishmaniasis in children, Mem Inst Oswaldo Cruz Rio de Janeiro 102(4): 497-500,28.

Brustoloni YM, Cunha RV, Dorval ME, Oshiro ET, Pontes ER, Oliveira AL, Hillebrand L, Ribeiro LF (2007). Comparison of conventional methods for diagnosis of Visceral Leishmaniasis in children of the center-west region of Brazil, Braz. J. Infect. Dis. 11: 106-109.

Cascio A, Calattini S, Colomba C, Scalomogna C, Galazzi M, Pizzuto M, Camili R, Gramiccia M, Titone L, Corbellino M, Antinori S (2002). Polimerase Chain Reaction in the Diagnosis and Prognosis of Mediterranean Visceral Leishmaniasis in Immunocompetent Children. Pediatrics 109: 27-27.

Ertabaklar H, Ozkan TA, Ozensoy S, Ozbel Y, Babur C, Esen B, Ugurlu M (2003). The nvestigation of Visseral Leishmaniasis of children in Corum. Turk. Parasitol. J. 27: 233-236.

Fissore C, Delaunay P, Ferrua B Rosenthal E, Giudice DP, Aufeuvre PJ, Fichoux LY, Marty P (2004). Convenience of Serum for Visceral Leishmaniasis Diagnosis by PCR. J. Clin. Microbiol. 42: 5332–5333.

Harms G, Schönian G, Feldmeier H (2003). Leishmaniasis in Germany. Emerg. Infect. Dis. 9: 03-023.

Kilic S (2002). Kala-azar and other Leishmania Infections. Infection Diseases and Microbiology Editors. Topçu WA, Söyletir G, Doganay M. Nobel Medical books, Istanbul. 1: 685-696.

Kilic S, Ozkan AT, Babur C, Tanir G, Schallig HD (2008). Evaluation of serological tests for the diagnosis of Visceral Leishmaniasis, Turk. J. Med. Sci. 38:13-19.

Lachaud L, Chabbert E, Dubessay P, Reynes J, Lamote J, Bastien P (2001). Comparısion of various sample preparatıon methods for PCR diagnosis of visceral leishmaniasis using peripheral blood. J. Clin. Microbiol. 39: 613-17.

Mathis A, Deplazes P (1995). PCR and in vitro cultivation for detection of Leishmania spp. in Diagnostic samples from human and dogs. J. Clin. Microbiol. 33: 145-1149.

Maurya R, Singh RK, Kumar B, Salotra P, Rai M, Sundar S (2005). Evaluation of PCR for diagnosis of Indian Kala-Azar and assessment of cure, J. Clin. Microbiol. 43: 3038-3041.

One World Health Organization (2006). Treatment in Leishmaniasis (Access date: www.oneworldhealth.org/diseases/leishmaniasis.php.

Ok UZ, Balcio lu I C, Ozkan AT, Ozensoy S, Ozbel Y (2002). Leishmaniasis in Turkey. Acta. Trop. 84: 43-8.

Osman OF, Oskam L , Zijlstra EE, Kroon NC, Schoone GJ, Khalil ET, El- Hassan AM, Kager PA (1997). Evaluation of PCR for diagnosis of Visceral Leishmaniasis. J. Clin. Microbiol. 35: 2454-2457.

Ozbel Y, Turgay N, Alkan MA, Babaoglu A, Ozensoy S, Babalıoglu N Karabuk (2002). The focus of zoonotic Visceral Leishmaniasis in West Black-Sea Region Turkey: Karabük, Parasitol. J. 26: 362-366.

Ozbilgin A, Ozdemir E, Balcıoglu IC, Girginkardesler N, Degerli K, Ok Uz Alkan MZ, Ozbel Y (1997). Pathological changes detected in bone marrow samples in patients with Visceral Leishmaniasis. Turk. Parasitol. J. 21:213-215.

Ozdener N, Kayar B, Koksal F, Akbaba M (2005). The answers for the consideration and suggestion forms of Leishmania Symposium Course, XIV National Parasitology Congress Summary Book. pp. 167-68.

Ozensoy S, Korkmaz M, Balcıoglu IC, Ozbel Y, Ertabaklar H, Rastgeldi S (2002). Zoonotic Visceral Leishmaniasis in Karaburun and Urla Region. Turk. Parasitol. J. 26: 234-38.

Ozensoy S, Ozbel Y, Gül MA, Ertabaklar H, Sakru N, Taylan AO, Hokelek M (2002). Polymerase Chain Reaction (PCR) method in samples collected from Human and Dogs for the diagnosis of Leishmaniasis. Turk. Parasitol. J. 26: 239-44.

Pearson RD, Sousa ADQ, Mandell GL, Bennett JE, Dolin R, Jeromino SMB (2000). Leishmania species: Visceral (Kala-azar), cutaneous and mucosal leishmaniasisIn. Douglas and Bennett's Principles and Practice of Infectious Diseases. Philedelphia: Churchill Livingstone pp. 2831-2844.

Piarroux R, Gamberelli F, Dunon H, Fontes M, Mary C, Toga B, Quilici M (1999). Comparasion of PCR with direct examination of bone marrow aspiration, myeloculture and serology for diagnosis of visceral leishmaniasis in immuno compromised patients. J. Clin. Microbiol. 32: 746-749.

Reithenger R, Quinnell RJ, Alexdar B, Davies CR (2002). Rapid detectıon of Leishmania infantum infection in dos comparative study using an immunchoromatograpic dipstick test, Enzyme Linked Immunosorbent and PCR. J. Clin. Microbiol. 40: 2352-356.

Schallig HDFH, Schone GJ, Koron CCM, Hailu A, Chappıus F, Veeken (2001). Development and application of simple diagnostic tools for Visceral Leishmaniasis. Med. Microbiol. Immunol. 190: 69-71.

Schonian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HD, Presber W, Jaffe CL (2003). Diagnosis and characterization of Leishmania in local and imported Clinical samples. Diagn. Microbiol. Infect. Dis. 47: 349-58.

Silva SE, Gontijo FMC, Pacheco SR, Brazil PR (2004). Diagnosis of human Visceral Leishmaniasis by PCR using blood samples spotted on filter paper. Gen. Mol. Res. 3: 251-257.

Sundar S, Rai M (2002). Laboratory Diagnosis of Visceral Leishmaniasis. Clin. Diag. Lab. Immunol. 9: 951–958.

Unat EK, Yücel A, Altas K, Samastı M (1991). Unat's Medical Parasitology. I.U Cerrahpasa Medical Faculty PublicationsIstanbul, pp. 570- 9 (In Turkish).