Indirect immunofluorescent antibody test for the rapid diagnosis of melioidosis

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Chure is the only reliable method available at present for the diagnosis of melioidosis. Though serological tests have been described, their value in routine diagnosis is controversial. An indirect immunofluorescent assay (IFA) was therefore evaluated to determine its use in the diagnosis of melioidosis. Whole cell antigen prepared from a laboratory isolate of *Burkholderia pseudomallei* was used to assay IgG and IgM antibodies. Fourteen of the 22 (63.6%) culture proven cases had IgM antibodies while only 10 (45.5%) had IgG antibodies. Negative predictive value of IgM assay was 92 per cent. Positive predictive value was 100 per cent if both IgM and IgG were considered together. The present study done on a limited number of samples suggests that IFA may be useful in routine diagnosis of melioidosis.

Key words Diagnosis - indirect immunofluorescent assay - melioidosis

Melioidosis though reported from different parts of India, is still believed to be underdiagnosed and underreported. Melioidosis is endemic in Thailand and Malaysia and with increasing travel and transport there is a chance for this infection to spread to non-endemic areas. Based on clinical features alone melioidosis cannot be differentiated from infections caused by other organisms. Since the mortality associated with this infection can be as high as 80 per cent in untreated cases, early and specific laboratory diagnosis is crucial. Culture is the only reliable method available at present. Other techniques including serology, antigen detection and polymerase chain reaction (PCR) have been evaluated for rapid diagnosis. Conflicting reports exist as to the applicability of these tests for routine diagnosis. A recent study has shown that immunofluorescent assay is both sensitive and specific and also has prognostic value.

The main disadvantage of serological tests is the presence of antibodies either cross-reacting or produced during subclinical infections in healthy individuals in endemic areas leading to false positive reports. False negativity in acute infection is another problem with currently available serological tests. There are not many studies from India evaluating serological tests, where the actual extent of the problem of melioidosis is as yet unknown. We therefore evaluated an indirect immunofluorescent antibody assay (IFA) for the diagnosis of melioidosis.

Serum samples were collected from 22 patients, who were culture positive for *Burkholderia pseudomallei*, attending Christian Medical College and Hospital (CMCH), Vellore during 1994-2000. Serum samples were also collected from 108 apparently healthy individuals and 208 patients with fever but negative by diagnostic tests for typhoid fever, tuberculosis, and malaria.

An isolate of *B. pseudomallei* obtained in the Department of Microbiology, CMCH, Vellore from the...
synovial fluid of a patient, was used to make antigen by Ashdown’s method with minor modification. Overnight culture of this isolate on tryptcase soy agar (DIFCO, USA) was washed thrice in 0.03 M phosphate buffered saline (PBS) pH 7.4 and resuspended in PBS to obtain a turbidity matching Browne’s opacity tube No.4. This suspension was then spotted on clean glass slides, air-dried and fixed in acetone for 10 min. Serum samples were diluted in PBS to give an initial dilution of 1:16 and doubling thereafter, before applying to antigen spots. The slides were incubated at 37°C for 30 min. After washing, fluorescein isothiocynate (FITC) tagged anti-human globulin (Dako, Denmark) was applied and incubated for 30 min at 37°C. Each sample was tested separately with \( \gamma \) chain specific and \( \mu \) chain specific conjugates. The slides were read using an epiluminant fluorescent microscope (Diaplan-Leitz, Germany). Definite fluorescence of the whole bacterium or its outline was taken as positive. One serum sample known to be positive by ELISA test was chosen as positive control. Positive and negative controls were used for each run. The person reading the test was blinded to the status of the individual; two different persons read the test independently.

Among the 22 culture proven patients, 14 (63.6%) had detectable IgM and 10 (45.5%) had detectable IgG antibodies (Table). A titre of 32 was chosen for calculating performance indices because this titre was best able to differentiate infected from uninfected individuals. Thirteen (6%) patients with PUO and five (4.6%) of healthy individuals had IgM antibodies in titres of 32 or above. Of the many serological tests evaluated for the diagnosis of melioidosis, none has been found to be fully reliable. This study done in a non-endemic area shows the sensitivity and positive predictive values of IFA for detecting IgM antibodies, using whole cell as antigen, to be 59 per cent and 72 per cent respectively which is lower than earlier reports. However, as reported earlier, we also found that IgM assay has better diagnostic indices compared to IgG assay. The negative predictive value of IgM assay was 92 per cent in this study. Good negative predictive values of serological tests have been reported previously. If both IgG and IgM are considered together, the specificity and positive predictive values improve. These findings are comparable to those obtained using other serological tests like indirect haemagglutination test (IHA) and ELISA. IHA using purified antigen has better performance characteristics, but this test has not been evaluated properly or widely available.

We observed antibodies in normal healthy individuals which could be due to antibodies that cross react with lipo poly saccharide (LPS) of \( B. pseudomallei \). The positivity rate observed among those with undiagnosed fever was higher than that observed in healthy individuals as was observed by others also. Whether some of the cases with undiagnosed fever in this area can in fact be melioidosis, needs further investigation. Though in absence of internationally acceptable and standardized

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<th>IgM positive</th>
<th>IgG positive</th>
<th>Both positive</th>
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<tr>
<td></td>
<td>( \geq 16 )</td>
<td>( \geq 32 )</td>
<td>( \geq 16 )</td>
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<tr>
<td>Patients (n=22)</td>
<td>14</td>
<td>13</td>
<td>10</td>
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<tr>
<td>Controls (n=108)</td>
<td>8</td>
<td>5</td>
<td>11</td>
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<tr>
<td>PUO (n=208)</td>
<td>19</td>
<td>13</td>
<td>16</td>
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**Diagnostic indices* (%)**

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<tr>
<td>Sensitivity</td>
<td>59</td>
<td>46</td>
<td>36</td>
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<tr>
<td>Specificity</td>
<td>95</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>72</td>
<td>59</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>92</td>
<td>89</td>
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* Calculation based on 32 as cut-off titre. PUO, pyrexia of unknown origin
antigens and methods, comparison between serological studies done at different locations is difficult, it appears from the present study that IFA may prove to be useful in the routine diagnosis of melioidosis. It is necessary to standardize procedures for antigen preparation and serum testing before firm recommendations can be made.

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References


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