Evaluation Of Diagnostic Utility Of Modified Faine's Criteria In Leptospirosis- Experience From A Tertiary Care Hospital.

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Abstracts: Background & Objective: Leptospirosis is an emerging infectious disease, for the diagnosis of which clinical, epidemiological and laboratory parameters may be evaluated as per modidified Faine's criteria suggested by several authors. The objective of this study was to validate the utility of modified Faine's criteria in the diagnosis of leptospirosis. Methodology: This study was carried out at a tertiary care hospital in Bengaluru, India from January 2011 to April 2012. Blood, urine and paired sera from one hundred patients with clinical suspicion of leptospirosis were collected. Relevant clinical and epidemiological details of these patients were also obtained as per modified Faine's criteria. Blood and urine samples of these patients were subjected to Dark Field Microscopy (DFM) and culture, whereas, their sera were subjected to Immuno chromatography (IgM Leptocheck), IgM Enzyme Linked Immuno Sorbent Assay (IgM ELISA) and Macroscopic Slide Agglutination Test (MSAT). All the leptospira seropositive samples were subjected to Microscopic Agglutination Test (MAT) which was also used as the gold standard to validate all the aforementioned serological tests and modified Faine's criteria. Results: Positive Predictive Values (PPV) of all the aforementioned serological screening tests and modified Faine's criteria were calculated. PPV of IgM Leptocheck, MSAT, IgM ELISA and modified Faine's criteria were found to be 14.3%, 6.5%, 8.7% and 21% respectively. Conclusion: The diagnosis of leptospirosis (both laboratory & clinical) is an uphill task. A high index of suspicion is needed in endemic areas & leptospirosis must be considered when a patient presents with acute onset of fever, headache & myalgia. From the results obtained in our study, it seems that modified Faine's criteria may not be as useful a diagnostic tool as it has traditionally been thought to be. More studies should be carried out to evaluate its diagnostic utility.[Bhatia M NJIRM 2015; 6(4):20-26]

Key Words: Leptospirosis; modidified Faine's criteria; MAT.

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Introduction: Leptospirosis is an emerging infectious disease which is associated with wide spectrum of manifestations ranging from subclinical infection to a severe syndrome of multi-organ infection with high mortality[1,2].The commonly followed case definition, which is also recommended by the WHO and International Leptospirosis Society prescribes that any person presenting with acute onset of fever, headache and body aches associated with severe muscle tenderness particularly in calf muscles, haemorrhages including sub-conjunctival haemorrhage, jaundice, cough, breathlessness and haemoptysis, oliguria, signs of meningeal irritation should be suspected as a case of leptospirosis and investigated[3]. A suspect, who tests positive in any of the screening tests such as dipstick, lateral flow or latex agglutination test should be considered as a probable case. Successful isolation of Leptospires from clinical specimens, a four-fold or higher rise in titre or seroconversion in paired MAT or a positive Polymerase Chain Reaction (PCR) is considered as confirmatory evidence of current leptospiral infection[3]. Owing to shortcomings of laboratory tests in establishing early diagnosis of leptospirosis, the World Health Organization (WHO), introduced Faine's criteria which includes the scoring of clinical, epidemiological and laboratory parameters of patients (Parts A, B and C respectively) [4].This criteria has been simultaneously modified and validated by Brato et al and Shivkumar et al, who recommended addition of abdominal symptoms, local factors (like rainfall) and newer investigations in the total scoring respectively[5,6]. The present study was carried out to validate the utility of modified Faine's criteria in diagnosing leptospirosis.

Material and Methods:

A descriptive study was carried out in a tertiary care hospital (catering only to beneficiaries

covered under Employee's State Insurance Act, 1948) in Bengaluru, India from January 2011 to April 2012. One hundred patients attending OPDs or admitted in Paediatric & Medicine wards were included in the study. The inclusion criteria for selecting these patients were: (a) Patients with undiagnosed fever of ≤ two weeks in duration. (b) Patients with fever and jaundice.(c) Patients with fever and associated manifestations such as headache, myalgia, rashes , conjunctival suffusion, oliguria/anuria & features suggestive of meningitis. Patients on antibiotic (Crystalline Penicillin & Tetracyclins etc) treatment & those less than one year in age were excluded from the study. Relevant clinical & epidemiological data (including occupational history) was obtained as per the proforma formulated in accordance with modified Faine's criteria recommended by Shivkumar et al as shown in Table 1[6]. Appropriate scores were assigned to patients taking into account clinical, epidemiological and laboratory parameters. A score of 26 or more when using Part A or Part A+B or 25 or more using Part A+B+C was presumed to be a case of current leptospirosis[6]. Institutional ethical approval was obtained and the following samples were collected:

Table 1: Modified Faine's criteria as per the recommendations of Shivkumar et al [6]

Part A: Clinical data	**Score**
Headache	2
Fever	2
If fever, temperature 39°C or more	2
Conjunctival suffusion (bilateral)	4
Meningism	4
Muscle pain (especially calf muscle)	4
Conjunctival suffusion + meningism + myalgia	10
Jaundice	1
Albuminuria or nitrogen retention	2
Part B: Epidemiological factors	
Rainfall	5
Contact with contaminated environment	4
Animal contact	1
Part C: Bacteriological and laboratory findings	
Isolation of leptospires in culture	Diagnosis certain
IgM ELISA positive	15
Macroscopic Slide Agglutination Test (MSAT) positive*	15
Microscopic Agglutination Test (MAT) single high titre*	15
MAT rising titres (paired sera)*	25

*Only one of these tests to be scored

Blood: Five ml of intravenous blood samples were collected aseptically from patients between seventh and tenth day of fever in sterile vacutainers coated with ethylene diamine tetra acetic acid (EDTA) for Dark Field Microscopy (DFM) and culture.

Urine: Freshly voided mid-stream urine samples were collected from patients between tenth to fourteenth day of fever in sterile universal containers containing equal amount of PBS with pH 7.2.

Sera: Two ml of intravenous blood samples were collected aseptically in plain vacutainers on two occasions (obtained one week apart on seventh and fourteenth day of fever respectively) for serological assays like IgM Leptocheck, IgM ELISA, MSAT & MAT. Hemolysed, clotted, contaminated, viscous/turbid specimens were rejected. Serum samples were stored at -20˚C if not processed immediately. Repeated freeze-thawing was avoided. No preservatives were used for storing serum samples.

The following procedures were carried out on the samples collected:

Dark Field Microscopy (DFM)

Blood: Blood sample (treated with an anticoagulant) was centrifuged at 1000g for 15 minutes. About ten µl of plasma was placed on a thin microscopic slide and cover slip was placed. The wet films were examined under DFM under low & high power magnifications (x100 & x400). If no leptospires were seen, the plasma was centrifuged at 3000-4000g for 20 minutes. The supernatant was removed carefully and a drop of sediment was examined microscopically as above[7].

Urine: A part of freshly voided urine was centrifuged at 3000 g for ten minutes. A drop of deposit was then placed on a thin microscopic slide & a cover slip was added to prepare a wet film & examined under DFM under both low & high power magnifications (x100 & x400) respectively[7].

Culture: Commercially available Ellinghausen-McCullough-Johnson-Harris (EMJH) semisolid mediumbase and Leptospira enrichment (BD-Difco) were used to prepare the culture medium. Selective EMJH medium was prepared by adding 100µg/ml of 5-Fluoro Uracil (Rolex Chemical Industries) to the original medium for inoculating urine samples.

Blood: Few drops of blood were inoculated into sterile capped plastic centrifuge tubes containing about 5 ml medium and incubated at 28- 30°C for four to six weeks[7,8].

Urine: Urine diluted in PBS 7.2 was inoculated into sterile capped plastic centrifuge tubes containing selective EMJH medium. Subcultures were done into EMJH medium without 5-Fluoro Uracil within 48 hours to minimize the inhibitory effect of selective agent on leptospires and was incubated at 28-30ºC for four to six weeks[7,8].

Examination of the cultures: The cultures were examined for signs of growth i.e. turbidity, haze or a ring of growth (Dinger's ring) and by using dark field illumination initially on day one, three and five followed by seven to ten days interval up to six weeks[5].When the tube inoculated with a specimen became contaminated before 6 weeks, filtration of the medium was done using a 0.22µm membrane filter using syringe filters (Millipore India Pvt. Ltd.). The filtrate was then inoculated into a fresh medium. Lightly contaminated cultures were sub-cultured into selective EMJH medium[9].

Reporting of the results: Cultures were incubated for four to six weeks before reporting them as negative. If all the tubes inoculated with a specimen became contaminated beyond retrieval before six weeks, it was reported as contaminants isolated in culture. All the positive cultures werereported as "Leptospires isolated"[9].

Serology:Immunochromatography(IgMLeptocheck, IgM Enzyme Linked Immuno Sorbent Assay (IgM ELISA) and Macroscopic Slide Agglutination Test (MSAT) obtained from Zephyr Biomedicals, J. Mitra& Co. Pvt. Ltd. and Bio-Rad respectively were performed on all the sera samples. These tests were carried out according to manufacturer's instructions. All the serum samples tested positive by Leptospira serological tests were sent to Regional Medical Research Centre (Indian Council of Medical Research), WHO collaborating centre for diagnosis, reference, research & training in leptospirosis, Port Blair, Andaman and Nicobar islands (India) in cold chain to be tested by Microscopic Agglutination Test (MAT). The serovars used were: *Australis, Bankinang, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes*&*Hardjo*. Sera with titres >/= 1:80 were interpreted as positive by MAT[10].

Statistical analysis: Positive Predictive Values (PPV) of IgMLeptocheck, IgM ELISA, MSAT and modified Faine's criteria were calculated using MAT as the gold standard.

Results: Out of one hundred cases, sixty were factory workers, thirty were school going children & ten were home bound. All 100 patients presented with fever, however twenty presented with high grade fever, thirty one with myalgia , thirty one with signs of renal dysfunction twenty with bilateral conjunctival suffusion ,twenty four with jaundice, fifteen with meningism and ten with head ache in various combinations as shown in Table 2.

Table 2: Table showing clinical profile of patients**

Clinical Manifestations	No. of cases
Fever/High grade fever (>39°c)	100/20
Myalgia (especially of calf muscles)	31
Signs of renal dysfunction (oliguria/albuminuria/raised blood urea nitrogen)	31
Jaundice	24
Bilateral conjunctival suffusion	20
Meningism	15
Headache	10

******Table has been formulated in accordance with modified Faine's criteria recommended by Shivkumar et al[6]

Sixty out of 100 patients presented during rainy seasons (May-August & October-November). Only five cases gave positive history of contact with

contaminated environment. None of the cases gave positive history of animal contact.

None of the blood & urine samples showed positivity by DFM. Leptospires were isolated from urine of a one year old child & identified as *Leptospira inadai* by PCR performed at PD_ADMAS (Project Directorate on Animal Disease Monitoring and Surveillance), Hebbal, Bengaluru.

Sixty three patients gave paired sera. The serum samples (acute &/or convalescent) of forty nine of these cases were tested positive for leptospirosis by IgM Leptocheck, MSAT & IgM ELISA in various combinations (eleven by IgM Leptocheck, MSAT &IgM ELISA; thirty three by MSAT & IgM ELISA; three by IgM Leptocheck & MSAT & two by IgM Leptocheck & IgM ELISA) as shown in Table 3.

Table 3: Table showing the positivity of leptospira serological tests in various combinations

Positivity by IgM Leptocheck MSAT & IgM ELISA	Positivity by MSAT & IgM ELISA	Positivity by IgM Leptocheck & MSAT	Positivity by IgM Leptocheck & IgM ELISA	Total
11	33	3	2	49

Out of 100 cases, IgM Leptocheck detected seropositivity in sixteen cases (16%), MSAT in forty seven cases (47%) & IgM ELISA in forty six cases (46%) respectively.MAT detected two out of sixteen (12.5%) IgM Leptocheck seropositive cases, three out of forty seven (6.4%) MSAT seropositive cases & four out of forty six (8.7%) IgM ELISA seropositive cases as depicted in Table 4.

Table 4: Table showing the overall positivity & concordance (with MAT) of leptospira serological tests

Serological assay	No. of seropositives (%)/Concordance with MAT (%)
IgM Leptocheck	16 (16%)/ 2 (12.5%)
MSAT	47 (47%)/ 3 (6.4%)
IgM ELISA	46 (46%)/ 4 (8.7%)

Samples of all the 49 cases tested seropositive were subjected MAT. Only sera of four of these cases were tested positive by MAT [three of which were tested positive for serovar *Australis* & one for two *serovars* (i.e) *Grippotyphosa* & *Pyrogenes*.

None of the leptospira seronegative cases (fifty one cases) satisfied modified Faine's criteria. Out of the remaining 49 leptospira seropositive cases, only nineteen (38.8%) satisfied modified Faine's criteria. However, sera of only 4 out of these 19 patients was tested positive by MAT.

The Positive Predictive Values (PPV) of IgM Leptocheck, MSAT, IgM ELISA and modified Faine's criteria were calculated using MAT as the gold standard and were found to be 14.3%, 6.5% , 8.7% and 21% respectively as shown in Table 5.

Table 5: Table showing Positive Predictive Value of IgM Leptocheck, MSAT, IgM ELISA & modified Faine's criteria using MAT as the gold standard

Name of the test/criteria	Positive Predictive Value(%)
IgM Leptocheck	14.3%
MSAT	6.5%
IgM ELISA	8.7%
Modified Faine's criteria	21%

Discussion: Leptospirosis is difficult to distinguish from dengue, malaria, influenza & many other diseases characterized by fever, headache & myalgia[11].The differential diagnosis of leptospirosis depends on the epidemiology of acute febrile illnesses in the particular area. The diagnosis of leptospirosis in humans is almost entirely dependent on laboratory findings[12]. The most frequently used diagnostic approach for leptospirosis has been that of serology[11].

Faine had introduced a criteria for diagnosis of leptospirosis on the basis of clinical, epidemiological and laboratory data (Parts A, B and C respectively)[6]. A presumptive diagnosis of leptospirosis may be made if:

(i) Part A or Part A+B score = 26 or more (Part C laboratory report is usually not available before fifth day of illness; thus it is mainly a clinical and epidemiologic diagnosis during early part of disease)

(ii) Part A+B+C = 25 or more. A score between 20 and 25 suggests a possible but unconfirmed diagnosis of leptospirosis.

Brato, et al, in 1998 recommended modification of the WHO Faine's criteria by adding abdominal pain or diarrhea in the clinical features (Part A) with a score of four[5]. As per modified Faine's criteria recommended by Shivkumar et al, only epidemiological and laboratory criteria (Parts B and C) were modified and no modification was made in the clinical criteria (Part A)[5]. The reasons for modification suggested were as follows:

(i) Most of the leptospirosis is reported in monsoon and post-monsoon period. Therefore they have suggested rainfall separately to be adjusted in the scoring criteria of Part B.
(ii) Microscopic agglutination test (MAT) is the Gold Standard test, but it is complicated and less sensitive compared to newer tests like IgM Enzyme Linked Immuno Sorbent Assay (IgM ELISA) and Macroscopic Slide Agglutination Test (MSAT). IgM ELISA and MSAT are simple, sensitive tests and can be used to diagnose current leptospirosis. Thus, they have been included with appropriate score.

The difficulties in utilizing MAT are due to the following reasons[6]:

(a) The antibody titres rise and peak only in second or third week. Thus, paired sera are required to demonstrate four-fold rise of titre.
(b) The test is complicated requiring dark-field microscopy and cultures of various serovars, which may not be available in smaller laboratories.

The advantage of including simple diagnostic tests (IgM ELISA or MSAT) in modified Faine's criteria is that it helps in diagnosing milder forms of leptospirosis which are associated with low clinical score (Part A). Suggestion of modification of existing Faine's criteria appears justified; however further evaluation is required[6].

In our study, Positive Predictive Values (PPV) of IgM Leptocheck, MSAT & IgM ELISA (taking MAT as the gold standard test) were found to be extremely low (14.3% , 6.5% & 8.7% respectively).

The reasons for obtaining such low PPV could be due to the various diagnostic pitfalls of MAT as follows[13]:

(1) The antibodies may not be detectable when the causative strain is not represented in the panel (of serovars used for MAT) or only a low titre is found with a serovar that antigenically resembles the absent causative serovar.
(2) It is never possible to be sure that the panel is complete since, new, unidentified leptospires may cause the disease.

Many studies have shown that Immunochromatography (ICT), MSAT & IgM ELISA which are used for diagnosing leptospirosis have much higher PPV[12, 14-17]. In most of these studies, only sera from cases of leptospirosis (confirmed by MAT) were subjected to various rapid leptospira serological tests like IgM ELISA, ICT & MSAT & validity of these tests was evaluated. However, in the present study, sera from clinically suspected cases of leptospirosis were first screened by rapid leptospira serological tests & then all the seropositive samples were subjected to MAT for confirmation of diagnosis. This also probably explains the lower PPV in the results obtained in the present study.

Factors that can increase the positive predictive value of a diagnostic test include screening a population at high risk of developing the disease and increasing the specificity of the screening test[18]. This study was carried out in a tertiary care hospital which did not cater to general population and therefore, the prevalence of leptospirosis in the target population might have been low. This in turn could have resulted in low PPV of the various serological tests which were used in our study. Also, the sensitivity and specificity (as per manufacturer's guidelines) of IgM Leptocheck , IgM ELISA and MSAT were 91.2% and 52.8%, 99.22% and 99.84% & 45% and 76.5% respectively. With the exception of IgM ELISA, all other serological tests used in the present study had low specificity. This could well be another reason for obtaining low PPV of leptospira serological tests.

None of the 100 cases were tested positive for leptospirosis by DFM. However, DFM has not been accepted for diagnostic purposes; as it is

considered insensitive and the results are non specific[1].

Leptospires could be isolated from only one patient. This could be because culture is known to give low isolation rates & is time consuming[1]. This isolate was identified as *Leptospira inadai* by PCR. Both acute & convalescent serum samples of this patient were tested positive by IgM Leptocheck, MSAT & IgM ELISA.

In the present study, none of the 51 leptospira seronegative cases satisfied modified Faine's criteria & only 19 out of the remaining 49 seropositive cases satisfied this criteria. This can be explained by the fact that most of the patients obtained low scores when clinico-epidemiological parameters were evaluated.

The PPV of this criteria in diagnosing leptospirosis (taking MAT as the gold standard) was found to be 21%. This could be due to low PPV of the rapid serological tests performed in our study.

A major drawback of our study was that we could not calculate the sensitivity, specificity and Negative Predictive Value (NPV) of modified Faine's criteria as only 49 out of 100 seropositive samples were subjected to MAT. Also, owing to the various diagnostic pitfalls of MAT enlisted earlier, its usage as gold standard for validating various serological tests used for screening of leptospirosis and modified Faine's criteria is questionable. From the results obtained in our study, it seems that modified Faine's criteria may not be as useful a diagnostic tool as it has traditionally been thought to be. However, one should be cautious while interpreting these findings owing to the various short comings of this study enlisted earlier. More studies should be carried out to evaluate the diagnostic utility of this criteria.

Conclusion: The diagnosis of leptospirosis (both laboratory & clinical) is a challenging task. A high index of suspicion is needed in endemic areas & leptospirosis must be considered when a patient presents with acute onset of fever, headache & myalgia. Further evaluation of diagnostic utility of modified Faine's criteria is need of the hour.

References:

1. Prasad SR, Rajini M. Leptospirosis: An Overview. The Journal of the Academy of Clinical Microbiologists 2008;10(2):89-97
2. Levett PN. Leptospirosis.Clin Microbiol Rev 2001 April;14(2):296-326
3. Singh SS. Clinical Manifestations. In:Leptospirosis Laboratory Manual. Regional Medical Research centre, Port Blair,WHO Country office for India:WHO;2007. p.22-26
4. Faine.S. Guidelines for the control of Leptospirosis. WHO offset publication 1982:67
5. Brato DG, Mendoza MT, Coredero CP, Leptospirosis Study Group. Validation of the WHO criteria using the MAT as the gold standard in the diagnosis of leptospirosis. PJMID 1998;27:125-128.
6. Shivakumar S, Shareek PS. Diagnosis of leptospirosis utilizing modified Faine's criteria. JAPI 2004 Aug;52:678-79
7. Sehgal SC. Leptospirosis on the Horizon**.** The National Medical Journal of India 2000;13(5):228-30
8. Betty AF, Daniel FS, Alice SW, editors. The Spirochetes. In: Bailey &Scott's.Diagnostic Microbiology. 12th ed. St.Louis, Missouri: Pub : Mosby Elsevier; 2007. p.539-40
9. Sharma KK, Kalawat U. Early diagnosis of leptospirosis by conventional methods: One year prospective study. Indian J Pathol Microbiol 2008; 5:209-11
10. Carlos RRC, Eduardo PB. Pulmonary complications of leptospirosis.Clin Chest Med 2002;23:469-78
11. Mary DB, David AA, Sandra LB, Christopher WW, Tin A, Richard AS et al. Evaluation of Four Commercially Available Rapid Serological Tests for Diagnosis of Leptospirosis. J Clin Microbiol 2003 Feb;41(2):803-09
12. Pimjai N, Piyada W, Wimol P, Ornnalin L, Paijit W. A Comparitive Evaluation of Different Methods for the Serological Diagnosis of Leptospirosis. J Trop Med Parasitol 2000 December;23(2):59-65
13. World Health Organization. Human Leptospirosis: Guidance for diagnosis, surveillance and control [Book on the internet].World Health Organization;2003 http://whqlibdoc.who.int./hq/2003/2003/WHO_CDS_CSR_EPH_2002.23.pdf

14. Brandao AP, Camargo ED, Silva ED, Silva MV, Abrao RV. Macroscopic Agglutination Test for rapid diagnosis of human Leptospirosis. J Clin Microbiol 1998 Nov;36(11):3138-42
15. Hamid RH, Gholamreza A, Seyyed SE. Comparison of two ELISA methods for the laboratory diagnosis of acute leptospirosis. Iran J Med Sci 2010; 35(2):116-21
16. Sekhar WY, Soo EH, Gopalakrishnan V, Devi S. Leptospirosis in Kuala Lumpur and the comparitive evaluation of two rapid commercial diagnostic kits against the MAT test for the detection of antibodies to Leptospirainterrogans. Singapore Med J 2000; 41(8): 370-75
17. Tanvi P, Summaiya M, Parul P. Seroprevalance of leptospirosis in south Gujarat region by evaluating the two rapid commercial diagnostic kits against the MAT test for detection of antibodies to Leptospira interrogans. National Journal of Community Medicine 2011; 2(1):64-70
18. Carniero I, Howard N, editors. Prevention Strategies. In: Introduction to epidemiology. 2nd ed. Maidenhead, Berkshire: Pub: Open University Press; 2011.p.137-54

Conflict of interest: None
Funding: None
Cite this Article as: Bhatia M, Umapathy B L, Navaneeth B V, Evaluation Of Diagnostic Utility Of Modified Faine's Criteria In Leptospirosis- Experience From A Tertiary Care Hospital.. Natl J Integr Res Med 2015; 6(4): 20-26