Abstract

Background
Brucellosis is endemic in Iran, and is seen in all age groups, including children. Its diagnosis in childhood needs a high index of suspicion. The diagnostic methods currently in use need evaluation for analytical performance.

Objective
The present study tries to evaluate the hematological indices, serologic markers of inflammation and symptoms in patients suspected of brucellosis. Furthermore, the results of three routine methods are compared: PCR, blood culture and Wright agglutination test.

Methods
Symptoms of patients were asked by questionnaire in 48 children. Hematological indices of the CBC test as well as results of CRP, ESR, blood culture, Wright test and PCR were also recorded. Analytical performance of those 3 tests was calculated.

Results
Nine out of 48 patients were positive for brucellosis by PCR, seven of which being positive for Wright test and 2 for culture. Fever and arthralgia were seen in 88.8% and 77.7% of PCR-positive cases, respectively. According to hematological findings 3 of PCR-positive patients (33.3%) had anemia and 2 (22.2%) showed leukopenia. Elevation of ESR was observed in 5 (55.5%), and CRP was positive in 7 such cases (77.7%).

Conclusions
Clinical symptoms, CBC parameters and laboratory markers of systemic inflammation cannot be considered reliable criteria for diagnosis of childhood brucellosis. We suggest usage of PCR rather than blood culture and Wright test for diagnosis in suspected pediatric cases, due to low sensitivity of both culture and Wright test.

Key words
Brucellosis, Children, Diagnosis, Polymerase Chain Reaction, Culture

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Introduction

Brucellosis, as a zoonotic disease caused by *Brucella* species, occurs in various animals and humans worldwide. *Brucella* spp are gram-negative aerobic intracellular rods affiliated with Proteobacterium (1,2). The human beings are usually affected via contact with corpses of infected animals or by consumption of contaminated dairy foods, and rarely through inhalation of aerosols(3).

The world health organization estimates that the annual incidence of human brucellosis is around 500000, and *B. melitensis* is the most prevalent species (4).

Although the incidence of childhood brucellosis may be considered low, but there are reports from some endemic areas in which 20-30% of brucellosis cases are seen in children, with clinical manifestations ranging from minimal symptoms to high morbidity and even death (5, 6).

A six-years epidemiologic study of brucellosis in our area (Yazd city, central part of Iran) revealed that 745 patients were diagnosed with brucellosis, and the highest number of infected patients were children <= 12 years old (27.7%). When the symptoms of infected patients were reviewed it was found that the most common presenting symptoms and physical findings with active brucellosis were fever (89%), chills (63%) weakness and malaise (57%), and headache (47%). In addition mild anemia, leukopenia and relative lymphocytosis were common in all of the studied cases (7).

Analytical performance of the current diagnostic methods is somewhat questionable. Nowadays, laboratory tests for diagnosis of brucellosis include culture, polymerase chain reaction (PCR), and serologic tests which detect anti-*Brucella* antibodies, the most accepted of them being tube Wright agglutination test. It has long been assumed that definite diagnosis of brucellosis is made when the causative organism is grown on culture of blood, bone marrow, tissues or body fluids (such as cerebrospinal fluid, joint fluid, or urine). Since cultures have low sensitivity (around 40-70%) and require long incubation time (up to 6 weeks), it cannot be regarded as the gold standard, although it is presumed by many (1).

It seems that exact diagnosis of the disease should be based on sum of the entire findings in the patient, including history, symptom, physical signs, hematologic abnormalities (such as anemia, thrombocytopenia, pancytopenia, and leukopenia), bacteriologic diagnostic methods, serologic tests and molecular studies (6, 8).

In a study on 102 brucellosis-suspected patients, PCR and blood culture with blind subculture of all culture-negative cases at 7, 14, 21 and 28 days were done, and it was found that 41 cases (40.2%) had bacteremia. It was concluded that the BACTEC 9120 system is able to correctly detect all of the traditional culture-positive cases (9).

The present study follows two aims: firstly, evaluation of the three routine laboratory methods for diagnosis of brucellosis-suspected children, and secondly, to describe the symptoms, hematological findings and traditional inflammation markers in them.

Materials and Methods

In this descriptive study, 48 children (<15 years) clinically suspected of brucellosis and referred by physicians to the laboratory were taken under investigation. A physician collected some relevant clinical data. Then, 10 ml of venous blood was drawn for diagnosis of brucellosis using PCR, Wright agglutination tube test (by the kit from Pasteur institute, Iran), blood culture (on biphasic Castaneda medium), complete blood count (CBC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) tests. We tried to draw
samples at the time of fever, but if impossible, a longer (more than the usual 28 days) incubation was applied for blood culture. A blind subculture was performed weekly, using *Brucella* agar medium containing sheep blood. Any positive blood culture was further tested for oxidase, urease and Gram staining for final diagnosis of presence of *Brucella*. For the PCR test, EDTA-blood was kept at -20°C until the time of white blood cell (WBC) lysis using Miller technique. DNA extraction was done by reagents in the PCR kit (Pouya Zist Tech, Iran). The primer used was B4 (5'-TGG GGT TGC CAA TAT CAA-3') and B5 (5'-CGC GCT TGC CTT TCA GGT CTG-3') which amplifies a 223 bp fragment on a gene that encodes a 31 kDa *B. abortus* antigen (MWG-biotech, Germany). Selection of the primer in our study was based on its proven suitability for use on human blood samples which was shown by some authors (12, 13, 14).

To determine hematological indices, we used the ABX micros 60 hematology analyzer (ABX, Japan). ESR was determined by Westergren method which uses undiluted citrated blood, and semi-quantitative CRP test was done using slide agglutination kit (Bionik, Iran). The following formulas were used for determination of analytical performances of 3 routine *Brucella* tests: Sensitivity =True positive/ (True positive + False negative) Specificity =True negative/ (True negative + False positive) PPV = True positive/ (True positive + False positive) NPV= True negative/ (True negative + False negative). After collecting data, statistical analysis was performed by SPSS 16.

## Results

Among 48 suspicious cases (31 male and 17 female), 9 (18.7%) were diagnosed as having brucellosis based on assumption of PCR as the gold standard (Table I). PCR was positive in 9 cases, Wright agglutination test in 7 (14.6%), and blood culture in only 2 cases (4.2%). Note that all Wright-positive and culture-positive cases were also positive for PCR technique (Table II).

Analyzed data collected from clinicians showed that the most common symptoms of infected patients were fever in 8 (88.9%) and arthralgia in 7 (77.8%), followed by other common brucellosis symptoms such as chills, malaise, sweating and headache (Table III). When hematological findings were reviewed it was found that 3 patients (33.3%) had anemia and 2 (22.2%) had leukopenia. Other CBC indices were unremarkable. The ESR was elevated (>20 mm/h) in 5 patients (55.5%), and 7 cases (77.8%) showed positive for CRP. Based on PCR as a gold standard, the sensitivity of Wright agglutination test and blood culture were 66.7% and 22.2% respectively. The specificities of these 2 tests were 97.4% and 100% respectively. The predictive value of positive for the Wright agglutination test and blood culture were 85.7% and 100% respectively. The predictive value of negative for the Wright agglutination test and blood culture were 92.7% and 84.8% respectively.

### Table I: Children suspected for brucellosis, according to sex (n=48)

<table>
<thead>
<tr>
<th>Result of PCR</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6 (19.3%)</td>
<td>3 (17.6%)</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>25 (80.6%)</td>
<td>14 (82.3%)</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>17</td>
<td>48</td>
</tr>
</tbody>
</table>
Table II: Comparison of 3 laboratory diagnostic tests for brucellosis

<table>
<thead>
<tr>
<th>Tests</th>
<th>+ # (%)</th>
<th>- # (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright test</td>
<td>7 (17.1)</td>
<td>41 (82.9)</td>
</tr>
<tr>
<td>Blood culture</td>
<td>2 (4.2)</td>
<td>46 (95.8)</td>
</tr>
<tr>
<td>PCR</td>
<td>9 (18.7)</td>
<td>39 (81.3)</td>
</tr>
</tbody>
</table>

Table III: Brucellosis major symptoms among infected patients (n=9)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Positive # (%)</th>
<th>Negative # (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Both of them</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
</tr>
</tbody>
</table>

Discussion

Like some other Asian countries, childhood brucellosis is common in our country due to consumption of unpasteurized milk and milk products, mainly cheese (6,7,8).

Although the number of case in our study was limited, the results are in agreement with others (8, 9, 10) and previous epidemiological investigations in our area (7). In general, hematologic abnormalities of mild anemia and leukopenia have been frequently associated with acute brucellosis. However, they are not reliable criteria for diagnosis and follow-up of brucellosis. This is because there are some other infectious diseases such as typhoid fever, tuberculosis and malaria which may present with the same signs (8,10). As a result, microbial detection tests together with serological and molecular techniques are necessary.

Since only 2/9 of blood samples from suspected persons were positive for culture, we agree with many other researchers who do not accept blood culture as a gold standard, because it has very low sensitivity and needs a long waiting period. Also, Wright agglutination test cannot be a reliable test because of some false-negative.

In a study by Morata et al, 34 non-blood specimens from human brucellosis cases were assessed, in whom PCR test was positive in 33 (97%) but only 29.4% showed positive culture. Also, 11.4% of the patients had negative Wright test or low antibody titer. Therefore, they recommended PCR as a helpful detecting method for brucellosis because of its high sensitivity, high speed and low contamination risk (11).

We may conclude that in the pediatric population PCR has the highest diagnostic yield, and must be regarded as the gold standard for diagnosis of brucellosis. Its added values are rapidity (hours vs. weeks when compared with culture), and easy availability in almost every equipped laboratory. It is useful also as a confirmatory test in Wright-positive suspected cases and for epidemiologic surveillance studies, although the cost may be quite high when applied on a large population. Another conclusion is that symptoms of patients and results of CBC parameters, ESR and CRP tests cannot be used as a reliable tool for diagnosis of brucellosis.

Acknowledgement

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Conflict of Interest

The authors have no conflict of interest.
Reference