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RESEARCH ARTICLE

Early diagnosis of leptospirosis using conventional techniques: A study in a tertiary care hospital, Mysore, South Karnataka, India

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Abstract

Leptospirosis is an emerging worldwide zoonotic disease and it has become a serious threat to the people of developing countries. Here we have evaluated a commercially available IgM ELISA kit with an Immuno Chromatographic Test (ICT) and also analyzed the clinical and laboratory findings of the seropositive cases. ICT and IgM ELISA were performed on 265 serum samples obtained from patients with signs and symptoms of leptospirosis from August 2013 to October 2014. Out of the 265 samples 140 were positive with ELISA, 10 were positive with ICT. The sensitivity of ELISA was 92.1% (140/152), and sensitivity of ICT was 11.8% (18/152). ICT gives less subjective results than IgM ELISA. Our study showed that adults were more infected and the majority of seropositive cases occurred from August-October. Fever, head ache and hepatomegaly were the predominating clinical symptoms and thrombocytopenia the commonest laboratory finding among the seropositive cases. This data also suggests that there is strong association between the occupation of the people and the incidence of leptospirosis since majority of the *Leptospira* antibody positive cases were farmers. Lack of proper diagnostic tests and unawareness of the disease has contributed to the under-reporting of leptospirosis. All persons with fever for >7 days should be screened for leptospirosis with IgM ELISA. IgM ELISA can serve as a very good alternative to the unaffordable tests like MAT and PCR.

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Introduction

Leptospirosis is a zoonotic infection with multiorgan involvement caused by the pathogenic strains of *Leptospira* spp that affects both humans and animals (Levett, 2001). It may occur as an endemic or sporadic disease. It is the most wide spread zoonotic disease in the world (WHO, 2003). The disease gets transmitted to human beings in the form of occupational hazards, avocational hazards and recreational hazards (Vinetz, 2001). The high risk groups include agricultural labourers, sewage workers, animal handlers etc. (Leptospirosis Manual, In accordance with National Guidelines NCDC and Department of Medicine, GMC, Surat). Contact with contaminated soil, vegetation or water or with the body fluids of infected animals result in human infections (Smythe, 2008). It occurs worldwide, but most commonly in tropical and subtropical areas with heavy rainfall (WHO, 2003). During rainy seasons and also during harvest times the risk of leptospirosis increases as there are chances of frequent contact with infected rat populations (WHO, 2003).

The incidence of Leptospirosis in developing countries is found to be 10 - 100/1,00,000 cases per year. According to this data, India should report 0.1 - 1.0 million cases per year, but less than 10,000 cases are reported. There is paucity of such data from Karnataka which makes this study a stepping stone in providing more updated information on leptospirosis incidence in South Karnataka. Only four states (Kerala, Gujarat, Tamilnadu and Maharashtra) report

more than 500 cases per year. Andaman and Nicobar Islands, Andhra Pradesh, Assam, Goa, Delhi, Karnataka, Orissa, Puducherry, and Uttar Pradesh also report cases (Shivakumar, 2013;Sumana,2014). This doesn't mean that the incidence of leptospirosis is less in these parts of India. Leptospirosis is under reported due to lack of awareness of the disease and unavailability of proper diagnostic tests. Leptospirosis is rapidly emerging as a public health problem in developing countries due to the improper living and sanitary conditions. Agriculture is the main source of income to the people of Karnataka, mainly sugarcane and paddy cultivation in South Karnataka. The water logged fields, the handling of animals and the use of community ponds in which both humans and animals bath contribute to the human infections.

Material and Methods

A total of 265 patients visiting a tertiary care center in Mysore from August 2013 to October 2014, inclusive of all age groups and occupations were included in this study.

A semi structured questionnaire was prepared for collecting their personal data and to analyze the risk factors associated with leptospirosis: living conditions, over- crowding, direct contact with animals and contact with contaminated water. Serum samples collected from AFI subjects were tested serologically for anti-Leptospira IgM antibodies by a quantitative enzyme linked immunosorbent assay (ELISA) from PanBio, Brisbane, Australia and by Immuno chromatographic Test (ICT) from Bio Standard Diagnostics, Haryana. The test procedure was performed according to the protocol provided along with the kit. The results were interpreted according to the manufacturer's instructions. Negative and positive controls were kept with each test run. Cut-off was calculated and reporting of results was done as positive, negative and equivocal as per the manufacturer's guidelines provided along with the kit. The patients' demographic data, underlying diseases, clinical manifestations and laboratory data were retrospectively analyzed.

Results

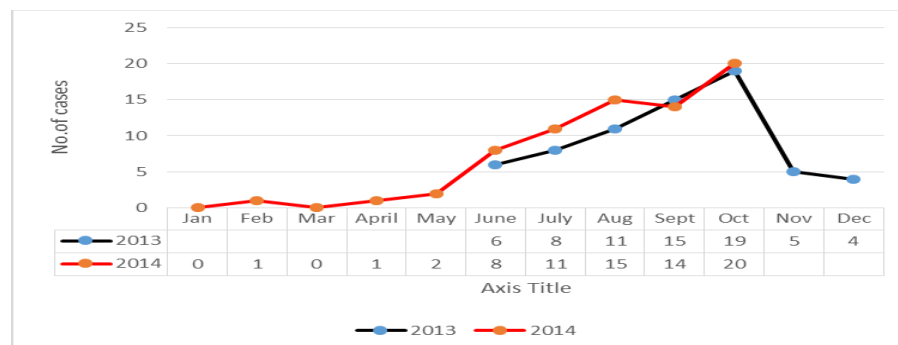
ICT and IgM ELISA were performed on 265 serum samples obtained from patients with signs and symptoms of leptospirosis from August 2013 to October 2014. Out of the 265 samples 140 were positive with ELISA, 10 were positive with ICT. Comparison of ICT and IgM ELISA was done. Results of ICT and IgM ELISA are presented in Table 1. Alternative diagnosis was made in 95 samples, which were negative for Leptospira antibodies by ICT and IgM ELISA, as shown in Table 2. Among these 95 samples the commonest diagnosis was Dengue fever (75.7%), followed by gastritis (11.5%) malaria (8.4%), scrub typhus (2.1%), enteric fever (1.05%) and RTI (1.05%). The combined efficacy of ICT and IgM ELISA was 94.7% (144/152), sensitivity of ELISA was 92.1% (140/152), sensitivity of ICT was 11.8% (18/152) and the correlation between ELISA and ICT was 45% (119/265). The number of adults affected was higher as compared to children in this study. Among the 140 patients positive for Leptospira antibodies, 94 were males and 46 were females. The majority of the cases presented in the months of August to October, coinciding with the monsoon and post-monsoon season Fig1. Among the Leptospira antibody positive cases, most of them were farmers which makes it more evident that contact with contaminated water and animals contribute to the disease.

Table.1 Correlation of ICT and ELISA assay

Test Result	No. of patients
ICT(+)/ELISA(+)	6
ICT(-)/ELISA(-)	113
ICT(+)/ELISA(-)	0
ICT(-)/ELISA(+)	134
ICT(+)/ELISA(equivocal)	4
ICT(-)/ELISA(equivocal)	8
TOTAL	265

Table.2 Alternative cases diagnosed in the study

Co-infection	No. of cases
Dengue fever	72
Gastritis	11
Malaria	8
Scrub typhus	2
Enteric fever	1
RTI	1
TOTAL	95

Fig.1 Occurrence of serologically positive cases of leptospirosis in 2013 and 2014

Analysis of the signs and symptoms of the patients' positive for leptospiral antibodies, fever was the symptom that was registered in most proportion 100% (140/140). Table.3 shows the clinical profile of the subjects' positive for *Leptospira* antibodies. The laboratory investigations presented a picture as follows:-thrombocytopenia 76.4 % (107/140) was predominating followed by elevated transaminases 47.1% (66/140), leukocytosis 32.9% (46/140), elevated alkaline phosphatases 21.4% (30/140), elevated serum bilirubin 18.6% (26/140), elevated serum creatinine 11.4% (16/140), decreased hemoglobin level 7.1 % (10/140) and decreased PCV in 2.8 % (4/140). The urine analysis presented a characteristic feature of albuminuria in 2.8% (4/140) of the seropositive study subjects.

Table:3 Clinical symptoms of the patients positive for *Leptospira* antibodies.

Clinical Symptoms	No. of cases
Fever	140(100%)
Headache	111(79.2%)
Hepatomegaly	59(42.1%)
Vomiting	50(35.7%)
Myalgia	37(26.4%)
Anuria	35(25%)
Pain Abdomen	27(19.3%)
Conjunctival suffusion	24(17.1%)
Splenomegaly	17(12.1%)
Jaundice	27(10.1%)
Altered sensorium	11(7.8%)

Discussion:

The number of cases being reported is very less either due to the unawareness of the infection or due to lack of proper diagnostic facility. Leptospirosis in its early stage mimics other tropical infections, so there arises a need of educating both medical professionals and the general public about the disease and thus early medical intervention will be possible decreasing the mortality rates.

There are several studies evaluating different diagnostic techniques used for early diagnosis of *Leptospira* infection. IgM ELISA test is a sensitive and specific test for diagnosing patients in acute phase of illness as IgM antibodies appear in the first week of illness allowing the diagnosis to be confirmed and thereby administering appropriate treatment modality (Karande et al., 2003; Kumar et al., 2012; Bharadwaj et al., 2002 ; Deodhar and John, 2011.). This is similar to the present study where sensitivity of IgM ELISA was 92.1% where as that of ICT was 11.8%. But this is in contradiction to several other studies in which they have summarized that in terms of results and utility, lateral flow matched IgM ELISA and suggested lepto lateral flow as a more suitable and convenient test or suggested that both ICT and ELISA were not sufficiently accurate for the diagnosis of acute leptospirosis (Sehgal et al., 2003; Blacksell et al., 2006).

Rainfall and occupation plays an important role in the dissemination of the disease to human beings (Vimala et al., 2014 and Ko et al., 1999). In our study, the highest incidence occurred from August to October and these months were characterized by heavy rainfall and most of the subjects were farmers which added to the transmission of the disease. Fever and head ache were the predominating symptoms among the subjects as mentioned in certain other works (Bharadwaj et al., 2002; Vimala et al., 2014).

Conclusion:

Serological diagnosis by Microscopic Agglutination test (MAT) is the gold standard in leptospira diagnosis but it is not universally available. Though it has a high degree of specificity, identifies serogroups and is accepted worldwide, it is expensive, time consuming and labour intensive. *Leptospira* IgM antibodies can be easily be diagnosed using IgM ELISA as it appears in the first week of illness. We recommend that all persons with fever for >7 days should be screened for leptospirosis with IgM ELISA. IgM ELISA can serve as a very good alternative to the unaffordable tests like MAT and PCR.

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Conflict of interests:

The authors declare that they have no conflict of interest.

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