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Diagnostic significance of fibrin(-ogen) degradation products in cerebrospinal fluid in childhood meningitis

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Fibrin(-ogen) degradation products (FDP) was determined in cerebrospinal fluid (CSF) of 106 children suffering from meningitis (35 tuberculous, 37 pyogenic, and 34 non-bacterial meningitis) by using a locally developed test kit (DMR-FDP test kit). Generally diagnosis of different meningitis is made clinically, supported by routine examination of CSF (CSF-RE). The sensitivity of the test kit is 2 μg/ml fibrinogen equivalent. FDP content is determined semi-quantitatively by the doubling dilution of CSF and described as 0, 2, 4, 8, 16, 32, 64, 128, 256, and 512 μg/ml. Overall FDP content in CSF was ranging from 0-512 μg/ml. It varied with the type of meningitis: 0-8 μg/ml in non-bacterial meningitis, and ≥16 μg/ml in bacterial meningitis. CSF-FDP content was always ≤8 μg/ml in all of non-bacterial meningitis cases (34/34; 100%). In 35 TBM cases, 34 cases have 16-64 μg/ml, 1 case 128 μg/ml. In 37 pyogenic meningitis cases, 10 have 64 μg/ml and 27 have ≥128 μg/ml. Statistically, CSF-FDP level of 0-8 μg/ml has 100% sensitivity for non-bacterial meningitis; 16-64 μg/ml has 97.1% sensitivity for TB meningitis; and ≥128 μg/ml has 72.9% sensitivity for pyogenic meningitis. This study could define a cut-off point: CSF-FDP content, >8 μg/ml for bacterial meningitis and ≤8 μg/ml for non-bacterial meningitis and could also differentiate a meningitis case into different types: (i) nonbacterial meningitis when CSF-FDP ≤8 μg/ml and bacterial when ≥16μg/ml, (ii) tuberculous when 16-64 μg/ml, and (iii) pyogenic meningitis when ≥128 μg/ml. In conclusion, determination of FDP content in CSF by DMR-FDP test kit greatly facilitates the differential diagnoses of meningitis in children and it will be of great benefit to the clinicians, particularly at the health centers where and/or when laboratory facilities for CSF-RE are inefficient. The CSF-FDP should be measured routinely in children with meningitis and is suggested to be included in CSF-RE as an additional biochemical parameter to other conventional tests.

INTRODUCTION

Routine examination of cerebrospinal fluid (CSF-RE) is a common laboratory procedure in day-to-day clinical practice. It is particularly useful in meningitis for the etiological diagnosis as bacterial (pyogenic or tuberculous) and nonbacterial (viral, fungal, others) to be followed by an appropriate and prompt treatment. Provisional diagnosis of meningitis is made clinically and etiologic diagnosis follows after CSF-RE. Etiological confirmation by microbiological, immunological and DNA techniques is done only when importantly indicated in selected cases for various constraints.

The presence of FDP in CSF has been reported in meningococcemia, fulminant pyogenic and viral meningitis, sub-arachnoid hemorrhage and intraventricular hemorrhage [1, 2, 3, 4, 5]. In these studies the emphases were made only from the aspects of the clinical severity and associated DIC being

occurred in these cases. Reports on CSF-FDP in relation to CSF-RE findings are very few and it was never been described from diagnostic aspect in meningitis worldwide. Moreover, CSF-FDP has never been studied in tuberculous meningitis. The reasons for such knowledge gaps are many but scarcity of cases available and expensiveness of FDP determination are commonly and importantly included.

We have been previously reported the presence of FDP in CSF in association with CSF-RE findings [6]. That report highlighted that abnormality in CSF-RE (such as increased protein content, decreased sugar and chloride content and increased cell counts) is associated with presence of FDP in CSF. We have also suggested CSF-FDP to be used as an alternative approach when CSF-RE is not feasible or available for any reasons. Based on the previous findings the present study is conducted with the following main objectives: (i) To determine FDP content in CSF from children with meningitis either pyogenic, tuberculous or others (non-bacterial meningitis such as viral, fungal, parasitic) diagnosed clinically and supported by CSF-RE findings; and (ii) To evaluate the role of CSF-FDP determination in the diagnosis of different forms of meningitis.

MATERIALS AND METHODS

Study design

A cross-sectional, analytical, hospital- based, collaborative study was conducted between Pathology Research Division, DMR (Lower Myanmar), and Medical Units and Clinical Pathology Laboratory of Yangon Children Hospital (YCH).

Methodology

One-hundred and six children admitted for signs and symptoms of meningitis were entered into the study in one year study period. CSF-RE was indicated and lumbar puncture was done as routines after getting an informed consent. One millilitre of venous blood was also collected in a clean test tube and separated for serum.

Preparation of DMR-FDP test kit

DMR-FDP test kit was prepared as described previously at the Pathology Research Division, DMR (Lower Myanmar) [7].

In brief:

Coagulase positive strain *Staphylococcus aureus* is subcultured on tryptic soy agar overnight. Bacterial colonies are collected from the plate by rinsing with normal saline in a large test tube. The bacterial suspension is washed twice with saline and thrice with distilled water. Then it is deep-freezed and dried by lyophilization to make powdered form. Each test kit contains 10 mg bacterial powder (clumping factor) which is reconstituted with one ml of distilled water. The sensitivity of the test kit is 2 μg/ml of FDP.

Routine examination of CSF (CSF-RE)

CSF-RE was done conventionally [8]. It contains tension at the time of tapping, colour or appearance visually, visual formation of coagulum on overnight standing, total and differential white cell count by special counting chamber using white cell pipette, biochemical tests for sugars (copper reduction method), proteins (turbidimetric method) and chloride content (colourimetric method).

Determination of total FDP in CSF

FDP was determined in the CSF as described previously [6, 9]. Briefly: A drop of CSF is placed on a black tile (or glass slide) and mixed thoroughly with a drop of suspension from the test kit. Clumping reaction was observed in a few seconds in positive samples indicating presence of FDP >2 μg/ml (i.e., sensitivity of the test).

Doubling dilution was done in positive samples into 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and so on by using distilled water or normal saline. The highest dilution which shows clumping was taken and it was multiplied by 2 μg/ml. The result is the FDP

content of CSF.E.g., When the highest dilution with clumping reaction is 1:16, the FDP content is 16x2 = 32 µg / ml.

Determination of total FDP in serum

FDP in serum was determined similarly as described above [10, 11, 12, 13].

Diagnosis of meningitis

The clinical diagnosis of meningitis was made by thorough history taking and physical examination. The aetiological diagnosis was based on the CSF-RE findings and CxR evidences.

(a) Pyogenic meningitis:

- Straw colour / turbid in appearance
- Total WBC count is greatly increased (50 - numerous / cu.mm)
- All polymorph or polymorph predominance in differential count
- Total protein content is greatly increased (100 - >200 mg%)
- Sugar content is greatly reduced (<50% to absent)
- Chloride content reduced (about 120 mg% or less than 100 mg%)
- A septic foci may or may not be present

(b) Tuberculous meningitis

- Clear colourless / opalescent in appearance
- Total WBC count increased (50-500 / cu. mm)
- Lymphocyte predominance and some polymorphs in differential count
- Total protein content is moderately increased (100->200 mg%)
- Sugar content reduced (<50% to absent)
- Chloride content reduced (about 120 mg% or less than 100 mg%)
- CxR finding may or may not be positive for tuberculosis
- Tuberculin test may or may not be positive
- History of TB contact present or not

(c) Viral and other meningitis

- Clear and colourless in appearance
- Total WBC count increased (about 5 per cu.mm)
- Lymphocyte predominance in differential count
- Total protein content slightly increased (about 10 mg%)
- Sugar content normal (>50%)
- Chloride content normal (120+ mg%)

RESULTS

Clinical and CSF-RE findings have established the etiological diagnosis in a total of 106 children as follows:

(i) 37 pyogenic
(ii) 35 tuberculous
(iii) 34 non-bacterial

CSF-FDP was detected in 97 cases, not in 9 cases. It ranged from 4–512 µg /ml and distributed as follows:

FDP content	Total cases	Pyo-genic	Tuber-culous	Non-bacterial
0 µg/ml	9	0	0	**9**
4µg/ml	7	0	0	7
8 µg/ml	18	0	0	**18**
16 µg/ml	20	2	**18**	0
32 µg/ml	6	2	**4**	0
64 µg/ml	18	6	**12**	0
128 µg/ml	17	**16**	1	0
256 µg/ml	10	**10**	0	0
512 µg/ml	1	**1**	0	0
Total	106	37	35	34

i CSF-FDP ≤ 8 µg/ml was found only in nonbacterial meningitis cases (n=34)

ii CSF-FDP 16-64 µg/ml was found only in bacterial meningitis cases (n=44) 34 tuberculous; 10 pyogenic;

iii CSF-FDP 128-512 µg/ml was found only in bacterial meningitis cases (n=28) 27 pyogenic; 1 tuberculous;

Table 1. CSF-FDP content in different meningitis

Meningitis	FDP content (μg/ml)									
	Total	0	4	8	16	32	64	128	256	512
Pyogenic	37	0	0	0	2	2	6	**16**	**10**	**1**
Tuberculous	35	0	0	0	**18**	**4**	**12**	1	0	0
Nonbacterial	34	**9**	**7**	**18**	0	0	0	0	0	0
Total	106	**9**	**7**	**18**	**20**	**6**	**18**	**17**	**10**	**1**

(1) All of 34 nonbacterial meningitis cases have CSF-FDP ≤ 8 μg/ml.

(2) 34 of 35 tuberculous meningitis cases have CSF-FDP 16-64 μg/ml; one case has 128 μg/ml.

(3) 27 of 37pyogenic meningitis cases have CSF-FDP≥ 128 μg/ml; ten cases have 16-64 μg/ml.

Table 2. Serum FDP in different meningitis

Meningitis	FDP content (μg/ml)									
	0	2	4	8	16	32	64	128	256	512
Pyogenic	4	5	9	6	6	4	1	1	1	0
Tuberculous	6	2	5	9	4	4	2	1	2	0
Nonbacterial	9	5	15	2	2	1	0	0	0	0
Total	**19**	**12**	**29**	**17**	**12**	**9**	**3**	**2**	**3**	0

Table 3. CSF-FDP vs serum FDP in different meningitis

Meningitis		Serum FDP								
		0-8 (μg/ml)			16-64 (μg/ml)			128+ (μg/ml)		
		P	T	NB	P	T	NB	P	T	NB
CSF FDP 0-8 (μg/ml)	P	0			0	0		0		
	T		0						0	
	NB			31			3			0
CSF FDP 16-64 (μg/ml)	P	9			1					
	T		17		10	7			0	
	NB			0			0			0
CSF FDP 128+ (μg/ml)	P	15	5		0	3		2	2	
	T								1	
	NB			0			0			0
Total		24	22	31	11	10	3	2	3	0

CSF-FDP and serum FDP level has no significant correlation (p>.7631)

P = Pyogenic meningitis

T = Tuberculous meningitis

NB = Non-bacterial meningitis

77/106 cases (72.6%) have serum FDP within normal range (2-10 μg/ml); 16-64 μg/ml was seen in 24/106 cases (22.6%); 5/106 cases (4.8%) have serum FDP 128-256 μg/ml. 32 μg/ml is maximum for non-bacterial meningitis (Table2).

Summary of the findings

1. CSF-FDP is significantly correlated with CSF-appearance, -WBC count, and –protein.
2. CSF-FDP 8 μg/ml is the cut-off point for bacterial meningitis either pyogenic or tuberculous.
3. CSF-FDP <8 μg/ml predicts non-bacterial meningitis.
4. CSF-FDP 16-64 μg/ml predicts tuberculous meningitis.
5. CSF-FDP >128 μg/ml predicts pyogenic meningitis.
6. CSF-FDP 0-8 μg/ml has 100% sensitivity for non-bacterial meningitis; 16-64 μg/ml has 97.1% sensitivity for TB meningitis; >128 μg/ml has 72.9% sensitivity for pyogenic meningitis.
7. Sensitivity of CSF-FDP >128+ μg/ml for pyogenic meningitis will be increased to 98.2% when CSF-appearance is considered together. All cases of pyogenic cases in this group have CSF-appearance opalescent / turbid; no xanthochromic.

DISCUSSION

No study has been described the usefulness of FDP determination in CSF in the diagnosis of different forms of meningitis worldwide. Previous studies reported the presence of small or late fragments of fibrin degradation such as D- and E-fragments, and D-dimers since the emphases were made only from the clinical severity, prognosis and DIC (disseminated intravascular coagulation) aspects; never from the diagnostic emphasis [14, 15, 16, 17, 18].

This study could describe diagnostic implication of determination of total FDP in CSF in different forms of meningitis since DMR-FDP kit used in this study detects all fragments of fibrin degradation (not only late fragments but also early fragments like fragments A, B, X, Y, fibrinopeptides, fibrin monomers, and fibrin polymers) [19, 20, 21].

CSF-RE is a common laboratory practice for various disorders involving nervous system including meningitis. Patients suspected of having meningitis always should have a specimen of CSF (usually by lumbar puncture) and examined in the laboratory as soon as possible. Prompt identification of the causal organism is important because until an exact aetiological diagnosis has been made the proper antimicrobial therapy cannot be prescribed. A small range of biochemical tests is usually undertaken for CSF-RE. Hence, biochemical investigation of the CSF is usually less important diagnostically than simple inspection for appearance (colour, turbidity, spontaneous clotting or coagulum) and cytological examination (red cells, white cells, others) [22]. Only when appropriate and indicated, microbiological investigations and serological tests for syphilis are carried out.

Although CSF-RE is a common, easy, important and useful laboratory procedure, it has some laboratory and diagnostic pitfalls and disadvantages such as:

(A) The specimen must be dispatched to the laboratory at once; delay may result in the death of delicate pathogen such as meningococci, disintegration of leucocytes and the reduction in the CSF-sugar. Specimen should not be kept in the refrigerator, which kills *H. influenzae.*

(B) The presence of blood is the main cause of an abnormal colour. Normally no red blood cells should be present. Some may be introduced as a result of trauma whilst obtaining the fluid. Xanthochromia (yellow colour) may be due to altered hemoglobin several days after a subarachnoid hemorrhage, large amount of pus, a very high protein content and jaundice. Small numbers of red cells also give fluids an opalescent appearance. If traces of substances such as alcohol are mixed with the fluid during its collection some opalescence may result. Spontaneous clotting (coagulum) occurs when there is an excess of fibrinogen in the specimen, usually associated with a very high protein concentration.

(C) CSF-cell count is done using a wbc count pipette in a special counting chamber. Leishman's stain is used for differential count when cell count is increased. CSF-wbc count may be undetectable in some cases of bacterial meningitis, particularly in children, in immunocompromised patients, and if antibiotics have been given before lumbar puncture.

(D) CSF-sugar is carried out by any of the usual blood sugar methods. If obvious pus is present, being indicated for bacterial meningitis, CSF-sugar content provides little additional information. CSF-sugar determination does not reliably distinguish between different forms of infective meningitis, because the result may be normal in any form. More importantly, CSF which has become contaminated during laboratory sampling may show a fall in CSF-sugar content if kept at room temperature. Streptomycin given intrathecally shortly before CSF sampling may interfere with copper reduction methods.

(E) The CSF-protein is increased in the presence of blood and pus. If either of these is apparent on visual inspection or by microscopical examination of the specimen no further information is provided by

CSF-protein content and the laboratory staff should not be unnecessarily exposed to potentially dangerous infected material. Although turbidimetric method used for CSF-protein is simple and quick, turbidity is affected by temperature, time, presence of red cells and bacteria.

(F) Normal CSF-chloride content is usually affected by plasma concentration. If there is much vomiting, particularly in TBM before specific treatment became available, the plasma chloride falls and the CSF value follows it.

As it has been described elsewhere [6, 7, 9, 11, 12], DMR-FDP test kit has many advantages. It needs neither special skill nor equipment to perform, is simple and easy to interpret, and rapid enough to be accomplished even at the bed-side or in lumbar puncture room, and more importantly, highly economical.

This study has clearly demonstrated that CSF-FDP:

(i) is detected in more than 95% of meningitis cases
(ii) 8 μg/ml is a cut-off point for bacterial and non-bacterial meningitis
(iii) 16-64 μg/ml predicts tuberculous meningitis
(iv) 128 μg/ml and above predict pyogenic meningitis
(v) has overall sensitivity 90%
(vi) has sensitivity 100% for non-bacterial meningitis: 97% for TBM and 73% for pyogenic meningitis
(vii) was formed mainly by local fibrinolysis, not by simple diffusion from the plasma since serum FDP level is within normal range in most of the cases.

Thus CSF-FDP content measured by DMR-FDP test kit strongly and reliably predicts aetiologic diagnosis of meningitis occurring in the children. In conclusion, determination of CSF-FDP is suggested in children with meningitis particularly in health centers where or when laboratory facilities for CSF-RE lack and it should be included as an additional test to other routine biochemical tests of conventional CSF-RE.

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