MATERIAL AND METHODS
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The present study was carried out in the Department of Paediatrics, M.L.B. Medical College, Jhansi, in collaboration with the Department of Biochemistry, M.L.B. Medical College, Jhansi, over a period of 11 months from May 1981 to March 1982. Children from birth to 12 years of age admitted in the Paediatric ward, were selected for this study. Cases were grouped as:

A: Controls - children not manifesting any neurological disorder.
B: Cases of tuberculous meningitis.
C: Cases of pyogenic meningitis.
D: Cases of encephalitis.
E: Cases of grand mal epilepsy.

Selection of controls -

Eight children from birth to 12 years, not manifesting any neurological disorders were taken as control for the present study. Children from birth to 12 years of age recovering from upper respiratory infections or bronchopneumonia or primary tuberculosis who had not suffered in the past with convulsions,
meningitis or encephalitis or liver diseases were taken as control subjects.

Selection of cases of tuberculous meningitis -

Fifteen cases of tuberculous meningitis were taken for the present study. The diagnosis of tuberculous meningitis was based on history, clinical findings and CSF examination. In most of the cases X-ray chest was done to find out the evidence of primary focus.

Selection of cases of septic meningitis -

Seven cases of septic meningitis were taken for the present study. Diagnosis of septic meningitis was also based on history, clinical and CSF examinations. Besides biochemical examination of CSF, smear for gram staining and CSF culture and sensitivity was also done in each case.

Selection of cases of encephalitis -

Nine cases of encephalitis were taken for the present study. Diagnosis of encephalitis was based on a short history of fever, generalised convulsions followed by unconsciousness. Clinically these children presented with varying degrees of neurological deficit and some had a fatal outcome. The diagnosis was substantiated by a normal CSF examination in all the cases.
Selection of cases of grand mal epilepsy -

Nine cases of grand mal epilepsy were taken for the present study. The diagnosis of grand mal epilepsy was based on history and clinical findings - i.e. children presenting with generalized convulsions, usually with tonic and clonic phases of the muscular spasms associated with loss of consciousness and usually recovering within 24 hours. In addition, CSF examination, X-ray chest, X-ray skull and montoux test were also done in a few cases to rule out secondary or organic epilepsy, whenever indicated.

Children suffering from tuberculous or pyogenic meningitis, or encephalitis or grand mal epilepsy were treated as follows :-

a) **Tuberculous meningitis** - The cases of tuberculous meningitis were treated with anti-tubercular treatment and steroid. Intravenous fluids, anticonvulsants and drugs to reduce raised intra-cranial tension such as glycerol and mannitol were used as and when required.

b) **Septic meningitis** - The cases of septic meningitis were treated with broad spectrum antibiotics and later specific antibiotics were started after culture and sensitivity. In addition, other supportive treatment was given as in tuberculous meningitis.
c) Encephalitis - The cases of encephalitis were treated by intravenous fluids, broad spectrum antibiotics and steroids. Other supportive measures were given as in cases of tuberculous meningitis.

d) Epilepsy - The cases of epilepsy were generally treated by giving anti-convulsants such as sodgardinal, diazepam or other anticonvulsants were given in addition or substitution as and when required. In addition other supportive measures were given as and when required.

An attempt was made to follow the cases of tuberculous or pyogenic meningitis or encephalitis at five to ten days interval thrice during and following recovery.

During each follow-up a detailed clinical examination and CSF examination was done. Other investigations were done as and when necessary. In each case besides name, age, sex, address, antenatal, natal and postnatal history, socio-economic status, developmental history and dietary history following facts were recorded.

Immunization status -

History of immunization was taken from the parents or family members. For smallpox and B.C.G. vaccination confirmation was made by careful inspection of scar marks. For polio and DPT vaccination, however,
verbal statements were relied upon and confirmed from records whenever available.

Present, past and family illnesses -

A detailed account of the history of present illness, past and family history was taken in each case. Special emphasis was given to obtain the history of onset of disease, history of fever, headache, vomiting, unconsciousness, convulsions and paresis or paralysis of any part of body. Efforts were also made to find out the various important past illnesses, such as definite history of primary tuberculosis, measles, pertussis, ear discharge, head injury or convulsions which could have precipitated or helped in diagnosis of present illness.

An enquiry was made about the history of any familial illness such as tuberculosis and convulsion.

Physical examination -

A thorough clinical examination was done in each case. General physical examination included, a general appearance of the patient, for evidence of pallor, anaemia, any significant lymphadenopathy and for any obvious septic focus in the body. Anthropometric measurements were recorded in each case and special emphasis was given to record the serial head circumference to evaluate the possibility of developing hydrocephalus.
Central nervous system was examined in full
detail. Level of consciousness was graded into 4 stages —
stage I — stupor, stage II — light coma, stage III —
deep coma and stage IV — patient flaccid and apneic.

The child's posture was examined and was
described as normal, decorticate or decerebrate.

Speech and gait of the child were observed, if
possible. Sign of meningeal irritation such as neck
rigidity, kernig's sign and brudzinski's sign were
elicited. Fundus examination was carried out in each case.

Cranial nerves specially optic nerve, oculomotor
nerve, trochlear nerve, abducens nerve and facial nerves
were examined in detail.

Each group of muscles was examined for bulk,
tone, coordination and involuntary movements.

Perception to fine and coarse touch, pain,
temperature, position and joint sense, vibration and
cortical sensations were evaluated, whenever possible.

Superficial reflexes such as corneal,
conunctival, pupillary, abdominal, cremastric and
planters and deep tendon reflexes such as biceps,
triceps, supinator, knee and ankle jerks were examined
in detail. Besides these, the child was examined for
the presence of cerebellar and extrapyrimidal signs.
Thorough systemic examination was made to detect any abnormality in cardiovascular, digestive and respiratory systems.

Laboratory investigations viz. haemoglobin, leucocyte count (total and differential), erythrocyte sedimentation rate, CSF examination for tension, colour, coagulum, protein, sugar, chloride and cells was carried out routinely in each and every case. Grams staining, culture and sensitivity of the CSF were done in each case. Radiological and other relevant investigations were performed if necessary.

Blood was collected by venepuncture. CSF was collected after doing lumbar puncture. Samples were stored at 4°C in refrigerator. Magnesium and calcium levels were estimated within 48 hours of sample collection. All the glasswares were stored in chromic acid cleaning solution and rinsed just before use and were used wet.

I - Determination of serum and CSF magnesium level.

Serum and CSF magnesium were determined by colorimetric method using titan yellow reagent, described by Neill and Neely (1956).

**Principle** - Titan yellow gives a red colour with magnesium. Neill and Neely modified earlier methods by using gum ghatti as the colour stabilizer and including calcium in the standard. Calcium intensifies the colour
so this allows for the effect of calcium present in the serum.

Reagents:

1. **Sodium tungstate (10%)**: -

   10 gm of sodium tungstate was dissolved in 100 ml of distilled water to prepare 10% solution.

2. **Gum ghatti (0.1%)**: -

   0.1 gm of powdered gum ghatti in a muslin bag was suspended in 100 ml of distilled water for 24 hours to prepare 0.1% gum ghatti.

3. **Sulphuric acid (2/3 N)**: -

   18 ml of concentrated sulphuric acid was added carefully, with mixing, to about 90 ml of distilled water. Then the volume was made to 100 ml to prepare 2/3 N sulphuric acid.

4. **Titan Yellow (0.05%)**: -

   0.1 gm of powdered dye was dissolved in 200 ml of distilled water to prepare 0.05% solution of titan yellow.

5. **Sodium hydroxide (4 N)**: -

   1.6 gm of sodium hydroxide was dissolved in 100 ml of distilled water to make 4 N sodium hydroxide.
6. **Stock standard solution**: -

8.45 gm of MgCl\(_2\), 6H\(_2\)O was dissolved in distilled water and made upto a litre.

7. **Standard solution for use (5 ug/ml)**: -

1 ml of stock solution was diluted in 200 ml with distilled water. This contains 5 micrograms of magnesium per ml.

8. **Calcium chloride solution (0.05 mg/ml)**: -

13.88 mg of calcium chloride was dissolved in distilled water and made upto 100 ml. This solution contains 0.05 mg calcium per ml.

**Procedure**: -

1 ml of serum was diluted with 5 ml of distilled water and proteins were precipitated by adding 2 ml of 10% sodium tungstate and 2 ml of 2/3 N sulphuric acid. This was centrifuged for 10 minutes at 3000 revolutions per minute. 5 ml of the supernatant fluid was taken in a test tube. To this were added in turn 1 ml of distilled water, 1 ml of gum ghatti, 1 ml of 0.05 percent titan yellow and 2 ml of 4 N sodium hydroxide.

At the same time 1 ml of calcium chloride and 5 ml of distilled water was pipetted in a test tube marked as blank, and 1 ml of calcium chloride and 2.5 ml
of the working standard and 2.5 ml of distilled water
were kept in another test tube marked as standard.
To these test tubes were also added in turn 1 ml of
distilled water, 1 ml of gum ghatti, 1 ml of 0.05 percent
titan yellow and 2 ml of 4 N sodium hydroxide.

Standard and unknown were read in colorimeter
against the blank, using a green filter (520 millimicrons).

Calculation :-

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\text{Mg. magnesium per 100 ml serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 2.5
\]

II - Determination of serum and CSF calcium level :

Serum and CSF calcium was determined by Trinders
technique (1960), using calcium kit supplied by Span
Diagnostics, Private Limited, Udhna (Surat), India.

Principle :- Calcium present in the serum or CSF is
precipitated with naphthal hydroxamic acid. The
precipitate is then dissolved by the addition of EDTA
reagent. This solution, on addition of color reagent,
forms a colour complex which can be measured colorimetrically.
Only a small excess of naphthal hydroxamic acid, as a
precipitating agent, is required for precipitating
calcium and hence the precipitate does not require
washing.
Reagents:

1. Calcium reagent:

   250 mg of naphthal hydroxamic acid was dissolved by warming in 100 ml of water containing 5 ml of ethanolamine and 2 gm of tartaric acid. To this was added 9 gm of sodium chloride dissolved in 500 ml of water and then diluted to 1 litre with water. If a precipitate was formed, it was filtered through a whatman No. 40 or 43 paper.

2. EDTA Solution:

   2 gm of disodium ethylenediamine tetra acetic acid was dissolved to 1 litre of 0.1 N sodium hydroxide.

3. Colour reagent:

   60 gm of ferric nitrate (Fe(NO$_3$)$_3$ 9 H$_2$O) was dissolved in 500 ml of water. To this was added 15 ml of concentrated nitric acid and was diluted to 1 litre with water.

Calcium standard:

   125 mg of dry calcium carbonate was dissolved in 40 ml of 0.1 N hydrochloric acid and was diluted to 500 ml with distilled water. This contained 5 mEq of calcium per litre.
Procedure:

Test: 0.2 ml of serum was pipetted into a centrifuge tube. To this was added 5 ml of calcium reagent and mixed.

Standard: 0.2 ml of calcium standard was pipetted into a centrifuge tube. To this was added 5 ml of calcium reagent and mixed.

Blank: -

5 ml of calcium reagent was taken in a centrifuge tube. These tubes were allowed to stand for 30 minutes at room temperature and then centrifuged at 3000 - 4000 revolutions per minute for 10 minutes. The supernatant was decanted by slowly inverting the tubes and placing them inverted for 5 minutes on filter paper to drain completely.

1 ml of EDTA solution was added to each test tube. Each test tube was shaken well to suspend the precipitate. The mouth of each tube was covered with an aluminium cap and kept in boiling water bath for 10 minutes with occasional mixing to dissolve the precipitate completely. Then the tubes were allowed to cool and 3 ml of colour reagent was added to each tube and mixed well.

The optical densities of test (T), standard (S) and Blank (B) were measured at 450 nm or using blue filter against distilled water as reference blank to set zero.

Calculations: -

Calcium in mEq/litre = \( \frac{(T) - (B)}{(S) - (B)} \times 5 \)