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Clinical Profile of Bacterial Meningitis in Children and Comparative Inter-Alia Analysis of Various Microbiological Tests

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Abstract

Acute Bacterial meningitis is life-threatening and neurologically debilitating infectious disease. We studied the clinical profile,organisms involved in bacterial meningitis in children and compared tests on Cerebrospinal Fluid (CSF) Latex Agglutination Test (LAT), Polymerase Chain Reaction (PCR), gram stain (conventional), cyto-tek centrifuge cytospin gram stain to culture which is the gold standard .Methods: Ours was a observational cross-sectional study (age range 3 to 12 years) conducted in a tertiary care hospital, New delhi, India for one year. Total of 101 patients were enrolled and divided in 3 age groups *viz* <1year, 1-5years, >5years. Fever was the most common presenting symptom in all groups (84.2%). Refusal to feed, headache, altered sensorium, vomiting, blurring of vision were significantly associated with bacterial meningitis in all age groups. Cranial nerve palsies and neck rigidity were significantly higher in older children. Age<5years, low socioeconomic status, overcrowding and smoke exposure were identified as risk factors for meningitis. 8 children died within 48 hours of admission in our study the rest had a normal recovery. CSF culture was positive in 35.6% cases with streptococcus *pneumoniae* being the most common organism. PCR performed best for the diagnosis of meningitis but it has its limitations. Cytospin gram stain showed positivity in 65% cases which much higher compared to conventional gram stain. Cytospin gram stain was a viable low-cost alternative for early diagnosis of meningitis in low income countries like ours.

Keywords: Clinical features; Culture; PCR; Cytospin gram stain

Introduction

Acute Bacterial Meningitis (ABM) is a neurologically debilitating infectious disease, being a major cause of childhood morbidity and mortality. Mortality rates of 5% have been recorded in the developed world, but the numbers may be as high as 30% in the developing world. [1]. In India, bacterial meningitis constitutes 1.5% of pediatric hospital admissions and the mean case fatality rate is 16% [2]. Worldwide *Haemophilus influenza* type B (HiB) is the most common pathogen for meningitis in children. This led to introduction of vaccination against this organism resulting in fall in its incidence. However, there has been conflicting data regarding the most common organisms causing meningitis in Indian children. Also, there has not been widespread vaccination directed against *Haemophilus influenza type B (HiB), Streptococcus Pneumoniae, Neisseria Meningitidis* under the national immunization program in India. Most of these infections have been reported retrospectively in case of an outbreak or anecdotally [3].

Cerebrospinal Fluid (CSF) culture is considered the gold standard (due to high specificity) for diagnosis of ABM but has certain technical difficulties. Many children report to hospital after being treated with inadequate doses of antibiotics [4]. This prior administration of antibiotics may lead to a negative CSF culture. Also, culture results are not available earlier than 3-4 days and organisms causing meningitis are difficult to culture. Gram staining and bacterial antigen tests also permit a rapid diagnosis but have limited sensitivity. Molecular methods like Polymerase Chain Reaction (PCR) are pathogen specific and also have limited availability. Therefore, a rapid and sensitive tool is needed to diagnose ABM and improve the management of patients. In a previous study it was seen that cytospin slide centrifuge could concentrate CSF samples increasing the sensitivity of CSF gram stain by 2-3 logs compared to unconcentrated and conventional centrifuge smears. This would help expedite the diagnosis and improve the management of patients with meningitis. We studied the clinical profile, causative organisms of meningitis cases in children over one year and also did inter-alia analysis of CSF cytospin centrifuge (gram stain) to gram stain (conventional), culture, PCR and Latex Agglutination Test (LAT).

Objectives

- To study the clinical profile of patients with bacterial meningitis in children between 3months-12years.
- To identify the most common organism that is identified in our population.
- Comparative analysis of various diagnostic tests like latex agglutination, gram stain, cytospin gram satin with regards to the gold standard i.e. culture.

- Ours was a cross-sectional observational study in a tertiary care hospital, Safdarjung hospital New Delhi, India.

Inclusion criteria

Children in the age group 3 months- 12 years presenting to the emergency department with signs and symptoms of pyogenic meningitis i.e. fever (>38^{0}C), altered sensorium, neck rigidity, headache, vomiting, blurring of vision, focal neurological deficits, refusal to feeds, seizures along with CSF findings of>5 neutrophils/HPF, glucose<40 mg/dl or CSF glucose <2/3rd of random blood glucose, proteins>45 mg/dl.

Exclusion criteria

- Patients with alternative diagnosis and a normal CSF picture or mild pleocytosis.
- Patients with tubercular meningitis.
- Patients with a prolonged history or cerebral malaria were excluded.

Complete blood count, electrolytes, renal function tests, liver function tests and urine examination for all patients. Consent for lumbar puncture was taken from parents.CSF was collected under strict aseptic precautions and sent for cytology, biochemical analysis for sugar and protein estimation, CSF gram stain by conventional and cytospincytocentrifuge technique, latex agglutination for capsular organisms, CSF PCR and culture.

CSF culture (0.5 ml) to detect bacterial growth was done by Bact Alert 3D automation. Positive growth was confirmed by bacteria demonstration by subculture into Chocolate agar, blood agar and Mac conkeys agar. Latex agglutination was performed using Wellcogen bacterial antigen kit for detection of specific antigens of certain organisms. CSF culture was taken as gold standard for diagnosis of ABM. CSF PCR was done by commercially available kits.

Literature Review

Cytospin cyto centrifuge

Cyto-tek (cytospin) centrifuge, SakuraFinetek, U.S.A.inc,Torrance, CA 90504 is a self-contained instrument engineered to transfer cells from suspension on to a glass slide through the process of cytocentrifugation. The cytotek centrifuge has 12 specimen holder capacity and cell dispersion on the glass slide in a specific, square area. All the cells from the specimen were deposited evenly on the glass slide, therefore screening of the slide is simplified. 1 ml of CSF was transferred in autoclavable screw capped 15 ml centrifugation plastic tubes and equal volumes of 5% sodium hypochlorite was added within the biosafety class II cabinet. The tube caps were tightly closed and shaken for 1 min on a vortex mixer. The mixture was allowed to stand for 5 minutes to react with the decontaminating agent and then 1 ml of the mixture was dispensed each into two assembled cytotek chambers (1 ml disposable units) for preparation of duplicate smears. All samples were centrifuged in the cytotek centrifuge (cytospin) at 2500 rpm for 10 minutes as specified by the manufacturer [5]. After centrifugation, the slides were carefully removed from the cytotek chamber, air dried for 2 minutes and heat fixed for 1minute by passing through flame. The smears were stained by grams staining method. The patients were treated as per standard protocol.

Data analysis

Analysis was carried out by using SPSS software version 16.0. Accrement analysis with kappa coefficient will be done to evaluate without gold standard. If we have to analyze the test with respect to the gold standard then, standard tests like sensitivity, specificity, positive predictive value, negative value and accuracy was used.

Results

Out of 9216 admissions in pediatric department of the hospital, 101 were identified as ABM which was 1.09% of cases. The included children were divided into three groups for analysis, <1year, 1-5years, >5years. The majority number of cases were in the age group <1year (41.6%). 32.7% were in the age group 1-5 years and 25.7% in the age group >5years. There was no significant difference between the three age groups [6]. Sex ratio in the 3 groups too was comparable. Analysis of presenting complaints showed that fever (<7days) was a universal complaint among all the age groups, 84.2% of all the cases. Refusal to feed, headache, altered sensorium, vomiting, blurring of vision were significantly associated with bacterial meningitis ($p<0.05$). However, certain symptoms like altered sensorium, vomiting, blurring of vision and headache was more apparent symptoms in older children (>5 years) when compared to infants. Symptoms like focal deficits and blurring of vision was not seen in children in the age groups 1-5 years. Listlessness/ malaise/feed refusal, seizures and altered sensorium were commonly seen in younger children (<5years) with meningitis. The risk factors that were predisposed to meningitis- among these age less than 5 years, overcrowding, low socioeconomic status and exposure to smoke at home were most prominent.

On examination, most of the children had a normal nutritional status (75.2%). Severe malnutrition (grade 3 and 4) was reported in 10/101 (10%). Although age specific distribution of weight for age shows no statistically significant difference. When the three groups were compared, they did not differ with regards to physical signs of malnutrition (vitamin deficiencies), change in level of consciousness and motor system changes (muscle strength, deep tendon reflexes and muscle tone). Hypertension, abnormal fundus and cranial nerve palsies were predominantly seen in children older than 5 years. Neck stiffness and neck signs were significantly higher in older children (80.6% and 65%). Sensory deficits were not seen in any of the cases. 8 children (7.9%) died within 48 hours, 6 were in the age group<1 year, one each other two groups. 2 cases each in the age group <1year and>5years developed intracranial complications (both had hearing abnormalities). Rest all had a normal recovery.

Of all the meningitis cases, 36/101(35.6%) cases showed CSF culture positivity byBact Alert. In 32/36 (88%) *Streptococcus pneumoniae* was isolated, one each case of *E. coli, meningococcus, Staphylococcus aureus* and *alpha hemolytic*

streptococcus was isolated. *H.influenzae* was not isolated in any of the cases. Out of all the cases, PCR was positive in 39/101 (38.6%). Latex agglutination test was positive in 39/101(38.6%) of cases. Conventional gram stain was positive in only 7/101(6.9%) cases. Cytospin gram stain was positive in most number of 56/101(55.4%) cases. Each of these tests was compared to each other and to the gold standard (CSF culture).

Comparing with the gold standard, Cytospin gram stain showed a sensitivity of 65.65% which was significantly higher than conventional gram stain (sensitivity-11.1%).The sensitivity, specificity for each of the tests. Comparing cytospin gram stain with latex agglutination, there is 61.4% concordance in the two tests in the diagnosis of bacterial meningitis and 38.6% discordance in the two tests and the kappa value is significant (0.009). When cytospin gram stain was compared to PCR there was 53.5% concordance between the two tests and 46.6% discordance between the two tests. Here too kappa value was significant (0.082).

Discussion

Acute bacterial meningitis is a medical emergency, which warrants early diagnosis and aggressive therapy. The choice of empirical antimicrobial therapy for bacterial meningitis is based on the most common pathogen prevalent in a particular geographic area and age group and its antibiotic sensitivity pattern.

The incidence of ABM reported in our study was 1.09%. Most children who suffered from meningitis were<5years i.e 75% of the total cases, when compared to older children (5-12 years). 42.5% of their ABM were infants. Sex distribution of cases did not show any significant difference. However 70% meningitis cases in males which could be due to referral bias.

Short history of fever (<7days) was the universal presenting symptom among all the age groups. But presence of fever is not sufficient to diagnose bacterial meningitis since it is a very nonspecific symptom. Refusal to feed, seizure and vomiting were the majority of constellation symptoms seen in the<1-year age group. Whereas, headache was most commonly seen in>5year age group (76.9%). Altered sensorium is more common in the 1-5years (57.6%) and>5years (69.2%) age group. This is in contrast by the study bychinchankar who reported altered sensorium in majority of their cases (98%). This could possibly be due to majority of children being infants in our study. Seizures were more commonly seen in children up to 5 years of age. But one must remember that children in this age group can present with seizures due to other illness like febrile seizures, cerebral malaria, hypocalcemia etc. Focal neurological deficit did not have any statistical association with any age group and usually signified some intracranial complication a similar number of patients reported with fever (35%-97%) and seizure (53%-94%) meningitis. Complaints like refusal to feed, headache, altered sensorium, vomiting, blurring of vision and focal neurological deficit were found to be statistically significant in the diagnosis of meningitis in children in their study.

Signs of vitamin deficiency, hypertension due to raised intracranial tension were seen in minority of cases. Motor and sensory system abnormalities were not seen in any cases.

Cranial nerve palsies were seen in 23.1% and fundus abnormalities in 11.5% casesall of whom were above 5 years age reported in 16.8% cases.Neck signs were mostly seen in the age group 1-5years (45.5%) and>5years (80.8%). Meningeal signs were elicited in 26% cases but they were all below 5years which contrasts our study reported neck pain to be present in 15% cases. This variability could be due to heterogeneity of our cases and difference in reporting our cases. Alteration in mental status was seen in 34.6% cases which is in contrast reported in 98% cases. This could be due to difference in assessment of both the studies (AVPU scale vs GCS).

About 35.6% of the CSF cultures were positive. Most of the cases were isolated from the age group 1-5years (45.5%). CSF culture positivity rates have been variable reported 47.5% positivity, isolated the causative organism in 50% cases. Some changing trends in etiological agents for community acquired acute bacterial meningitis have been reported worldwide in recent times. The most common organism isolated in our study was *Streptococcus.*

We performed many other tests on CSF like latex agglutination, PCR, gram stain with and without cytospin. PCR was found to have a very high sensitivity and specificity in concordance with other studies but the main limitation for its use is its limited availability and high cost. The PCR test has the ability to pick up cases which were not positive by conventional techniques like culture and gram stain.

Latex agglutination has an advantage of being a rapid diagnostictest, easy to perform and can test for various microorganisms at a time but has a poor sensitivity and specificity compared to PCR. In contrast to our results found 2/3rd of their patients were positive for latex agglutination.Its utility, hence is being questioned in various published studies which could be due to changes in the epidemiology related to the use of vaccines for capsulated organism.

Gram stain has a poor sensitivity but good specificity for bacterial meningitis and being a rapid, accurate bedside test is still used. The yield of bacteria on gram stain depends on several factors like the number of organism's present, prior use of antibiotics, technique used for smear preparation, staining technique and observer's skill and experience.

When gram stain was done with cytospin (cyto-tek cytocentrifuge), the positivity improved to 55.4%, they recorded a high yield on gram stain with cytospin use. A cytospin also provides several other diagnostic benefits like good preservation of morphology of cells and bacteria and increased rate of detection of bacterial pathogens especially in partially treated pyogenic meningitis, which can mimic tuberculous meningitis posing a diagnostic dilemma for clinicians. Comparing cytospin gram stain with PCR, there was 53.5%concordance between the two tests and 44.6% discordance. The cytospin centrifuge provides a simple, rapid and effective means of concentrating CSF for gram stain reported cytospin centrifuge to be effective in detecting microorganisms and in increasing the number of

bacteria found per smear as compared to unconcentrated smears. So, it is a useful supplement to conventional gram stain. Even 0.5ml or less of specimen is enough to do the test and aids in early diagnosis of bacterial meningitis.

Conclusion

Children presenting with complaints of fever, refusal to feed, altered were seen in younger children whereas older children had fever, neck rigidity and cranial nerve palsies. Younger age, low socioeconomic status, overcrowding and smoking exposure were the most important risk factors. The most common etiological agent isolated was *Streptococcus pneumoniae*. CSF culture was positive in only 36.5%. CSF PCR performed best amongst the tests studied for diagnosis of bacterial meningitis. But, cytospin gram stain was found to have a high sensitivity and specificity compared to conventional techniques. It is a viable alternative in low income countries and aids in the early diagnosis of bacterial meningitis.

Availability of data and materials

The data published in this study is original to the author and the raw data is available with the first author as he is the primary author of this study.

Author contribution

All the authors have equally contributed to the manuscript.

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