CEREBROSPINAL FLUID N-ACETYL NEURAMINIC ACID ESTIMATION FOR EARLY DIAGNOSIS AND DIFFERENTIATION OF BACTERIAL MENINGITIS

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ABSTRACT

Cerebrospinal fluid (CSF) analysis for free, bound and total N-Acetyl Neuraminic Acid (NANA) as well as serum NANA was done in 68 patients of bacterial meningitis, of which 37 cases were of pyogenic meningitis and 31 of tuberculous meningitis. Ten patients were included in the control group. The free NANA levels were increased in only pyogenic meningitis, independent of protein levels but the bound form increased with the increase in CSF proteins. The increase of free NANA in CSF of pyogenic meningitis patients was not related to the cell count or sugar content in CSF or to the duration or severity of illness. This finding can be of great help in differentiating cases of pyogenic meningitis, particularly partially treated patients, who may have ambiguous pictures of CSF analysis, from the cases of tuberculous meingitis.

Key words: *N-Acetyl Neuraminic Acid, Sialic-acid*

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Bacterial meningitis, including tuberculous meningitis is the most important cause of neurological disease in childhood. The importance of early lumbar puncture for the examination of cerebrospinal fluid (CSF) in these cases and specially in all children with minimal signs suggestive of meningitis, can not be overemphasized. Nowadays, CSF is frequently modified by antibiotics administered prior to the patient's admission to the hospital. The total cell count is lower, the cell types are modified and organisms may neither be seen nor cultured.

N-Acetylneuraminic acid (NANA) is a constituent of gangliosides and glycoproteins which are known to be present in significant quantity in brain. The metabolism of these compounds is closely associated with the many physiological functions and pathological conditions of the nervous system(1). Interest has been focussed on the NANA content of the easily accessible CSF in the hope that it may prove to be useful in diagnosis of pyogenic meningitis.

Most of the studies done previously have been limited to one or other form of NANA in either serum or CSF alone in one or other neurological disorder(2-7).

The present study was undertaken with the following aims: (*i*) To establish the normal values of serum and free, bound and total CSF NANA in children; (*ii*) To determine alterations in levels of free and bound NANA in CSF; (*iii*) To establish diagnostic significance of different fractions of CSF NANA; (*iv*) To correlate the duration and severity of disease with free and bound NANA in CSF; and (*v*) To correlate the protein, sugar and cell content in CSF and the type of organism causing meningitis with the free and bound NANA in CSF.

Material and Methods

The present study was carried out on 78 patients of bacterial meningitis admitted in the Pediatric Ward of Gandhi Medical College and Associated Kamla Nehru Hospital, Bhopal. Thirty seven patients of pyogenic meningitis and 31 of tubercular meningitis were included in the study group while 10 children not suffering from any organic neurological disorders served as controls.

The diagnosis of bacterial meningitis was made on the basis of clinical history and examination, CSF analysis (color, pressure, proteins, sugar, chloride, cell count and type); Gram staining and culture for pyogenic organisms, and Ziehl Neelsen staining for acid fast bacteria.

The free and total NANA was estimated according to thiobarbituric acid assay of Warren(8). The results were analyzed statistically using the tests of significance.

Results

In pyogenic meningitis, the free, bound and total CSF NANA values (*Table I*) were significantly higher when compared with the controls ($p < 0.001$). The serum NANA was also raised above the control value but the difference was not significant ($p > 0.05$).

In tuberculous meningitis, the bound and total CSF NANA was raised when compared with the controls but the free component was not significantly different ($p > 0.05$) as was the serum value. The serum and CSF NANA values did not vary significantly in various age groups and among both the sexes in normal as well as meningitis patients.

The bound and therefore total CSF NANA was increased both in pyogenic and infrequently in tuberculous meningitis. The bound CSF NANA increased with the increase in total proteins in CSF and this was significant ($p < 0.05$) both in pyogenic and tuberculous meningitis (*Tables II & III*).

No significant difference was found between the CSF NANA values and duration of illness (*Tables IV & V*). In both pyogenic and tuberculous meningitis the values of NANA in CSF and serum in patients who reported in the early phase of the disease did not differ significantly ($p > 0.05$) from those who came later in the illness.

Similarly, there was no correlation of

TABLE I--*CSF Free, Bound and Total and Serum NANA Levels in Control and Meningitis Cases*

Diseases	No. of cases	CSF NANA (μmol/L) Mean + SD			Serum NANA (mg/dl)
		Free	Bound	Total	Mean ± SD
Control	10	19.9± 7.7	28.5± 17.4	48.4±18.3	56.8± 7.4
Pyogenic meningitis	37	177.9±129.5**	259.6±190.5**	437.4±281**	65.0±25.4
Tuberculous	31	23.7± 11.7	83.4± 46.9*	107.1±45*	57.1±13.8

* = Significant ($p < 0.05$) ** = Highly significant ($p < 0.001$)

TABLE II--*Correlation of CSF NANA with Proteins in Pyogenic Meningitis*

CSF proteins (mg/dl)	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
40- 100	5	112.4 ± 67.8	104.8 ± 58.8	217.2 ± 105.8
101- 200	15	169.9 ± 100.7	166.4 ± 56.4*	336.6 ± 118.4
201- 500	14	149.4 ± 84.5	310.2 ± 136.6**	459.6 ± 176.6*
501-1000	3	460.6 ± 154.9*	747.5 ± 12.8**	1208 ± 115.4**

* = Significant ($p < 0.05$) ** = Highly significant ($p < 0.001$)

TABLE III--*Correlation of CSF NANA with Proteins in Tuberculous Meningitis*

CSF proteins (mg/dl)	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
≤ 40	2	28.6 ± 6.9	20.1 ± 6.3	48.7 ± 13.2
41 -100	14	22.3 ± 13.0	81.6 ± 29.4*	103.9 ± 24.9*
101-200	12	25.6 ± 11.1	98.2 ± 60.0	123.8 ± 58.6
201-500	3	17.8 ± 1.2	77.6 ± 26.4	95.4 ± 28.3

* = Significant ($p < 0.05$)

TABLE IV--*Correlation of CSF NANA with Duration of Pyogenic Meningitis*

Days of presentation	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
Same day	4	163.3 ± 94.9	233.1 ± 117.7	396.4 ± 200.7
1 - 3 days	13	185.1 ± 109.8	212.2 ± 86.1	397.6 ± 119.9
4 - 7 days	8	129.5 ± 68.3	167.5 ± 43.2	297.0 ± 88.6
8 - 14 days	6	280.3 ± 213.0	439.3 ± 315.6	719.6 ± 499.6
≥15 days	6	134.6 ± 63.0	214.2 ± 136.1	348.8 ± 173.6

CSF NANA values with the outcome of illness. As shown in *Tables VI & VII* the free, bound or total CSF NANA in patients who improved from the illness did not differ significantly from those who developed sequelae or died due to the disease.

Out of 37 cases of pyogenic meningitis in the study, causative organisms could be demonstrated in 20 (54%) cases. The organisms isolated were pneumococci (6 cases), streptococci (3 cases), *H. influenzae* (4 cases), Proteus (3 cases), *Staph. aureus* (2 cases) and Klebsiella and pseudomonas (1 case each) as shown in *Table VIII*. The highest value of free CSF NANA was seen in *Pseudomonas pyocyaneus* infection.

TABLE V--*Correlation of CSF NANA with Duration of Tuberculosis Meningitis*

Days of presentation	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
<7	1	21.71	13.82	35.53
8-15	8	27.9 ± 14.9	75.1 ± 34.2	103.0 ± 29.6
16-30	17	21.8 ± 11.1	86.2 ± 33.8	108.6 ± 36.2
>30	5	25.3 ± 8.5	100.9 ± 77.7	126.2 ± 71.9

TABLE VI--*Correlation of CSF NANA with the Outcome in Pyogenic Meningitis*

Outcome of illness	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
Improved	24	142.8 ± 83.8	243.5 ± 181.6	386.3 ± 220.4
Improved with sequelae	5	123.9 ± 50.7	163.8 ± 80.1	287.7 ± 95.8
Expired	8	258.1 ± 132.9	300.4 ± 166.9	558.5 ± 262.5

TABLE VII--*Correlation of CSF NANA with outcome in Tuberculosis Meningitis*

Outcome of illness	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
Improved	7	20.9 ± 2.6	76.1 ± 27.8	96.9 ± 27.1
Improved with sequelae	14	28.1 ± 15.1	95.4 ± 59.9	123.5 ± 55.7
Expired	10	20.2 ± 8.6	74.3 ± 35.2	94.5 ± 27.9

Proteus, streptococci and pneumococci also showed high free CSF NANA.

Discussion

The free, bound and total CSF NANA was significantly higher in pyogenic meningitis when compared with the controls ($p<0.001$) whereas in tuberculous meningitis only the bound and total CSF NANA was raised significantly in comparison to controls.

The increase in free NANA levels in CSF of pyogenic meningitis has been reported by most of the authors(2-5,7). It is conceivable that the enzyme neuraminidase elaborated by some of the pyogenic organisms may be responsible for this increase. The free CSF NANA levels were found to be high in pseudomonas, proteus, streptococcal and pneumococcal meningitis. Of the pyognic organisms identified in the present study pneumococcus, streptococcus and pseudomonas pyocyaneus are

TABLE VIII--*Correlation of CSF NANA with Causative Organisms*

Caustive organism	No. of cases	CSF NANA (μ mol/L) Mean ± SD		
		Free	Bound	Total
Streptococci	3	292.8 ± 142	472.7 ± 142.8	765.5 ± 280.6
Pneumococci	6	219.7 ± 72.3	203.8 ± 11.3	423.4 ± 168.0
Staph.aureus	2	61.2 ± 2.0	95.6 ± 5.0	156.8 ± 53.0
Pseudomonas	1	493.5	664.6	1158.1
Klebsiella	1	48.4	351.5	399.8
H. Influenzae	4	46.0 ± 22.8	175.6 ± 10.5	222.5 ± 25.4
Proteus	3	359.3 ± 39.5	172.3 ± 72.2	531.6 ± 11.7
Mycobacterium	9	22.1 ± 12.4	76.2 ± 30.9	117.8 ± 59.5

known to elaborate neuraminidase which splits and terminal sialic acid (or non-reducing) end of various sialoglycoproteins and gangliosides increasing the free CSF NANA(2,9-11). The free CSF NANA levels in Klebsiella, *H. influenzae* and *Staph. aureus* meningitis were not significantly higher than controls. Among these *H. influenzae* has been shown not to elaborate the enzyme neuraminidase(2, 9, 11).

The glycoprotein present in infected CSF may comprise major substrate for neuraminidase. Neurons contiguous with the meninges and bathed in infected CSF may constitute another substrate for neuramidase and a source of free CSF NANA. These cells are rich in NANA bearing glycoproteins and gangliosides(2).

The bound and the total CSF NANA was raised in tuberculous meningitis also but the free component was not different significantly from controls, as was the serum value. Most of the earlier workers too have not found any significant difference between free CSF NANA levels in patients with ubercular meningitis and the controls(3-6).

The bound and therefore total NANA was increased both in pyogenic and infrequently in tuberculous meningitis. This is in accordance with the observations made by some of the earlier workers(2,3,6,7) and may be explained on the basis of increased protein content especially of the sialic acid containing immunoglobulins usually found in the CSF of these cases(2-4). In fact, in our study the bound CSF NANA increased with the increase in total CSF proteins and this was significant ($p<0.05$) both in pyogenic and tuberculous meningitis (*Tables II & III*).

The rise in bound CSF NANA was found not due to gangliosides but is the result of sialoglycoproteins(3). The increased protein values seen in the CSF as well as the reported increase of immunoglobulins in CSF in bacterial meningitis(2) support this interpretation. The fact that sialic acid is bound chiefly to the glycoproteins, *i.e.,* alpha-1 and alpha-2 globulins in CSF explains the increase in bound and consequently in total NANA with the increase in CSF proteins. Similar observations have been made by earlier workers(2-4,7) in cases of pyogenic meningitis. Balasubramanian *et al.*(3) have shown that the sialoglycoproteins of both control and pyogenic CSF are similar with respect to their molecular weight and charge, which can apparently be applied to the proteins in

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tuberculous meningitis also(4).

No significant difference was found between the CSF NANA values and duration of illness, in both pyogenic as well as tuberculous meningitis. In one of the previous studies(7) the CSF sialic acid levels were significantly higher in patients who reported in the early phase of the disease as compared to those who came later in the illness. In our study, however, the levels of free and total CSF NANA in both pyogenic as well as tuberculous meningitis did not differ according to their days of illness thus serving as important tool for diagnosis of the partially treated cases also, who reported later in illness.

The CSF NANA values were also not related with the outcome of the disease. These values did not differ significantly whether the patient recovered from the illness with or without sequelae or died due to it, whereas in a previous study(2) the percentage of free out of total CSF NANA was related to coma and cure. These authors found that fewer patients in the low free CSF NANA range manifested coma than in the high and the cure was more common in the low range groups.

Out of 37 cases of pyogenic meningitis in the present study, causative organisms could be demonstrated in 20 (54%) cases only. This can be explained by the fact that antibiotics administered prior to the patients' admission to the hospital frequently modify the CSF(5).

The free sialic acid in pyogenic and tuberculous meningitis were not much different from those of non-meningitis cases in one of the earlier studies(6), although the total sialic acid was found raised in both types of meningitis. They found no evidence of raised free sialic acid at any stage during follow up, even in cases with persistent presence of pathogen and concluded that from a practical standpoint measurement of free CSF, sialic acid offers little as an aid for diagnosis of pyogenic meningitis and its differentiation from other types of meningitis.

The increase of CSF NANA in pyogenic meningitis was useful for cases reporting in the early phase of illness, thus improving the chances of early diagnosis for management and prevention of complications. The partially treated cases also documented increase in free CSF NANA despite ambiguous CSF picture in some. Determination of free sialic acid levels in CSF will thus be of help in the early diagnosis of and differentiation between pyogenic and tuberculous meningitis. It has, perhaps, a few disadvantages; one is that in meningitis caused by certain pyogenic bacteria which do not elaborate enzyme neuraminidase(2) the high free CSF NANA cannot be expected(2,4). Another disadvantage is that in mixed infection of both pyogenic and tuberculous organisms free CSF NANA will indicate only the pyogenic infection.

It has, however, the advantages that in those cases where the result of CSF culture are negative due to partial treatment, or otherwise, the increase in free CSF NANA persists.

Estimation of NANA in CSF can, therefore, serve as a useful test for diagnosis of doubtful cases of meningitis with ambiguous CSF picture.

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NOTES AND NEWS

NATIONAL PEDIATRIC NEPHROLOGY UPDATE-1991

The IV National Pediatric Nephrology Update under the auspices of Indian Pediatric Nephrology Group of IAP, Department of Pediatric Nephrology, Institute of Child Health and Hospital for Children, Madras; Department of Pediatric Nephrology; Childs Trust Hospital, Madras and Tamil Nadu Chapter of IAP will be held on *24th and 25th August 1991* at Madras. The delegate fees will be Rs. 150/- for bonafide students and Rs. 200/- for others (add Rs. 5/- for outstation cheques).

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