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Full Length Research Paper

KAtex antigen-detection test as a diagnostic tool for latent visceral leishmaniasis cases

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Kala-azar is an infectious disease that mostly affects children under 5 years old, and, in untreated cases, is fatal. There are several routine serological tests employed to diagnose the disease, but these tests have disadvantages. Parasitological diagnosis tools are accurate but invasive, while molecular tools are expensive and not commonly used. We report that the newly developed kAtex test is a rapid, non invasive and simple tool for the detection of visceral leishmaniasis (VL), capable of detecting and distinguishing between recovered, subclinical and latent cases. The study was carried out in endemic areas of Kalibar and Ahar districts, East Azerbaijan province, Northwest Iran. Urine and Sera samples were collected from 313 children under age 5 years old from the endemic area. Additionally, sera and urine of 18 confirmed VL patients were studied. Urine samples were tested by kAtex kit to detect VL antigen. All sera samples were also tested by other serological tests such as DAT, IFA, and ELISA (IgG and IgM). kAtex tests were positive for 10 out of 313 examined urine samples of children and 2 positive samples for ELISA-IgM, but were negative all for other serological tests. During clinical follow up of the kAtex positive individuals, three of them developed VL disease, with DAT titers for these individuals reaching 1/3200. The results show that kAtex is an effective screening test for latent infection of VL. The test discriminates between active disease, sub clinical and latent infections. Its results can complement DAT for the diagnosis of unconfirmed visceral leishmaniasis cases.

Key words: KAtex, visceral leishmaniasis, diagnosis, Iran.

INTRODUCTION

The trypanosomatid parasite of the genus *Leishmania* is the etiological agent of a variety of disease manifestations, collectively known as leishmaniasis. Leishmaniasis is prevalent through out the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world) and South and Central America (new world). It is estimated that approximately 12 million people are currently infected and a further 367 million are at risk of acquiring leishmaniasis in 88 countries (Desjeux, 2001). The annual incidence rate is estimated to be 1-1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis (VL); these are the two major clinical types of leishmaniasis (Desjeux, 2001; Musa et al., 2002; Cox, 2003).

The VL foci revealed in Iran seems to be an extension of the Mediterranean infantile form of leishmaniasis. Unlike cutaneous leishmaniasis (CL), which is extremely prevalent in Iran and has been long known to occur. Pouya (1950) was the first to report three human cases of VL (two children, one adult) from Tonekabon district in the North of the country. The second report was from the South of the country (Reid, 1952). Twenty-five cases were later reported from throughout Iran from 1949-1961. Twenty-five children with VL were detected in Fars province in the South from 1961-1968 (Gavgani et al., 2002; Jalayer, 1968), and nine cases (diagnosed clinically) were reported from Mashhad in 1969 (Yaghmai, 1969; Kadivar et al., 2000). Hashemi-Nasab et al. (1980) repor-

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ted 23 more VL cases from the Fars province between 1968 and 1972. Between 1968 and 1978 131 VL cases were reported from the Shiraz Medical University Hospitals (Davies and Gavgani, 1999; Soleimanzadeh et al., 1993; Hashemi-Feshareki et al., 1993; Behforouze et al., 1981; Nadim, 1978).

Since 1976, the number of annually reported cases has increased considerably, probably to some extent because of the introduction of IF AT for the diagnosis of VL in Iran in 1975, and DAT in about 1990 (Behforouze et al., 1976; Edrissian et al., 1978; Edrissian et al., 1981; Edrissian, 1996). Up to the end of 2003, more than 5594 VL patients had been diagnosed from 28 provinces in Iran and the number of affected cities had reached 113, together with hundreds of districts with thousands of villages. In Iran provinces, VL has been sporadic, with from 1 - 88 cases reported per province prior to 1993 (Nadim et al., 1992; Edrissian, 1997). By the end of 1997, the total number of diagnosed VL patients in the endemic provinces of Ardabil, Fars, and East Azerbaijan had reached 2200, 2000 and 175, respectively, with over 5200 for the whole country. The number of diagnosed VL cases in Boushehr province (which neighbors Fars) has also increased (Tahernia and Jalayer, 1968; Ziai et al., 1968; Edrissian et al., 1988; Kala-azar, 1990). In summary, VL is currently sporadic in all provinces of Iran and endemic within at least three provinces: Ardabil and East Azerbaijan in the north-west and Fars in the south. In the endemic provinces, over 50% of all VL patients have been children below 2 years old, and 90% have been children under 12 years (Edrissian et al., 1988).

VL is a severe systemic disease characterized by destructive infiltration of organs and high rates of fatality in symptomatic patients. Clinical manifestation includes fever, hepatomegaly and/or splenomegaly and pancytopenia (Nadim, 1978). However, most *L infantum* infections in people are subclinical, and cause the development of a transient humoral response followed by a protective cell-mediated immune response, but no clinical symptoms (Gavgani et al., 2002).

These symptoms are very similar to those seen in other diseases and laboratory confirmation is needed (mandatory) when the diagnosis is suspected. Bone marrow aspiration or biopsy followed by demonstration of leishmania parasites by microscopic and/or cultural examination is the most common diagnosis procedure (Siddig et al., 1989; Zijlstra et al., 1992). Although microscopic examination of bone marrow aspirate has been reported to be 62 to 93% sensitivity, it is an invasive method (Da silva et al., 2005) and isolation of parasites by culturing is time-consuming, expensive, and difficult (Weigle et al., 1987). Serological tests show a high degree of cross-reactivity and can not discriminate between past and current infections; also their sensitivity are low (Kar, 1995). In addition to the conventional microscopic, cultural and serological methods, in recent years numerous PCR-based diagnostic methods with a wide range of sensitivities and specificities have been described (Osman et al., 1997; Piarroux et al., 1994). However, its sensitivity for the detection of *Leishmania* DNA in the blood of parasitologically proven VL cases was only 70% (De Colmenares et al., 1995). The PCR positivity observed in these patients may be due to non-viable parasite. Similarly, PCR results for healthy endemic controls may be positive (Osman et al., 1997), which may lead to the erroneous conclusion that they suffer from VL.

Antigen detection is more specific than antibody-based immunodiagnostic tests (De Colmenares et al., 1995). This method is also useful in the diagnosis of disease in cases where there is deficient antibody production (as in AIDS patients) (Riera et al., 2004). Because of the conditions prevailing in endemic area, any sophisticated method cannot be employed on a wider scale. There is a need for a simple rapid and accurate test with good sensitivity and specificity, which can be used without any specific expertise. A promising ready to use Latex test (KAtex) based on detection of antigen in urine has been developed as a rapid test for use in difficult field conditions (Attar et al., 2001).

The KAtex test is simple to use, and economical: its two main advantages are the rapid capability of this test in diagnosis, especially when the patient is coming from far distances for the test and the fact that the performance of the test does not require any electric appliance. The main aim of this study was to investigate the utility of urine as a sample for the diagnosis of *Leishmania infantum* infection disease by KAtex.

MATERIALS AND METHODS

Study area

East Azerbaijan province region is bounded on the North by the Republics of Azerbaijan and Armenia, on the West by Turkey and Iraq, on the South by Kordestan province and on the East by Zanjan province. The climate of the study area is influenced by the Mediterranean Sea and the Orumiyeh Lake. There are four seasons in most of the region depending on the topography, and rainfall is plentiful. Apart from the mountainous areas, the land is mostly fertile with some covered by forest. The climate of the mountainous areas includes a snowy winter, a rainy spring and a mild summer. The temperatures vary considerably from season to season as well as from place to place with a minimum of - 20ºC and a maximum over 35ºC in some localities. Kalaibar district (in the Northeast of East Azerbaijan province) has an area of 4038 square km and contains 13 towns and 405 villages with a population of 82,124. The capital is located at 48°0.2'N and 38°51'E, and the district ranges from 40-2000 m asl.

Study population

From 35 villages belonged to endemic areas of Ahar and Kaleibar; located in province East-Azerbaijan; 385 urine and blood samples

Table 1. Number and percent of positive samples in different tests.

Test	Total sample (385		72 Arevious historic		18 Active patients	
	Pos	(%)	Pos	(%)	Pos	(%)
KAtex	38	9.8	28	38.8	14	77.7
DAT	71	18.4	54	75	16	88.8
IFA	60	15.5	42	58.3	15	83.3
ELISA-IgG	56	14.5	42	59.7	17	94.4
ELISA-IgM	35	9.9	25	34.7	13	72.2

Table 2. Intensity of antigenuria in 72 historic and active VL cases.

Age group	Grade 1+	Grade 2+	Grade 3+	Neg	Total
> 1 year	2	0	0	3	5
1 year	1	2	3	17	23
2 years	7	3	0	10	20
3 years	1	0	1	5	7
4 years	1	1	3	2	7
5 years	1	0	0	3	4
6 years	0	0	0	1	1
7 years	0	0	0	2	2
8 years	0	1	0	1	2
9 years	1	0	0	0	1
	14	7	7	41	72

were taken from children under one year old to nine years old as follows:

a) 313 cases from 35 endemic villages of Kalaybar and Ahar (included 72 new VL suspects: 54 cases who reported for follow-up and 18 cases parasitologic confirmed VL from Kalaybar and Ahar villages),
b) 57 non endemic healthy controls, and
c) 14 cases of confirmed toxoplasmosis (6 cases), pulmonary tuberculosis (3 cases) and brucellosis (3 cases).

385 Urine samples collected from all VL suspects, non VL cases and healthy individuals in sterile containers, also 385 blood samples were collected and tested by DAT, IFAT, and ELISA (IBL HAMBURG Co.) diagnostic tests. KAtex kit (Kalon Biological, UK CO) was used for diagnosis in 72 confirmed and suspected Kala-azar patients and for case finding among 313 children in the endemic area of Kalibar and Ahar, East Azerbaijan province, Northwest Iran. Results also compared with serologic testes (DAT, IFA, and ELISA-IgG and IgM) to finding of agreement and validity and reliability indices. The data from the endemic areas were recorded on forms modified for the project. The information was entered in the computer, checked for errors in data entry and stored in files. Results analyzed by SPSS ver12 software. Tow statistics methods (Mac-Nimar and Kappa agreement) were used for comparison of KAtex, ELISA (IgG and IgM), IFA and DAT results.

RESULTS

From 385 urine samples 38 samples (9.8%) were positive, by urinary latex test (KAtex) (Table 1). Most of positive samples were in male group. 23 male samples (5.97%) were positive against 15 female samples (3.89%). Also the most positive samples with latex test observed in 2 years old class; which contains 10 positive samples (3.86%) (Table 2). Also sampled urine from confirmed VL patients and with previous history of VL (cured VL cases), were tested with KAtex. The urine samples from 72 cases were positive in 28 patients (38.8%). 18 cases had active VL (confirmed with: clinical sign and parasitology). KAtex test was positive on 14 cases of confirmed VL patients (77.7%). For survey of agglutination intensity, the positive urine samples divided to four groups: grade 1+ (pos. +), grade 2+ (pos. ++), grade 3+ (pos+++) and negative. Negative position do not have any *Leishmania* antigens in urine therefore agglutination was not observed. Grade 3+ showed the maximum amount of antigen in urine and highest agglutination. From 72 cases (confirmed VL patients and with previous history of VL), 14 cases (19.4%) were grade1+, 7 cases were grade 2+ (9.7%), 7 cases (9.7%) were grade 3+ (Table 2).

In 18 urine samples taken from parasitology confirmed VL patient, 14 cases were positive with KAtex test: 7 cases (50%) were grade 3+, 3 cases (21.42%) were grade 2+ and 4 cases (28.57%) were grade 1+. Also most grade 3 + samples were observed in one year old and four years old

Table 3. Validity and reliability indices employed in tests.

Test	Reliability indices				Validity indices		
	Sen	SPe	PPv	NPv	e	NPo	PPo
KAtex	77.7	98.2	93.3	93.3	95.5	91.8	73.6
IFA	83.3	92.9	78.9	94.6	90.6	88.3	83.3
DAT	88.8	91.2	76	96.2	90.6	88.1	72.7
ELISA-IgG	94.4	87.7	70.8	98.0	89	86	70.8
ELISA-IgM	72.2	89	68.4	91.4	85.3	82.2	54.1

Sen = sensitivity; Spe = excellent specificity; PPv = positive predictive value; NPv = negative predictive value, e = efficiency; NPo = negative power; and PPo = positive power.

classes.

The percentage of KAtex positive cases was not significantly different between the fresh and frozen urine samples.

Validity and reliability indices

KAtex test in our study had sensitivity (77.77%) and excellent specificity (98.24%), positive predictive value (PPv) 93.3%, and negative predictive value (NPv) 93.3%. Also KAtex reliability indices were: efficiency 95.57%, positive power (PPo) 73.68%, and negative power (NPo) 91.80%. Other serologic test (ELISA IgG and IgM, DAT and IFA) had different validity and reliability indices (Table 3).

Comparison of KAtex with serologic tests

For comparison of KAtex with other serologic tests Kappa and Mac-nimar statistic methods were employed:

KAtex and DAT comparison

Comparison of diagnostic value of KAtex with DAT by Kappa and Mac-nimar statistic tests showed low agreement (PV =0.000, Kappa = 0.400) between these tests. Sensitivity of KAtex comparison with DAT estimated 36.6% and specificity 78.4% comparison with DAT. Positive and negative predictive value calculated 68.4 and 87.03% with statistical methods.

KAtex and ELISA-IgG comparison

Comparison of ELISA-IgG with KAtex diagnostic value showed sensitivity 41% and specificity 95% (Pv =0.013 and Kappa =0.421). Like DAT, ELISA-IgG had not good agreement with KAtex. For validity factors, efficiency was 87%, positive power (PPo) was 32% and negative power (NPo) was 89% in comparison of ELISA-IgG with KAtex.

KAtex and ELISA-IgM comparison

The advantage of this test is recognizing new infected people. In comparison of KAtex and ELISA-IgM we found good agreement between both tests. There is an agreement between these two tests. Pv = 0.549 and based on Kappa test the agreement percentage was 0.834. Comparison with ELISA-IgM, the sensitivity of KAtex was 88% and specificity became 98%. Positive predictive value was 81% and negative predictive value became 98.8%. For reliability indices, efficiency was 97%, positive power was 72% and negative power was 96%.

KAtex and IFAT comparison

In comparison of KAtex and IFA we could not find good agreement between both tests. Pv = 0.003 and based on Kappa test the agreement percentage was 0.420. Comparison with IFA, the sensitivity of KAtex was 40 and specificity became 95.6. Positive predictive value was 63.1 and negative predictive value became 89.6. For reliability indices, efficiency was 87%, positive power was 32.4% and negative power was 89.1%. See Tables 4, 5 and 6.

Cross reaction

14 individuals suffering from tuberculosis, toxoplasmosis, brucellosis from non endemic area, were examined by KAtex, ELISA-IgG, DAT, IFA and ELISA-IgM. KAtex had not any cross reaction with urine of tuberculosis, toxoplasmosis and brucellosis patients.

DISCUSSION

Leishmaniasis is endemic in the tropical and subtropical regions of 88 countries. There are an estimated 12 million cases worldwide; 1.5 to 2 million new cases occur every year, and 500,000 cases of VL occur every year (Altes et

Table 4. KAtex results compared with serologic tests.

Test		KAtex				
		Pos	Neg	Total	P.V	McNimar
ELISA IgM	Pos	31	4	35	0.549	0.834
	Neg	7	343	350		
ELISA IgG	Pos	23	33	56	0.013	0.421
	Neg	15	314	329		
DAT	Pos	26	13	38	0.000	0.400
	Neg	45	302	347		
IFA	Pos	24	14	38	0.003	0.420
	Neg	36	311	347		

Table 5. Validity and reliability indices of KAtex comparison with serologic tests (IFA, DAT, ELISA IgG and IgM).

Test	KAtex						
	Reliability indices				Validity indices		
	Sen	SPe	PPv	NPv	e	PPo	NPo
IFA	40	95.6	63.1	89.6	87	32.4	89.1
DAT	36.6	78.4	68.4	87.03	85.1	31.32	84.12
ELISA IgG	41	95	60	90	87	32	89
ELISA IgM	88	98	81	98.8	97	72	96

Sen = sensitivity; Spe = excellent specificity; PPv = positive predictive value; NPv = negative predictive value, e = efficiency; PPo = positive power; and NPo = negative power.

Table 6. Calculated Kappa in comparison of the tests (DAT, IFA, ELISA IgG, ELISA IgM and KAtex).

DAT				
0.697	IFA			
0.727	0.899	ELISA IgG		
0.420	0.414	0.418	ELISA IgM	
0.400	0.426	0.421	0.832	KAtex

al., 1991). Moreover, the number of new host populations, i.e., populations of immunodeficient HIV-infected patients, is increasing. Substantially, for quick treatment of patients, suitable and proper diagnosis is one of the most essential steps. Thus, researchers often try to find the best and the most exact diagnosis methods, apart form the type of the disease. Creating and development of replacing diagnosis methods which can be used in field conditions is one of the most important factors of controlling and preventing a lot of parasite infections such as visceral leishmaniasis. The diagnosis of VL is complex because its clinical features are shared by a host of other commonly occurring diseases, such as malaria, typhoid, and tuberculosis; many of these diseases can be present along with VL (in cases of co infection). The commonly used method for diagnosing VL has been the demonstration of parasites in splenic or bone marrow aspirate. The sensitivity of the bone marrow smear is about 60 to 85%. The risk of splenic puncture is fatal hemorrhage from a soft and enlarged spleen (Siddig et al., 1989; Zijlstra et al., 1992; Da silva et al., 2005). Culture of parasite can improve the sensitivity of detection of parasite, but leishmania culture is rarely needed in routine clinical practice. Contamination of the culture media by bacteria or yeast species or other fungi usually complicates the culture (Pintado and Lopez-Velez, 2001). Animal inoculation is not usually employed as a diagnostic test, since several months may be required to obtain a positive result (Fisa et al., 2002). For several decades, nonspecific methods, which depend upon raised globulin levels, have been used in the diagnosis of VL. Some of the tests used for detecting these nonspecific immunoglobulins are Napier's formol gel or aldehyde test and the Chopra antimony test. Since these tests depend upon raised globulin levels, results can be positive in a host of conditions (Aika et al., 1979). Lack of specificity, as well as varying sensitivities, renders them highly unreliable. Conventional methods for antibody detection included gel diffusion, complement fixation test, indirect hemagglutination test, IFA test, and counter-

current immunoelectrophoresis (Aika et al., 1979; Zijlstra et al., 2001). However, aside from practical difficulties at peripheral laboratories, the sensitivities and specificities of most of the above tests have been the limiting factors. Like most antibody-based tests, DAT may yield positive results for a long time after complete cure and thus has not proved to be of much prognostic value (Smrkovski and Larson, 1977). ELISA has been used as a potential serodiagnostic tool for almost all infectious diseases, including leishmaniasis. The technique is highly sensitive, but its specificity depends upon the antigen used. The sensitivity of ELISA is reported to range from 80 to 100%, but cross-reactions with sera from patients with trypanosomiasis, tuberculosis, and toxoplasmosis have been recorded (Aika et al., 1979; Smrkovski and Larson, 1977; Sundar et al., 1998).

Among the 385 samples taken from endemic areas of Ahar and kaleibar, 23 positive samples were from males and 15 positive samples were from females, which were in the ratio of two to one by means of frequency distribution, explained by other researchers (Edrissian, 1996). As it is shown in Table 2, 3.8% of the persons of 2 years old had positive latex test. In most of the kala- Azar cases reported, the amount of the infected people in 1-2 year old group is higher than the infected persons under one year old (Edrissian, 1996).

All of this village habits are tribes who have a large number of dogs and this point may be the cause of the high amount of positive samples. By an echoepidemiologic study performed in southern Spain, close relationship between the number of dogs and number of positive serums in school children have been observed (Cruz et al., 2002). Parasite intensity is probably well-correlated with antigen load in urine. The number of grade 3+ samples in 1 and 3 years old group was maximum, which was predictable because infection percentage in these groups was higher than other groups. Besides, it was confirmed by recent studies performed (Edrissian, 1996).

The probability of cross reaction between serologic tests and other infections, such as toxoplasmosis, mycobacterium's is high, but KAtex does not give any cross reaction with toxoplasmosis,tuberculosis, brucellosis (Aika et al., 1979; Smrkovski and Larson, 1977). For survey of probable cross reaction between leishmanias antigens and other pathogenic agent, 14 samples were taken from patient suffering from toxoplasmosis, tuberculosis and brucellosis. Our study has not seen any cross reaction between KAtex test and other pathogenic agent. This result confirmed other studies about probable KAtex cross reactions: KAtex results were negative in all PKDL case (primary study in Sudan) (Attar et al., 2001). These findings in our study and other researchers showed that KAtex tests are valuable only for VL diagnosis.

Our studies for finding validity and reliability indices with using urine of 18 parasitology confirmed VL active patients and non-endemic controls showed that KAtex has sensitivity (77.77%) and excellent specificity (98.24%), positive predictive value (PPv) 93.33%, and negative predictive value (NPv) 93.3%. Sarkari et al. (2002) has evaluated KAtex in laboratory trials, using urine collected from well defined cases and endemic and non-endemic controls. The test had 100 specificity and sensitivity between 64 and 100% (Sarkari et al., 2002). Attar et al. (2001) in a field trial in Sudan showed that the test was positive in all 15 microscopy-positive kala-azar cases (sensitivity 100%) and was negative in 41 of 45 bone marrow and/or lymph node smear negative clinical suspect cases of kala-azar (specificity 87.2%) (Attar et al., 2001). Evaluation of KAtex in Nepal (2004) showed low sensitivity (47.4%) against excellent specificity 98.7% amongst a control group of patients with similar symptoms in whom kala-azar was ruled out. These findings differ from other studies. They observed a significant increase in the KAtex sensitivity with the duration of fever, spleen size and the parasite intensity in the tissue aspirate; the first two probably reflect the duration of the illness. Parasite intensity is probably well-correlated with antigen load in urine (Rijal et al., 2004). These researchers mentioned that the low overall sensitivity is in contrast to earlier results by Attar et al. (2001) and unpublished data from Muzzafarpur, India and Sudan (Rijal et al., 2004). However, Attar et al. (2001) reported data from Brazil where only 16 of 25 confirmed kala-azar showed a positive KAtex (sensitivity 64%, specificity 95%). Rejal et al. (2004) mentioned low sensitivity in their study could possibly be explained by shorter duration of the disease in the patients presenting. Also, series of kala-azar patients contained a high number with low parasite intensity, 46.5% had grade 1+ or 2+ (Rijal et al., 2004; Riera et al., 2004).

Attar et al. (2001) in Sudan showed that KAtex, unlike the DAT, discriminates between active disease, sub clinical and past infections (Attar et al., 2001). In our study, from 313 healthy endemic residents, 10 cases were positive with KAtex and for the detection of asymptomatic or pre-patent cases of VL evaluation, we reported positive cases name to health centers of Ahar and Kalibar for follow up. After 15 days 2 cases, from reported KAtex positive cases increased in titter of antibody (DAT > 1/3200), and 8 remaining cases were negative. Evaluation for the detection of asymptomatic or pre-patent cases of VL were not carried out in population surveys before our study (Attar et al., 2001; Rijal et al., 2004; Riera et al., 2004; Singh et al., 2002).

These findings in our study, and taking into consideration that positive LST rules out the diagnosis of active visceral leishmaniasis, showed KAtex can screen latent class of VL and is useful to complete DAT results for the diagnosis of unconfirmed and pre-patent visceral leishmaniasis cases.

Primary studies showed KAtex results going to be ne-

Table 7. KAtex results of confirmed VL patients in deferent weeks.

Time	Grade 3+	Grade 2+	Grade 1+	Negative	Total
Week 1	7	3	4	4	18
Week 3	3	4	2	9	18
Week 12	0	0	3	9	12

gative in week 12 after infection (Attar et al., 2001). The follow-up of treated confirmed visceral leishmaniasis patients for 1-3 months have been shown conversion of KAtex results to negative. These findings are due to the rapid decline of the antigen level in the urine at week 12 resulting in conversion of the test before the end of the course of treatment (Attar et al., 2001; Sarkari et al., 2002). Other reports recommended the use of a competitive ELISA and rK39 for the prognostic evaluation of visceral leishmaniasis and the success of drug treatment (Aikat et al., 1979; Sundar et al., 1998).) Obviously, due to its simplicity, the KAtex may be more appropriate in this respect.

The animal experiments suggest that the amount of detectable antigen tends to decline rapidly following chemotherapy (Riera et al., 2004). In our study, urine samples of confirmed VL patients (14 samples), who were positive with KAtex, examined after 3 and 12 weeks again to finding time of KAtex results conversion to negative. Result of KAtex in week 3 showed decrease of grade 3+ cases from 7 to 3 and grade 2+ from 3 to 4 cases. Also for survey of cases in week 12, we could not access to 6 VL patient samples and because of chemotherapy effects after week 12 we had only 3 cases with grade 1+ results (Table 7).

Also our study showed highest agreement between KAtex and ELISA-IgM results (but not with other serologic tests), though we know IgM antibody increase in acute step of disease and decrease its titer rapidly. In this survey more KAtex positive results was in patients with recent infection and treated persons even with DAT positive had negative KAtex result. Moreover we could not fined good agreement between KAtex and other serologic tests like IFA, ELISA-IgG and DAT in our study. Study in sudan recorded sensitivity of 95.2% for KAtex. Test showed good agreement with bone marrow smear (Kappa coefficient = 0.65, P = 000), but like our study, poor agreement with serological tests (DAT, IFAT, ELISA, WB) (Attar et al., 2001). The important point in use of KAtex is boiling of urine for about 5 minute for false positive prevention. Our survey showed 49% false positive in KAtex results without boiling the urine. In other experiments, 23.7 % false positive without boiling sample was observed while no false positive after boiling was seen (Attar et al., 2001; Rijal et al., 2004). There were no differences with fresh and frozen urine samples using KAtex validity indices.

An antigen detection test like KAtex would, in principle, provide better means for diagnosis since antigen levels are expected to broadly correlate with the parasite load. Antigen detection systems are also an ideal alternative to the antibody detection systems in immunocompromised patients and more particularly with the growing number of HIV co-infected cases, especially in advanced cases where the immune response is impaired

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