Diagnosis of Major Bacterial Causes of Culture Negative meningitis in Children

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ABSTRACT

The study included 250 patients admitted to fever hospitals in Egypt with suspected clinical signs and symptoms of bacterial meningitis, their age ranged from six months up to eleven years. Cerebrospinal fluid (CSF) samples isolated from patients were transported to the central health laboratories for confirmation of diagnosis. Physical, cytological, chemical examination for glucose and total protein concentration. Gram stained smear, culture on blood and chocolate agar were done. 62 (24.8%) CSF samples were culture negative and no bacterial organisms could be detected by direct gram stain. All these samples were further examined by latex agglutination and RT-PCR. Samples which found to be culture positive were excluded from this study. 15 samples of them (24.2%) were positive by RT-PCR. 7 of them (46.6%) were Haemophilus influenza b (H. influenza b) and 8 samples of them (53.3%) were Streptococcus pneumoniae (S. pneumoniae). Out of 15 RT-PCR positive samples 12 of them (80%) were Latex positive, while 3 of them (20%) were Latex negative, one of the three was S. pneumoniae, while the others were H. influenza b by RT-PCR only. Out of 62 culture negative CSF samples 47 (75.8%) had a history of previous antibiotic use. 11 samples of them (23.4%) were positive by latex agglutination (5 samples were H. influenza b and 6 samples were S. pneumoniae) and 3 samples of them (6.3%) were positive by RT-PCR and negative by latex agglutination. Out of 15 RT-PCR positive samples, 8 of them were clear (53.3%), 3 of them were turbid (20%), and 4 of them were bloody (26.6%). 10 CSF samples out of the 15 samples (66.6%) had normal cytological profile, 6 samples (40%) had normal CSF glucose levels and 3 samples (20%) had normal total CSF protein concentration.

Keywords: Bacterial meningitis, CSF, Culture negative, RT-PCR and Latex agglutination test.

INTRODUCTION

Bacterial meningitis is a serious infection of the thin lining that surrounds the brain and spinal cord. The bacteria are transmitted from person to person through droplets of respiratory or throat secretions from carriers or cases. The most common symptoms are a stiff neck, high fever, sensitivity to light, confusion, headache and vomiting (1).

Suspected bacterial meningitis is a medical emergency, the mortality rate of untreated bacterial meningitis approaches a hundred percent. Even with optimal therapy, morbidity and mortality may occur. Neurologic sequelae are common among survivors (2).

Haemophilus influenzae type b (Hib), Streptococcus pneumoniae, and Neisseria meningitidis cause the majority of cases of acute bacterial meningitis (3).

Neisseria meningitidis, was previously reported as the major etiological cause of bacterial meningitis in Egypt. Recently the epidemiology has changed and Streptococcus pneumonia was described as the leading cause (4).

Immediate diagnostic steps must be taken to establish the specific cause so that appropriate antimicrobial therapy can be initiated (5). Initial diagnosis of bacterial meningitis can be made by clinical examination followed by a lumbar puncture showing a purulent spinal fluid. Sometimes the bacteria can be seen in microscopic examinations of the spinal fluid. The diagnosis is supported or confirmed by growing the bacteria from specimens of spinal fluid or blood, by agglutination tests or by polymerase chain reaction (PCR). The identification of the serogroups and susceptibility testing to antibiotics are important to define control measures. Appropriate antibiotic treatment must be started as soon as possible, ideally after the lumbar puncture has been carried. A range of antibiotics can treat the infection, including penicillin, ampicillin, chloramphenicol and ceftriaxone (6).

Although acute bacterial meningitis (ABM) can be detected by the presence of clinical signs of meningitis and abnormal CSF physical, chemical, cytological, bacteriological (culture and microscopy) and serological examination
results, some positive acute bacterial meningitis cases could be missed. Therefore; a normal CSF results may not necessarily exclude it and should not delay early institution of appropriate antimicrobial therapy\(^5\).

Availability of over-the-counter antibiotics, administration of antibiotics before performance of lumbar puncture, lack of microbiology resources for bacterial culture, and variable quality of microbiology services are among the reasons for culture negativity\(^6\).

A molecular examination is important for rapid diagnosis of acute bacterial meningitis and also to increase the sensitivity of bacterial meningitis detection\(^7\).

There have been a growing number of studies showing discrepancies between results of cultural methods and non cultural methods (latex agglutination and PCR) and suggested PCR to be part of the diagnostic methods\(^6\).

We aimed in this study at identifying the most accurate method(s) for diagnosis of clinically suspected, culture negative cases of bacterial meningitis by comparing the results of the Real-Time PCR test with that of the latex agglutination test, physical, chemical (glucose and protein levels) and cytological examinations of clinically suspected, culture negative cases of bacterial meningitis.

MATERIALS & METHODS

A- Study population:

This study was conducted on 250 childre who were admitted to fever hospitals in Egypt with suspected clinical signs and symptoms of bacterial meningitis, in the period between May 2012 to January 2013. Their age ranged from six months up to eleven years.

B- Sample collection and transport and processing:

- A volume of 5 ml of CSF was collected from patients by the neurologist through complete aseptic lumbar puncture technique.
- The macroscopic appearance of the CSF for color, aspect and presence of clots was recorded in a report.
- Each CSF sample was divided into 4 aliquots (for chemical, cytological, microbiological and molecular examination) with the patient name, age and hospital admission number on each aliquot and were transported in ice box to the central health laboratories for confirmation of diagnosis.

- All aliquots were processed immediately except those of molecular examination were refrigerated at -70 °C.

C- Sample examinations:

- Each CSF sample was subjected to:

1- Chemical examination: to detect
- CSF glucose level by glucose oxidase method. The technique of estimation and interpretation of CSF glucose level was done according to the standard methods; briefly, any sample with CSF glucose level > 40 mg/dL was reported as had a normal CSF glucose profile\(^8\).
- CSF total protein concentration by Trichloro acetic acid method. The technique of estimation and interpretation of CSF total protein concentration was done according to the standard methods; briefly, any sample with CSF total protein concentration <100 mg/dL was reported as had a normal CSF total protein profile\(^9\).

2- Cytological examination: to estimate of total and differential leucocytic count using a haemocytometer. The technique of cellular counting and interpretation was done according to the standard methods; briefly, any sample with total leucocytic count 1,000 WBCs/cu mm was reported as had a normal cytological CSF profile\(^10\).

3- Microbiological examination:
- The specimen was centrifuged for 15 minutes.
- The supernatant was stored at -70 °C for antigen detection by latex agglutination.
- The sediment was vigorously mixed in a closed tube using a vortex mixer (VELP Scientifica) and used for preparation of a Gram stained smear (to detect morphology of the present microorganisms)\(^11\) and platting out of a sheep blood agar plate and a chocolate agar plate supplemented with Iso Vitalex.
- The inoculated agar plates were incubated for 48 hours at 37°C with ~5% CO2.
- After 48 hours of incubation the inoculated plates were examined for presence of growth and the isolates (if present) were identified by the standard microbiological methods\(^11\).
- All CSF samples found to be culture negative were selected to be tested with latex agglutination and RT-PCR.

Latex agglutination test:

The supernatant taken from centrifuged CSF samples was tested for rapid detection of soluble antigens of the studied bacterial organisms (Haemophilus influenzae type b, Streptococcus pneumonia (AS Polyvalent “2”
for serogroups 6, 8, 9 & 10 and AS Polyvalent "3" for serogroups 11, 12, 14, 16), Neisseria meningitides groups A, C, Y, W135 and Neisseria meningitides group B using Remel Wellcogen Bacterial Meningitis Antigen Latex test kit (Therom Scientific Waltham, MA, U S A).

**Semi-quantitative Real-time PCR:**

1- **Extraction of bacterial DNA** from CSF was done using QIAamp DNA Mini Kits.

2- **Amplification and detection:**

- AccuPrime™ SuperMix II Invitrogen was used.

- The extracted DNA of each CSF sample was amplified in three separate tubes, each tube in a separate amplification run and targeting one of the three studied bacterial organisms using specific primers and probes for each organism as shown in table (1).

- With every TaqMan amplification run, a positive control containing target DNA from known N. meningitides, Hib., and St. pneumonea (proved to be PCR positive) was included, in order to verify Master mix functionality and performance. Also a negative control (DNA-free sample) was included to check that no external DNA contamination has occurred during preparation of master mix.

- A volume of 2ul of the extracted DNA was added to 12.5ul TaqMan PCR Master Mix (Applied Biosystems), 2ul forward primer, 2ul reverse primer, 2ul probe and 4.5ul sterile PCR grade water (Roche Diagnostics) in the PCR tube to reach a final amplification volume of 25ul.

- The DNA was amplified in Real-time BIORAD thermocycler (CFX 96™ Real time BIORAD), with the TaqMan system using the following cycling parameters: Heating at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min (These parameters are the same for the three targeted microorganisms).

- The Real-time thermocycler calculates the fluorescence readings to identify a baseline reading and detects any increase in fluorescence in each reaction well. The increase in fluorescence above a calculated background threshold indicates amplification of the target sequence. The PCR cycle number (out of 40) at which the measured fluorescent signal exceeds a calculated background threshold identifying amplification of the target sequence is called the cycle threshold (C<sub>T</sub>) value. If no increase in the fluorescence signal was observed after 40 cycles, the sample is assumed to be negative.

### Table (1): Primers and probes for fluorescent real-time PCR

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Primer/Probe Information</th>
<th>Working concentration uM</th>
<th>Final concentration nM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>St. pneumonia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autolysin A (lytA) gene&lt;sup&gt;(12)&lt;/sup&gt;</td>
<td>lytA-fwd: ACG CAA TCT AGC AGA TGA AGC</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>lytA-rev: TCG TGC GTT TTA ATT CCA GCT</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>probe-lytA: 5'-FAM-TGC CGA AAA CGC TGG ATA CAG GGA G -3'</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td><strong>N. meningitides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsule transport A (ctrA) gene&lt;sup&gt;(13)&lt;/sup&gt;</td>
<td>F753-fwd: TGTGTCCCGTATACGCCATT</td>
<td>3.75</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>R846-rev: GCCATATCCACAGATATACC</td>
<td>11.25</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>probe-Pb820i: AACCTTGAGCAA&quot;T&quot;CCATTTATCCT GACGTTCT</td>
<td>1.25</td>
<td>100</td>
</tr>
<tr>
<td><strong>H. influenza</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heamophilus protein D 3 (hpd 3) gene&lt;sup&gt;(12)&lt;/sup&gt;</td>
<td>hpdF822-fwd: GGTTAAATATGCGGATGTTGTG</td>
<td>1.25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>hpdR952-rev: TGCACTTTTACGCGGTTGTA</td>
<td>3.75</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>probe-Pb896i: TTGTGACACTCGGT &quot;T&quot; GGTAAAAGAAGTTGCAC</td>
<td>1.25</td>
<td>100</td>
</tr>
</tbody>
</table>
Statistical study:
Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples in normally distributed data and Mann Whitney U test for independent samples when data were not normal. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Agreement was tested using kappa statistic. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

- Out of 250 CSF samples taken from clinically suspected cases of bacterial meningitis, 188 (75.2%) were culture positive, 177 (70.8%) were Streptococcus pneumoniae, 9 (3.6%) were Haemophilus influenzae b and 2 (0.8%) were Neisseria meningitidis. Same results were found by gram stained smear.

- Out of 250 CSF samples, 62 (24.8%) were culture negative and no organisms could be detected by direct gram stain. These 62 samples were further examined by latex agglutination and RT-PCR. Samples which found to be culture positive were excluded from this study.

- Out of the 62 culture negative CSF samples 15 samples (24.2%) were culture negative and no organisms could be detected by direct gram stain. These 62 samples were further examined by latex agglutination and RT-PCR. Samples which found to be culture positive were excluded from this study.

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- Out of the 62 culture negative CSF samples 15 samples (24.2%) were culture positive by RT-PCR, 7 samples of them (11.3%) were H. influenzae type b and 8 samples (12.9%) were S. pneumoniae.

- y latex agglutination test; 50 samples out of 62 culture negative CSF samples (80.6%)were negative and 12 samples (19.4%) were positive, 5 samples of them (41.6%) were positive for H. influenzae b, and 7 samples (58.3%) were positive for S. pneumonia. All latex positive samples were Rt-PCR positive while 3 samples were Latex negative and Rt-PCR positive (2 samples were H. influenzae b and one sample was S. pneumonia). The relative correlation between the results of Latex test and Rt-PCR test was statistically highly significant (P value: <0.001) and the relative sensitivity, specificity, positive predictive value, negative predictive value and accuracy of latex test in relation Rt-PCR test were 80%, 100%, 100%, 94% & 95.17% respectively (table 2).

- Regarding CSF aspect; out of 15 culture negative RT-PCR positive samples, 8 samples of them (53.3%) had clear aspect and they were also Latex positive (3 samples were S. pneumonia and 5 samples were H. influenzae b), 3 samples of them (20%) were turbid (2 samples were S. pneumonia and 1 sample was H. influenzae b) and 4 samples of them (26.6%) were bloody (2 samples were S. pneumonia and 2 samples were H. influenzae b).

- The cytological examination of the CSF showed that out of 15 culture negative RT-PCR positive samples, 10 CSF samples (66.6%) had normal cytological CSF profiles (one sample with 500-1000 cells/ mm³, 8 samples with 100-500cells/ mm³ & one sample with < 100 cells/ mm³).

- The chemical examination of the CSF showed that out of 15 culture negative RT-PCR positive samples, 6 CSF samples (40%) had normal CSF glucose profiles (one sample with glucose level >100 mg/dL & 5 samples with glucose level 40-100 mg/dL) and 3 CSF samples (20%) had normal CSF total protein concentrations (CSF total protein concentrations were <100 mg/dL).

- History of Antibiotic use: 47 cases out of 62 (75.8%) culture negative clinically suspected cases of bacterial meningitis had history of antibiotic use before CSF sample collection; 11 cases of them (23.40%) proved to have bacterial meningitis by positive latex test and positive RT-PCR (5 samples were H. influenzae b and 6 samples were S. pneumonia) and 3 cases of them (6.38%) proved to have bacterial meningitis by only positive RT-PCR (2 samples were H. influenzae b and one sample was S. pneumonia), this relation was not statistically significant (P value = 0.090). There was only one culture negative clinically suspected case of bacterial meningitis without history of antibiotic use before CSF sample collection proved to have bacterial meningitis by positive latex test and positive RT-PCR (it was with H. influenzae b) (table 3).
Table 2: Relation between latex agglutination and RT-PCR

<table>
<thead>
<tr>
<th>Latex agglutination</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
</tr>
<tr>
<td>Latex -ve</td>
<td>50</td>
</tr>
<tr>
<td>Latex +ve</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 3: Relation between previous antibiotic use and latex agglutination results in RT-PCR positive cases.

<table>
<thead>
<tr>
<th>Antibiotic +ve</th>
<th>Antibiotic -ve</th>
<th>Latex +ve and Rt-PCR +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 12)</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>- H. influenzae b</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>- S. pneumonia</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>- N. meningitidis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibiotic +ve</th>
<th>Antibiotic -ve</th>
<th>Latex -ve and Rt-PCR +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 3)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>- H. influenzae b</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>- S. pneumonia</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>- N. meningitidis</td>
</tr>
</tbody>
</table>

DISCUSSION

*Streptococcus pneumoniae* and *Haemophilus influenzae* and *Neisseria meningitidis* are important agents of meningitis\(^{14,15}\). Identification of the microbiological cause of meningitis is important, as it enables pathogen-directed antibiotic therapy. To diagnose bacterial meningitis, CSF examination is mandatory (Matthijs et al., 2010). Conventional detection of bacteria in CSF is based on culture and phenotypic characterization. However, culture methods are time-consuming and have relatively low sensitivity, especially when antibiotics have been given to the patient prior to sampling\(^{15}\). The use of nucleic acid amplification tests, such as quantitative real-time polymerase chain reaction (qPCR), have enabled more sensitive and rapid detection of pathogens in CSF\(^{15,16}\).

In our study, 250 CSF samples were obtained from patients with suspected cases of acute bacterial meningitis. 62 samples of them (24.8%) were culture negative and at the same time, no bacterial organisms could be detected by gram stain.

Similar results were reported by Madhumita and Gupta\(^{17}\), who reported culture-negative cases of meningitis or a low CSF culture positivity, ranging from 6-50%. They suggested that the yield of bacteria on a Gram stain depends on several factors like the number of organisms present, prior use of antibiotics, technique used for smear preparation (centrifuged deposit, direct smear etc.), staining techniques and the observer’s skill and experience.

Various reasons are cited in the literature for a low yield of bacteria on culture: improper technique of lumbar puncture, delay in transport of specimens to the laboratory, non-availability of special media for specific pathogens in the emergency setting, autolytic enzymes in CSF, fastidious nature of pathogen and antibiotic treatment prior to lumbar puncture\(^{17}\).

Mani et al.\(^{18}\), reported that despite low Gram stain smear positivity from CSF samples and the fact that a negative Gram stain does not rule out infection, the importance of a positive smear cannot be over-emphasized, especially in developing countries where financial constraints limit the use of other rapid diagnostic tests to diagnose this potentially fatal infection. They reported that a simple Gram stained smear can offer an immediate clue to aid a diagnosis of pyogenic meningitis.

Van de Beek et al.\(^ {19}\), also stressed on the importance of the need for clinicians to know the causative organism in predicting the outcome and advocate Gram staining of CSF as a routine procedure for prompt identification of the pathogen. Also Kabra et al.\(^{20}\) stressed on a careful Gram staining and prompt bed-side inoculation of CSF samples on culture media by resident doctors.

On the other hand, Matthijs et al.\(^ {7}\), reported that (45%) of the CSF culture-negative patients had a positive Gram stain. Forty-four
percent of patients in this cohort were pretreated with antibiotics. Pretreatment with antibiotics decreased the yield of CSF Gram staining, from 56 to 52%.

In our study, out of 62 culture-negative CSF samples 15 were positive by RT-PCR; 14 of them gave a history of previous antibiotic use at the time of sample collection.

Out of the 12 samples which were positive by latex agglutination in our study; 11 samples gave a history of previous antibiotic use at the time of sample collection. This may explain the low yield of culture.

Similar results were reported by Claudio et al. (6), who found that the proportion of pretreated patients ranged from 0.0% to 50.0%.

Penelope et al. (23), reported that due to the early administration of antibiotics, meningococcal disease is increasingly difficult to diagnose by culturing, and recommended the use of real-time PCR to be a sensitive and specific tool for diagnosis, through a prospective comparison of real-time PCR, and standard culturing of cerebrospinal fluid.

Matthijs et al. (7) also reported that, the yield of CSF culture is lower in patients who have received antibiotic treatment before lumbar puncture.

Another study done by Kaplan (2), reported that isolation of a bacterial pathogen from the CSF culture confirms the diagnosis of bacterial meningitis. However, a negative culture of the CSF at a particular point in time does not preclude the development of meningitis hours or days later.

Out of the 62 culture negative CSF samples in our study, 15 (24.2%) were positive by real-time PCR. This may be due to the fact that PCR assay is not affected by antibiotic intake which damage bacteria, as the remaining DNA of dead bacteria can be detected.

Papavasileiou et al. (22), reported that (78.6%) of the positive CSF samples were detected only by PCR. Thomas-Cherian et al. (23), also reported that (45.4%) of negative culture and Latex Agglutination CSF samples, were positive by PCR only.

Claudio et al. (6), reported results of culture negative CSF samples with positive real-time PCR results. Sensitivity of real-time PCR was 100% for N. meningitidis, 97.8% for S. pneumoniae, and 66.7% for H. influenzae.

Specificity for the three organisms ranged from 98.9% to 100%. They found that the most important risk factor for being culture negative/RT-PCR positive was presence of antibiotic in CSF, which are widely available over-the-counter in

In addition, it is likely that some patients were given antibiotics by healthcare workers before CSF was obtained for culture.

Also, Xin Wang et al. (24), reported that the specificity for detection of its target organism was 100%, in both multiplex and singleplex real-time PCR.

Although the use of antimicrobial therapy before lumbar puncture affects the CSF culture and perhaps the Gram stain, conventional teaching has been that a pathogen still can be identified in the CSF in the majority of patients up to several hours after the administration of antibiotics. The time interval between antibiotic administration and negative CSF cultures may be shorter than appreciated for children who receive parenteral antibiotics (6).

Real-time PCR provides much more rapid results than culture, which is the gold standard. Additionally, the sensitivities for detecting the major bacterial pathogens associated with meningitis (Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae) equal culture. Importantly, in cases of meningitis where antibiotics are taken before cultures are obtained, PCR may be particularly advantageous as it can be positive, whereas culture is negative (25).

In our study, out of 15 RT-PCR positive samples 12 of them (80%) were Latex positive, while 3 of them (20%) were Latex negative. The 3 latex negative, RT-PCR positive CSF samples showed relatively high cycle threshold (CT) value of RT-PCR.

The false-negative Latex agglutination could be, possibly, because of low antigen titers in the CSF (17).

The clinical usefulness of the convenient latex antigen detection tests is limited as a negative test does not rule out infection and a false-positive test results, in a prolonged course of antibiotics, lengthened hospital stay and in some cases important clinical complications (17).

Nevertheless, several studies advocate the usefulness of Latex agglutination, especially in pretreated cases and to differentiate partially treated pyogenic meningitis from tuberculous meningitis. Latex agglutination test was found to be a simple, rapid procedure suitable for use as an adjunct laboratory test, though it needs to be interpreted cautiously in the context of the patient's clinical condition (17).

Mani et al. (18), also stressed that despite its drawbacks, in developing countries where many laboratories lack facilities for culture and other
elaborate investigations, latex agglutination can help establish the crucial diagnosis. They also reported that samples which did not show any evidence of the pathogen on either Gram's stain or culture, were positive by latex agglutination.

In our study the relative sensitivity of latex agglutination test in our study was 80%.

Matthijs et al.(7), reported sensitivities of latex agglutination testing of CSF samples from patients with suspected bacterial meningitis with no bacteria seen upon CSF Gram staining and negative CSF cultures, ranged from 78 to 100% for H. influenzae type b meningitis, 59 to 100% for pneumococcal meningitis, and 22 to 93% for meningococcal meningitis.

However, in a 10-year retrospective study of 176 children with culture-negative meningitis who were pretreated with antibiotics before lumbar puncture, none had a positive CSF latex agglutination result(26). In another study of 28 patients with negative CSF cultures who had clinical presentation and CSF parameters compatible with bacterial meningitis, CSF latex agglutination had a sensitivity of only 7% for detecting bacteria(27). A third study showed only 7 positive agglutination tests out of 478 CSF samples tested; all 7 patients had a CSF Gram stain showing the causative microorganism(28). A study of meningococcal meningitis patients showed a strong decline in the sensitivity of latex agglutination, from 60% for patients without antibiotic pretreatment prior to lumbar puncture to 9% for antibiotic-pretreated patients(29). The limited additional value of latex agglutination testing was also shown by Tarafdar et al.(28) and Nigrovic et al.(28).

In our study, 25 of 16 RT-PCR positive samples, the results of WBCs examination of 10 CSF samples (66.6%) were not concomitant with normal values in acute bacterial meningitis.

Similar results were reported by Kaplan(7), who found that CSF cellular findings were normal in (57%) of patients who underwent lumbar puncture for evaluation of possible meningitis. Matthijs et al.(7), also reported that although low CSF white blood cell counts do occur, especially in patients with septic shock and systemic complications; the majority of patients presenting with community-acquired bacterial meningitis have CSF parameters characteristic of bacterial meningitis.

Experimental pneumococcal meningitis studies also showed a relationship between a large bacterial CSF load, a lack of response of CSF leukocytes, and intracranial complications, probably indicating excessive bacterial growth and a lack of a CSF leukocyte response(30).

There are often more than 1,000 WBCs/cu mm, particularly neutrophils (PMNs) in bacterial meningitis. However, very early in the course (especially of meningococcal meningitis) there may be few or no cells or the cells may be mainly lymphocytes(31).

On the other hand, Mani et al.(18) and Madhumita and Gupta(17) reported that all the CSF samples had an elevated leukocytic count in CSF and were clinically compatible with a diagnosis of pyogenic meningitis.

Among culture-negative specimens, there was a strong relationship between the WBCs in CSF and the percent of CSFs that were RT-PCR positive. And that Prior administration of antimicrobial agents, particularly oral antibiotics, tends to have minimal effects on CSF cytology(6).

In this study, 15 RT-PCR positive samples, the results of sugar examination of 6 samples (40%) are not concomitant with normal values in acute bacterial meningitis, and the results of protein examination of 3 samples (20%) are not concomitant with normal values in acute bacterial meningitis.

Claudio et al.(6), recommended that CSF chemistry results, in pretreated patients, must be interpreted with caution. Receipt of antibiotics for ≥12 hours before lumbar puncture was associated with increased median CSF glucose concentration and decreased median CSF protein concentration.

The CSF culture may be negative in children who received antibiotic therapy before CSF examination. In such children, increased CSF cell count with a predominance of neutrophils, elevated CSF protein concentration, and/or decreased CSF glucose concentration usually are sufficient to establish the diagnosis of bacterial meningitis(2).

In our study we found that out of 15 CSF culture negative, RT-PCR positive samples, 8 of them (53.3%) had a clear aspect.

Ray and Rylance(31), also reported the incidence of meningitis with clear CSF, showing no cytological or biochemical abnormality in (9.7%) of cases. Also William et al.(32) reported a number of case studies of bacterial meningitis confirmed by CSF culture, in which an initial lumbar puncture yielded completely normal parameters.

CONCLUSION

Although culturing is still the gold standard for identification of the causative organism in acute bacterial meningitis and allows complete antimicrobial susceptibility testing of
isolates, RT-PCR has the added advantage of providing results more rapidly than culture also increases the diagnostic yield for bacterial meningitis and is ideal for incorporation into routine surveillance in a developing country; whereas the utility of PCR for predicting susceptibility profiles has yet to be widely validated.

Despite its drawbacks, latex agglutination test is a simple, rapid procedure suitable to be used as an adjunct laboratory test, but needs to be interpreted cautiously. It is the need of the hour to develop affordable indigenous latex agglutination kits, designed to detect the serotypes prevalent in our geographical area and also to help detect other prevalent pathogens.

CSF chemistry and cytology results in pretreated patients must be interpreted with caution.

REFERENCES


تشخيص الأسباب الرئيسية البكتيرية السلبية المزروعة للتهاب السحايا في الأطفال

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**وقسم الميكروبيولوجي، المعاليم المركزية بوزارة الصحة**

شملت الدراسة مائتان و خمسون مريضاً أدخلوا إلى مستشفيات المعاليم في مصر في وجود علامات وأعراض التهاب السحايا الجلوكومي السريري المبكر. وتراوحت أعمارهم من ثلاثة أشهر إلى أخذ عش عاماً. تم اجراء عيانات البلازما والشمعة من المرضى إلى مختبرات الصصة المركزية لتكدب التشخيص. تم جهود اجراء فحوصات فيزيائية، وفحص الدم، البلازما، والفحص الكيميائي للجلوكومي والتركيز الكلي للبروتين وصبغة الجرام، كما تم زرع العيانات على كل من أطباق آمار الدم و الشوكولاتي.

أعطت التحليلات تطور عينة ناقصة شوكي بنسبة (٢٤.٨%), نتيجة سلبية لأي كائنات بكترية، لكل من صبغة الجرام والزرع، وتم تحصيل هذه العيانات لكل لون الأميانتين من طرق تلازمنا (١٨٨) و ريل تايم بي سى. رগت استعمال العيانات التي تمت نتيجة إيجابية عن طرق الزرع من هذه الدراسة. من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق الريل تايم بي سى (١٦.٢%)، وحيد أن نسبة نقشها تهابية النizational من القطب بنسبة (٤٦.٤%). وثاني عيانات من البكتيريا الميكروب السحيبي السريع بنسبة (٣٥.٣%). من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق الريل تايم بي سى (٨٩.٤%)، وحيد أن نسبة نقشها تهابية الن战士职业ية من القطب بنسبة (٤٣.٠%). أظهرت ثلاث عيانات سلبية عن طريق تلازمنا (١٩.٤%)، وحيد أن نسبة نقشها تهابية الن战士职业ية من القطب بنسبة (٤٥.٣%). و выполنت نسبة في اختبار الأ inflammات بنسبة (٤٧.٩%)، من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق ريل تايم بي سى (٦٠.٨%). وذات نسبة في اختبار الأ inflammات بنسبة (٣٣.٢%). من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق ريل تايم بي سى (٤٥.٤%). وذات نسبة في اختبار الأ inflammات بنسبة (٢٢.٨%). من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق ريل تايم بي سى (٣٤.٢%). وذات نسبة في اختبار الأ inflammات بنسبة (٢٢.٢%). من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق ريل تايم بي سى (٥٢.٩%). وذات نسبة في اختبار الأ inflammات بنسبة (٢٣.٢%). من بين التحليلات سببت عينة ناقصة شوكي نتائج بريئة فيانة نتائج إيجابية عن طريق ريل تايم بي سى (٣٤.٢%). وذات نسبة في اختبار الأ inflammات بنسبة (٢٢.٢%). 

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