

Original Article

Rapid Test for Diagnosis of Bacterial Meningitis and Short Term Outcome in Children Under Five Years in Omdurman Hospital, Sudan

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ABSTRACT

In the Sudan, acute bacterial meningitis (ABM) remains a major threat for children under five. The objectives of this study were to determine the causative agents of acute bacterial meningitis by using rapid diagnostic techniques and study the short term outcome. During a six-month period, a total of 117 patients clinically diagnosed as ABM in Omdurman Children's Hospital, were investigated using latex agglutination test (serology) and polymerase chain reaction (PCR). The cerebrospinal fluid tested using serology was positive for ABM in 50 (42.73%) cases of whom 22 (44.0%), 18 (36.0%) and 10 (20.0%) cases were due to *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* (MCM) and *Streptococcus pneumoniae* (SPM), respectively. The PCR showed 58 (54.7%) positive cases of which MCM, Hib and SPM comprised 37 (63.79%), 11 (10.37%) and 10 (9.43%) cases, respectively. The PCR test proved to be a better diagnostic tool particularly for MCM ($p < 0.001$). In Toto, the study showed that the main causative agents of ABM in children under five were *Neisseria meningitidis* in 51% ($n=55$), *Haemophilus influenzae* type b in 31% ($n=33$) and *Streptococcus pneumoniae* in 18% ($n=20$). Ten of the patients

developed neurological complications and three cases died giving a case fatality rate of 2.8%. The need for rapid diagnostic tests to diagnose ABM in order to lower under-5 morbidity and mortality cannot be overemphasized.

Key Words: *Haemophilus influenzae* type b, meningitis, *Neisseria meningitidis*, *Streptococcus pneumoniae meningitis*, Sudan.

Introduction

Acute bacterial meningitis (ABM) is one of life threatening conditions affecting children and adolescents. Delay in prompt diagnosis and treatment could lead to permanent central nervous system (CNS) impairment. (1) Acute bacterial meningitis (ABM) remains a major cause of child morbidity and mortality in Africa. (2) Determination of the causative agents in ABM, leads to a better understanding of the pathogenesis, complications and treatment. (3)

Acute bacterial meningitis is a worldwide infection more prevalent in the developing countries where the incidence is in the range of 5 – 10/105/populations. (4) The most common organisms above one month are *Haemophilus influenzae* (HI), *Neisseria meningitidis* (MCM) and *Streptococcus pneumoniae* (SPM). (4) ABM has high incidence

in South Africa 76105/, which more or less like incidence in Middle East and in developed countries before era of vaccination.(4) In Kingdom of Saudi Arabia (KSA) the leading causes of Acute bacterial meningitis (ABM) are Hib, MCM, and SPM with incidence of 28%, 18% and 11% respectively.(5) After introduction of Hib vaccine in 1980, mortality started to decrease in developed countries because vaccine is widely used.(5) In Sudan the statistical records showed that Hib is the main cause of ABM in children under five in non-epidemic season.(6)

The introduction of Hib vaccine in routine immunization of children has lead to tremendous reduction in Hib meningitis and Hib related diseases in developing countries.(7) It is therefore mandatory for the policy makers and health care providers to have epidemiological data on ABM(8) The investigations of ABM in countries where the resources are limited is a real problem, particularly if indiscriminate use of antibiotics is prevalent. (9) Investigations of cerebrospinal fluid (CSF) for chemistry, cytology, culture, serology and (PCR) is well recognized.(10) PCR is useful test to detect organisms of ABM.(11) but it is has high sensitivity, specific for different type of serogroups of MCM. (12) PCR is costly, and still is not a substitute for CSF culture.(13) In Sudan, classical investigations on CSF such as culture, chemistry, cytology and gram stain are not helpful in most cases, because of indiscriminate misuse of antibiotics, that changes clinical picture and confuses the diagnosis. It is therefore essential to consider alternative tools for diagnosis of ABM. The objectives of this study were: to (a) use rapid diagnostic tools such as latex agglutination (serology) and PCR to determine the causative agents of ABM children under-5 years and (b) study the short term outcome of ABM after three months follow up.

Methods

The study was conducted in Omdurman Teaching Hospital (OTH) on a three-day-per-week basis. All

children admitted with clinical diagnosis of ABM were included. The case definition of ABM is acute onset of fever, refusal of feeding, vomiting, convulsions, bulging fontanelle and neck stiffness plus signs of meningeal irritation i.e. positive Kerning's sign, Brudzinski sign and/or tripod sign.(14,15) Children who were comatose, critically ill or whose guardians did not give consent were excluded. A pre-coded questionnaire on medical, social and family history was completed after obtaining informed verbal consent. Thorough clinical examination for each child was then conducted. Cerebrospinal fluid (CSF) was withdrawn for purpose of Latex agglutination (serology) and PCR looking for bacterial antigen according to manufacture instructions as follow:

By heating CSF at 100C for 5 minutes, centrifuged at 2000 rpm for 10 minutes, then test was performed on the supernatant. For suspected bacterial meningitis PCR in the CSF samples is usually done at National Public Health Laboratory. Approximately 250µl of each CSF sample was inoculated on sheep blood agar and chocolate agar (Oxoid, U.K) and incubated at 36°C in room air supplemented with 5% CO₂ for 72 h. Identification of organisms isolated from CSF was based on typical clonial morphology, oxidase reaction and serotyping.

A minimum of 50 µl of CSF of microscopy negative samples and 10 µl of microscopy-positive samples were used for extraction of DNA. The DNA was prepared using the QIAamp mini kit (QIAGEN, Hilden, Germany) according to the instructions of the manufacturer. Total DNA was eluted in 50 µl of AE buffer (QIAGEN) and stored at -20°C until amplification by the PCR machine. The amplification mixture of the N. meningitides PCR consisted of 21 of 10µl reaction mix (LightCycler Fast-Start master DNA, SYBR green; Roche Diagnostics, Germany), 4 mM MgCl₂, 0.5 µM concentrations of each primer, and 2 µl of template DNA in a final volume of 20 µl. Samples were amplified as follows: an initial denaturation step at 95°C for 10 min and

40 cycles of denaturation at 95°C for 0 s, annealing at 54°C for 10 s, and elongation at 72°C for 20 s. The temperature transition rate was 20°C/s. The Mx3000P QPCR System (STRATAGENE, USA) was used.

Simple tabulation of the data was performed using Einfo V6b software and the SPSS program was used for analysis with X² of 0.05 level of significance.

Results

During the six-month study period, a total of 117 cases were clinically diagnosed as acute bacterial meningitis. The majority of the patients received some sort of treatment – namely antibiotics before reporting to hospital. There were eight cases excluded from the analysis because they had negative CSF results. Almost half of the cases (n= 53, 49%) were under one year of age with males (54%) slightly more affected than females. The majority of the parents (73% of mothers and fathers) were illiterate or had primary education and lived in the outskirts of Omdurman (Table 1).

Table 1: Patients' characteristics

Criteria	<i>Niesseria meningitides</i>	<i>Haemophilus influenzae</i>	<i>Streptococcal pneumoniae meningitis</i>
Age (months):			
2 - 11	28	17	8
12 - 23	8	10	3
23 - 35	8	2	-
36 - 47	5	1	5
48 - 59	6	3	4
Total	55	33	20
Sex:			
Male	27	19	11
Female	28	14	9
Total	55	33	20
Mother's education (school years):			
0-4	18	17	7
5-8	18	12	7
9-12	19	4	6
>12	-	=	=
Total	55	33	20
Father's education (school years):			
0-4	16	13	7
5-8	22	12	9
9-12	17	6	4
>12	=	2	=
Total	55	33	20

All samples of CSF fluid taken from the patients looked turbid and were under tension. They were all tested using latex agglutination. Latex was positive in 50 (42.7%) cases, out of which 22 (44%), 18 (36%) and 10 (20%) cases were due to Hib, MCM and SPM, respectively. There was no significant difference in the sero-positivity between the three groups (p<0.73) as shown in Table 2.

Table 2: Number of cases in each type of meningitis according to latex agglutination and PCR

Lab test Organism	Latex		PCR		P. value
	Positive	Negative	Positive	Negative	
<i>Niesseria meningitides</i> (NM)	18	99	37	69	0.001
<i>Haemophilus influenzae</i> (Hib)	22	95	11	95	0.13
<i>Streptococcal pneumoniae meningitis</i> (SPM)	10	107	10	96	0.0997
Total	50	67	58	56	
P. value	0.073		0.001		

Out of 106 CSF samples tested using polymerase chain reaction (PCR), 58 (54.74%) were positive for bacterial meningitis; of which 37 (50.92%), 11 (10.37%) and 10 (9.43%) were due to MCM, Hib and SPM respectively. The difference in positivity between the groups is highly significant (p<0.001). Comparing serology to PCR, the latter seemed to be a better diagnostic tool in particular for MCM (p<0.001) (Table 2).

Table 3: Outcome of cases of acute bacterial meningitis

Outcome	n	%
Recovery	95	88.0
CP	1	0.9
Hydrocephalus	1	0.9
Defenses	2	1.8
Epilepsy	2	1.8
Haemiplegia	4	3.7
Death	3	2.8
Total	108	99.9

There were 19 cases with positive CSF result for both latex and PCR. These were 9 (7.7%) cases due to MCM and 5 (4.3%) due to each of Hib and SPM.

All cases of MCM were of the C sero-type, while

all cases of Hib were of the b sero-type.

Full recovery was seen in 95(88%) cases, 10 developed neurological deficits and 3 cases died: two due to Hib and one due to meningococcal meningitis (Table 3).

Discussion

The main aims of this study were to determine the common agents that causes of ABM using rapid tests and study complications of ABM in children under 5. In Sudan, the burden of ABM particularly that due to Hib and SPM is not well-defined.

The study showed that the leading causes of acute bacterial meningitis in under five children in Sudan were *Neisseria meningitidis* that constituted over half of the cases and *Haemophilus influenzae* type b which was responsible for nearly one third, while *Streptococcus pneumoniae* was diagnosed in 18.5% of the cases. This is in agreement with study done in Sudan few years ago by Esa, et al.,(16) where by using PCR for CSF, he found MCM in 27 (87.0%) cases. This make PCR is a sharp tool for diagnosis of ABM in country where indiscriminate medications with antibiotics were common.(16) Our findings were also consistent with study done in Niamey (Niger), where MCM was the most common cause of ABM.(17)

In this study the use of latex agglutination test showed that *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae* constituted 44.0%, 18.8% and 10.0% of cases respectively

These findings are in agreement with the study done in Kingdom of Saudi Arabia (KSA), some Asian countries as well as USA during the pre-vaccination era when Hib was the most common organism causing ABM in children less than one year.(5) This pattern of bacterial etiology is also reported in a study from Pittsburgh Hospital.(18)The etiology of acute bacterial meningitis in India, and previously in Sudan is similar to this study, where Hib, MCM and SPM were the main etiology.(6,19,20)

On the other hand the aetiology for acute bacterial meningitis by using polymerase chain reaction (PCR) showed that 37 (63.79%), 11 (18.96%) and 10 (17.24%)

cases were due to MCM, Hib and SPM, respectively.

This showed that PCR is useful, sensitive and lead to rapid detection of MCM which was in agreement with study done in China and Brazil.(12,21,22) PCR as a diagnostic tool for Hib revealed only 18.96% of cases, in comparison to CSF culture which revealed Hib in 31.25%, this differ from other studies where PCR is better than culture particularly when antibiotic already taken.(23)

It is very obvious, PCR is readily available, considered rapid test and usually considered by many authorities as first line for CSF investigation.(23)

The outcome in this study as follow: 95(85.4%) cases had full recovery, hemiplegia in 4(3.4%) cases, deafness in 2 (1.7%) cases, hydrocephalus in one (0.7%) case, epilepsy in two (1.7%) cases, cerebral palsy in one (0.9%) case and death in 5 (4.27%). The findings in comparison to meta analysis from all reports in English language from publications after 1995, which showed no sequale in 73.9%, bilateral defenses in 10.5%, Paresis in 3.5% and epilepsy in 4.2%.(24) The neurological sequale also was lower than that previously observed in Sudan by Salih following meningitis.(25) The overall case fatality rate was 6% (n =3), the case fatality for Hib, MCM was 9.09% and 5.55% respectively which was less than case fatality rate in Sudan previously, due to increase awareness and availability of health care facilities.(25)

The presentation of fever, convulsion and neck rigidity in agreement with previous studies in Sudan. (21)

Conclusion

In developing countries where indiscriminate use of antibiotics for febrile illnesses is prevalent, the classical investigations for CSF may not be helpful. Because of seriousness of acute bacterial meningitis and the urgency to treat, rapid diagnostic tools in particular PCR is highly recommended.

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