Serological Correlation of Clinically Suspected Cases of Leptospirosis in Valsad, South Gujarat

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Abstracts: Leptospirosis is a zoonotic infection with worldwide significance and a re-emerging public health problem in India. Since early 1980"s the disease has been reported from various states during monsoon months. It is endemic in Gujarat, Andamans, Kerala, Tamil Nadu, Maharashtra, Karnataka and is also reported from Andhra Pradesh, West Bengal, Orissa, Puducherry, Delhi and Uttar Pradesh. **Aim:** Present study was undertaken to identify cases of Leptospirosis by Laboratory techniques. **Materials and Methods:** 136 samples have been considered for serological study in which 30 samples were taken as controls and 106 samples were of clinically suspected cases of leptospirosis. The study was conducted from July – October 2013 at our Tertiary care Hospital situated in South Gujarat. Serum samples of suspected cases of leptospirosis were collected and tested by Immunochromatography, IgM ELISA and MAT. **Results:** 63.20% of sera found to be positive for leptospirosis in which immunochromatography and IgM ELISA showed 66.98% and 66.98% positivity respectively. **Conclusion:** Study reveals that combination of IgM ELISA and MAT offer most reliable method for diagnosis of Leptospirosis. [Patel P NJIRM 2014; 5(4) :22-24]

Key Words: IgM ELISA, Leptospirosis, Zoonosis

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Introduction: Leptospirosis is a potentially lethal zoonotic disease with a world-wide distribution. It is caused by Leptospira interrogans which is an aerobic spirochete with a wide range of rodents & animals (domestic and wild) as reservoirs[1,2,3]. It is caused by contact through broken skin and mucous membrane with fresh water, damp soil or vegetation contaminated by the urine of infected animals. Peak incidence is seen in rainy season in the tropical areas. High risk groups include farmers, veterinarians, abattoir workers and in those living in riverside communities and areas of high rainfall[4,5].

Leptospira species are responsible for a wide range of non specific clinical symptoms and the disease is often misdiagnosed [6,7]. Infection can progress rapidly from an apparently mild illness to life threatening conditions like renal failure, pulmonary haemorrhage, Meningoencephalitis and Weil's syndrome. Such conditions have a fatal outcome and have a poor prognosis in those who do not receive prompt treatment[2]. Thus it is important to have a time bound accurate diagnosis in a suspected case of leptospirosis in areas where this infection is prevalent so that antibiotic therapy can be initiated early in the disease[8].

The incidence rate ranges from 0.1-1/100,000 per year in temperate climates to 10-100/100,000 in tropics. The disease is endemic in South Gujarat since 1994 9,10 and the endemic districts are Valsad, Navsari and Surat.

Serological tests remain the mainstay in diagnosis for leptospirosis[11,12]. Diagnosis is based on demonstration of antibodies by Enzyme Linked Immuno Sorbent Assay (ELISA) and Macroscopic Agglutination Test (MAT).

Materials and Methods: This study was conducted from July to October 2013 at GMERS Hospital, a tertiary care hospital in Valsad, South Gujarat, India. During this period the Microbiology laboratory received 106 blood samples from suspected cases within the hospital and from health centres in neighbouring districts. Faine's criteria were used for selection of patients to be included in the study as follows: fever, headache, Jaundice, breathlessness, subconjunctival suffusion and signs of meningeal irritation. The patients were also screened to rule out Typhoid, Dengue and Hepatitis B.

Serum samples were tested for Immuno chromatographic test (Rapid Leptochek) and specific Anti Leptospira IgM antibodies using

Serion-Virion kit (classic Leptospira IgM) and PanBio Kit (Panbio diagnostics, Brisbane).

The test procedures were performed according to protocol provided by the respective kits. Positive and negative controls were kept with each test run.

MAT was performed if a paired serum is giving negative or equivocal results as compared to1st sample and if paired sera was not available. For MAT test, sample was sent to Government Medical College, Surat which is a reference centre for Leptospirosis.

For laboratory confirmation any one of the following criteria were used: 1) A titer of > 100 unit in IgM ELISA by Serion-Virion kit or ≥25 pan Bio units in Pan Bio Kit in a single sample, 2) Seroconversion in IgM ELISA / MAT from negative to a titre of at least100 units or 25 pan Bio unit for Serion Virion and PanBio Kits respectively 3) Four fold or greater increase in ELISA/MAT titer between acute & convalescent phase serum specimens obtained 2 weeks apart. 30 asymptomatic subjects were taken as control for this study.

Results: None of the control samples were positive by immunochromatography and IgM ELISA. 71(72.64%) sera out of 106 were positive in rapid Leptochek (immunochromatography) and 60(56.60%) sera were found to be positive in IgM ELISA for leptospira. 15 samples were sent for MAT test in which 7(46.6%) samples were positive. Total 67(63.20%) of sample were considered as confirmed positive for leptospirosis. These included 13 females and 54 males. The maximum numbers of affected persons were between 31 yrs.to 40 yrs. of age. Age group wise distribution is shown in Figure 1. Comparison to ELISA and MAT test with Immunochromatogarphy shown in Table 1.

Figure 1: Age Wise Distribution Leptospirosis Patients (N=67)

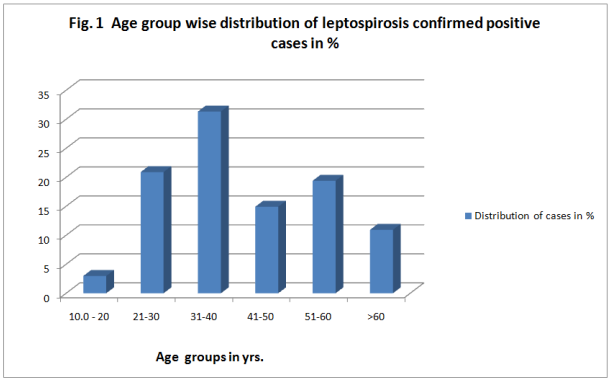

Table 1: Comparison of results of Rapid Leptochek with IgM ELISA and MAT (n=106)

Test Result	Pos. in IC	Pos. in IgM ELISA	Pos. in IC, Neg. in ELISA/MAT	Neg. in IC, Pos. in ELISA/MAT	Pos. in MAT test (n=15)	Confirm positive
No. of samples	71	60	3	4	7	67

Discussion: As leptospirosis is a common cause of acute febrile illness in the monsoon season in south Gujarat, early diagnosis is essential. In untreated cases disease progresses rapidly, leading to increase in morbidity and mortality. In our study prevalence of leptospirosis is 63.20% which is similar to study done by Bhardwaj *et al*[13]. People in this area are mainly dependent on agriculture and animal husbandry as their occupation. In agriculture main work is in rice fields and muddy soil is required for rice farming. There is over use of fertilizers making the pH of water and soil alkaline allowing *Leptospira* to survive for a longer time[13]. Farmers have to stand in these fields for a long time thus facilitating transmission of leptospirosis. It is observed that lack of awareness and late recognisation of symptoms of leptospirosis could be the reasons for higher prevalence of the disease in this region.

In our study males are more affected than female showing higher preponderance for leptospirosis which is similar to studies conducted by S.Shivkumar *et al*[14], Tanvi Panwala *et al*[15], and Smita B.Shekatkar *et al*[16]. The reason could be that farming is an outdoor activity and more males are involved in this. Commonly affected age group in our study is 26 to 45 yrs. which is similar to conducted by S.Shivkumar *et al.*

Detection of IgM antibodies by ELISA is now widely used in diagnosis of leptospirosis, which gives positive results earlier than MAT in acute phase of disease, helping in treatment timely[13]. MAT is a gold standard for diagnosis for leptospirosis[16], and is widely used as a reference test for leptospirosis. Several rapid assays have come up that are used for screening acutely ill patients. e.g. Immunochrmatography having good sensitivity and specificity

Conclusion: Serological tests remain the mainstay in diagnosis for leptospirosis, Immunochromatogralphy can be used as a screening test where facilities for ELISA/MAT are not available and combination of IgM ELISA and MAT offer the most reliable laboratory strategy to confirm leptospirosis.

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