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Detection of Igm To Leptospira Agent With Elisa and Leptodistick Method

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ABSTRAK

Sampel berupa serum diambil dari penderita demam yag dicurigai karena : leptospirosis,demam tifoid, malaria dan hepatitis. Pemeriksaan serum terhadap antibodi IgM spesifik untuk leptospira menggunakan pemeriksaan dipstik sederhana (leptodipstik). Sampel diperoleh dari bagian Mikrobiologi, pada rumah sakit universitas hasanuddin di makassar Indonesia, dan data hasil pemeriksaan sederhana ini dibandingkan dengan hasil prosedur diagnostik standar Rumah Sakit yang diterapkan oleh dokter di Rumah Sakit yang sama. Sebagai bagian dari penelitian ini, sebagian besar serum ,juga diperiksa dengan ELISA yang spesifik dan sensitif terhadap IgM leptospira pada Royal Tropical Institute di Amsterdam untuk dibandingkan dengan hasil pemeriksaan leptodipstik. Aplikasi leptodipstik memperlihatkan keberadaan antibodi IgM yang spesifik terhadap leptospira pada 21 dari 34 penderita yang telah didiagnosis leptospirosis dan 7 dari 368 penderita yang didiagosis selain leptospirosis. Hasil pemeriksaan dipstik memiliki korelasi yang baik dengan hasil pemeriksaan ELISA.

Kata kunci: IgM, ELISA,Leptodipstik, Leptospira.

INTRODUCTION

Leptospirosis is an acute febrile illness caused by infection with spirochaetal microorganisms of the genus leptospira of which more than 200 pathogenic strains are currently known (3). Natural hosts of pathogenic strains,which may cause infection in man, include wild animals, livestock and pets. Urine of infected animals is the main source of transmission and humans may become infected through wounded skin, mucous membranes and the conjunctivae. Exact incidence rates are not known for most regions of the world. The incidence of leptospirosis in wet tropical countries can be as 5-20/100.000 per year.

Leptospirosis may manifest as relatively mild flue-like symptoms or as a severe disease called Weil's syndrome and symptoms may include renal failure,liver impairment,meningitis and (lung) hemorrhages. The fatality rate of severe cases is high. The clinical diagnosis of acute leptospirosis may easily be missed or the disease may be confused with other major infectious disease including influenza,malaria,hepatitis,bacterial meningitis and viral hemorrhagic fever. Therefore, laboratory tests are important to establish the diagnosis of leptospirosis (1).

Several more or less complicated methods are available to make the laboratory diagnosis of leptospirosis. These include Microscopic Aglutination Test (MAT) and ELISA. Culture provide ultimate proof of leptospirosis but has a low efficacy. Recently two simple and robust serological methods were developed and evaluated. This is the leptodipstik [(4)], which provide a high sensitifity and specificity. The leptouria-test is the direct examination for leptospires in a patients urine-samples under a microscope, and is often used in some tropical hospitals as a diagnostic lab-test. In the literature this test is controversial since threads of fibrin and protein in a urine-sample can mimic leptospires which can give rise to false-positive results [(2)].

Several undetermined fevers can mimic the onset or presence of leptospirosis (such as typhoid fever, hepatitis, dengue fever and malaria) and are important possible diseases which can be confused with this disease. During a recent outbreak of leptospirosis in Nicaragua the disease was first mistaken for dengue fever [(5)]. This offers a good example for the importance of laboratory tests in confirming the clinical diagnosis.

MATERIAL AND METHODS

The serum samples were collected from Department of Microbiology at Hasanuddin University Hospital in Makassar, Indonesia. The total number of patients were 403. Of each patients was collected. In the leptospirosis group (n=179) were patients with clinical suspicion of leptospirosis at admission. Leptospirosis was suspected when patients; Suffered an acute febrile illness, myalgia (especially in the calves) conjunctival suffusion, icterohemorrhage, and hepatic disturbance. Showed renal involvement had a history of exposure to an infected animal or environment contaminated with animal urine. The other group (n=224) were patients which at admission had the suspicion of various other infectious disease including typhoi fever, malaria, hepatitis ad dengue fever or those with fever of unknown origin.

The direct examination for leptospires in a patient's urine-sample is referred to as the lepto-uria test. The test was performed at the Hasanuddin University hospital (according to standard procedures). The leptouria-test was performed on all patietns with suspicion of leptospirosis and on some patients with the suspicion of other than leptospirosis.

A *leptospira*-specific IgM ELISA test was performedon 237 out of 403 serum samples using routine procedure. The test is highly specific and sensitive [(7,8)], And is often used in the routine laboratory diagnosis of leptospirosis. The Igm ELISA was performed at the Royal Tropical Institute in Amsterdam, and the results were then used to compare with leptodipstik test result.

ELISA

The ELISA for detection of leptospira-specific IgM antibodies (IgM ELISA) was performed with antigen prepared from strain Wijnberg as described [(6,7)]. Sera with a titer of 1:80 or higher were considered positive.

Leptodipstick Test Procedures

The dipstick contains two horizontal bands: an antigen band consisting of broadly reactive specific antigen (lower band) and an internal control/upper band. The assay is based on the binding of leptospira-specific IgM antiboidies to the leptospira-antigen. Bound IgM antibodies are specifically detected with an antihuman IgM dye conjugate. The broadly reactive leptospira-antigen ensures the efficient detection of wide spectrum of leptospira

infections. The assay is performed by making a 1:50 dilution of serum (4 microliters) in the detection reagent (200 microliters) and incubating a wetted dipstick in this solution. Staining of the antigen band reveal the presence of specific IgM antibodies in the serum sample. The strength of the staining is important in the interpretation of the test result. A colored reference strip is used to compare the staining intensity which ranges from 0 (no reaction) to 4+ (best reaction). When evaluating the dipstick assay, a staining intensity of 2 + or higher are considered as a positive result.

Internal control

Each dipstick has an internal control band coated with anti-human IgM antibody. Coated antibody binds IgM molecules from the serum which are then stained by the detection reagent. The staining intensity of this iternal control band is rated 2+ for most of the sera tested, and is there to facilitate the interpretation of the assay results and also to make sure that a human serum is being tested each time.

Test and Storage Conditions

Dipstick incubation is performed in an air-conditioned room (23-27 ^{0} C) for 3 hours. During the air-drying period of the dipsticks 'cloudly' dipsticks are seen in the beginning of the study. This is suspect caused by air dryness, because the dipsticks were being air dried in the air-conditioned room. Thouroughly tapping all excess water droplets from the dipsticks before leaving them to be air-dried is the key solution.

All dipstick strips, dipstick vials are kept in the refrigerator (4 ^{0} C) for 1 hour before being used. All sera are kept in the freezer (-10 ^{0} C) for 1 hour before being used.

Statistical evaluation

The variation between the different tests chapter result; test comparisons was determined by calculating kappa values with the standard error of kappa. Values vary between 0 to 1, where kappa values below 0.40 represent a slight agreement;0.40 and 0.80 represent a fair to good agreement; > 0.80 represent almost perfect agreement beyond chance.

RESULT

Of the 179 patients with clinical suspicion of leptospirosis 27 (15.1%) had a diagnosis of leptospirosis (table.1). The diagnosis was based on the clinical symptoms in addition to microscopical observation of leptospires in a urine-sample (leptouria-test). About 84.9% of the patients with clinical suspicion of leptospirosis had a diagnosis other than leptospirosis. The predominant diagnosis was typhoid fever. Of the 224 patients with various other diseases 7 (3.1%) had a diagnosis of leptospirosis.

The clinical diagnosis was compared to the results of the leptospira-specific IgM ELISA test. Of the 16 patients with a final diagnosis of leptospirosis 12 (75%) had a positive IgM ELISA test result (table 2).

Of the 34 patients with a diagnosis of leptospirosis 21 (61.8%) had a positive reaction the leptodipstick test (table 3). Only 7 patients with a diagnosis other than leptospirosis had a positive lepto dipstick result.

Table 4 shows the comparison of the leptodipstick test results and the ELISA IgM test results (in the detection of leptospira-specific IgM antibodies) for these same samples. Comparison of the leptodipstick test result with IgM ELISA show an observed agreement of 0.97 (kappa coeficient=0.80, standard error of kappa=0.60).

Table 1. Clinical diagnosis using leptouria test

Patient groups	Final Diagnosis		Total
	Leptotospirosis	Other	
Leptospirosis suspect	27	152	179
Other Suspect	7	217	224
Total	**34**	**369**	**403**

Table 2. Clinical diagnosis in comparison to the leptospira-specific IgM ELISA Test

Final Diagnosis	IgM ELISA		Total
	Positive	Negative	
Leptospirosis suspect	12	4	16
Other Suspect	2	219	221
Total	**14**	**223**	**237**

Table 3. Clinical diagnosis in comparison to the leptodipstick test

Leptodipstick	Final Diagnosis		Total
	Leptotospirosis	Other	
Positive	21	7	28
Negative	13	361	374
Total	**34**	**368**	**402**

Table 4. Comparison between the leptodipstick test results and the ELISA IgM test results (in the detection of leptospira-specific IgM antibodies)

Leptodipstick	IgM ELISA		Total
	Positive	Negative	
Positive	13	5	18
Negative	1	218	219
Total	**14**	**223**	**237**

DISCUSSION

The diagnosis of leptospirosis was based on the clinical symsptoms and the result of the leptouria test. Of the 403 patients in this study 34 (8.4%) have been diagnosed with leptospirosis (table 1). In a leptospira specific IgM ELISA test, leptospirosis should be considered if a sero-conversion or a 4-fold titer is observed in paired sera. However when only single sera is available, a single raised titer equal to or more than 1:80 may be considered consistent with leptospirosis.

Diagnostic results have been compared to the leptospira-specific IgM ELISA test result in table 2., since this test is highly specific and sensitive and is often

used in the routine laboratory diagnosis leptospirosis. Of the 14 patients with a positive IGM ELISA 12 (85.7%) have a diagnosis of leptospirosis. For fear that doctors are biased by possible false positive leptouria test result it is important to compare the number of leptospirosis as a diagnosis with the number of negative IgM ELISA result. Of the 16 patients with a diagnosis of leptospirosis 4 (25%) have a negative IgM ELISA test result. This might indicate a slight bias for even more false-positive leptouria results.

When the results of the eptodipstick are put into consideration 21 (61.8%) of the 34 patients with a diagnosis of leptospirosis were found positive and 7 of the 28 (25%) patients with a positive dipstick result have a diagnosis other than leptospirosis (table 3).

Comparison the of leptodipstick results with IgM ELISA test results (table 4),shows a very good correlation (observed agreement of 0.97; kappa coeficient=0.80, standard error of kappa=0.60) for the 237 patients tested by both methods. From both literature [(4)], as well as results stated above, one can argue that the leptodipstick is equally sensitive and a specific as the leptospira-specific IgM ELISA test to be used as a laboratory test for the sero-diagnosis of leptospirosis.

CONCLUSION

The leptodipstick test as a laboratory test for the sero-diagnostic of leptospirosis is equally sensitive and specific as leptospira specific IgM ELISA test.

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