**MATERIAL AND METHODS**

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College Hospital, Jhansi U.P. The estimation of serum zinc and copper was done by Atomic Absorption Spectrometry in the laboratory of division of Endocrinology at Central Drug Research Institute (CDRI), Lucknow, U.P.

**Selection of cases:**

A total of 64 cases included in this study were divided into two groups.

**Control Group:** Ten cases of age and sex matched healthy children without any evidence of severe grade of malnutrition, hepatic, renal disorders or recent infection, were included in this group.

**Study Group:** Our study group comprised of 54 cases. Some of them had previous history of hepatic, renal or other infection depending on the nature of illness this group was further subdivided into bacterial, viral and protozoal infections. The diagnosis was comprised on the basis of history, clinical examination and investigations.
Selection of cases:

The detailed history and clinical examination was done in all cases. Patients with different type of infections were studied. The patients with lobar pneumonia (n = 6) had an X-ray picture consistent with the diagnosis and responded well to antibiotic treatment, raised ESR and leukocytosis.

The patients with Enteric fever (n = 10) had positive widal test. The patients with pyogenic meningitis (n = 6) had raised protein and decreased sugar and increase in polymorphs in CSF, and well responded to antibiotics.

The patients with primary pulmonary tuberculosis (n = 5) had positive Mantoux test raised ESR and lymphocytosis, positive X-ray chest.

The patients with tubercular meningitis (n = 7) had CSF findings consistent with TBM. ESR was raised and differential counts showed lymphocytosis.

The patients of postmeasles bronchopneumonia (n = 5) had positive X-ray chest finding of bronchopneumonia and positive H/O measles.

The patients of infective hepatitis (n = 5) had raised liver function tests e.g. - S.bilirubin, SGOT, SGPT, S. Alkaline phosphatase, Bile salt, Bile pigments.
The patients of Malaria (n = 10) had positive blood film for malarial parasite and well responded to antimalarial treatment.

Each group of infections was consistent with the diagnosis clinically as well as investigation findings.

**History** : Detailed history of each case was recorded from the parents or attendants on the planned sheet, which included name, age, sex, chief complaints, present illness, relevant past history, family history.

**Physical Examination** :

**General Examination** : General condition of the patient, pulse rate, respiratory rate, pallor, cyanosis, clubbing, icterus, lymphadenopathy, oedema, temperature, B.P. any skin changes, hairs.

**Anthropometric Examination** :

Height, length, weight, head circumference, mid arm circumference.

**Systemic Examination** :

Thorough systemic examination was done according to disease concerned. Respiratory system, CVS, CNS, abdominal system.

**Investigation** :

**Routine investigations** : According to the nature of ailments investigations were selected from the given list.
Blood: Hb%, TLC, DLC, ESR, GBP, MP was done from Department of Pathology of M.L.B. Medical College Hospital, Jhansi.

Urine: Albumin, sugar, microscopic examination, urobilinogen, bile pigment, bile salt, culture and sensitivity was done from the Department of Pathology of M.L.B. Medical College and Hospital, Jhansi.

Mantoux test - done from District T.B. Hospital, Jhansi.

Liver function test: Serum Bilirubin, SGOT, SGPT, Serum Alkaline phosphatase done from Department of Biochemistry, M.L.B. Medical College and Hospital, Jhansi. Estimation of Serum Zinc and Serum Copper of each selected case was done by Atomic Absorption Spectrophotometery (AAS) using PERKIN ELMER 1100B Model from The Division of Endocrinology, CDRI, Lucknow.

Collection of samples:

Five millitres of blood sample was drawn from antecubital vein, using stainless steel needles and acid cleaned, sterilized glass vials which were thoroughly washed with chromic acid and double distilled water. Serum was separated by centrifugation of samples at 3000 r.p.m. for half an hour and placed in thoroughly cleaned plain vials. Necessary precautions were taken to avoid contamination. Most of the samples were analysed on the same day, if not then they were kept in deep freeze until analysed.
Method of estimation of Copper and Zinc:

The estimation of serum zinc and copper was done by the method of Atomic Absorption Spectrophotometry using PERKIN ELMER 1100 B Model. Atomic Absorption Spectrophotometer, installed at the laboratory of Division of Endocrinology, Central Drug Research Institute (CDRI), Lucknow, U.P. Instrumental conditions for measurement of absorbance of the samples are listed in the table given below.

Atomic Absorption Spectrophotometry technique used in the present study was preferred because of its specificity, sensitivity, precision, simplicity and relatively low cost per analysis in comparison to other methods.

Table: Instrumental parameters for measurement of Serum Zinc and Copper by Atomic Absorption Spectrophotometer (PERKIN ELMER 1100 B)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>3247.5Å</td>
<td>2138.5Å</td>
</tr>
<tr>
<td>Slit width</td>
<td>100 µ</td>
<td>100 µ</td>
</tr>
<tr>
<td>Lamp current</td>
<td>3 mA</td>
<td>6 mA</td>
</tr>
<tr>
<td>Concentration to give 0.25 absorbance</td>
<td>2.3 µg/ml</td>
<td>0.7 µg/ml</td>
</tr>
<tr>
<td>Gas pressure in Tank</td>
<td>10 p.s.i.</td>
<td>10 p.s.i.</td>
</tr>
<tr>
<td>Support (air pressure)</td>
<td>15-18 p.s.i.</td>
<td>15-18 p.s.i.</td>
</tr>
<tr>
<td>Gas used</td>
<td>Acetylene</td>
<td>Acetylene</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.04 µg/ml</td>
<td>0.013 µg/ml</td>
</tr>
</tbody>
</table>
Introduction:

In 1860, Kirchoff and Bunsen, observed that the wave lengths of the dark Fraunhofer absorption lines in the solar spectrum coincided with the wave lengths of elemental lines in various emission spectrums. Based upon the assumption that atoms absorb light at the same wave lengths as they emit light, Kirchoff and Bunsen deduced the presence of several elements in the solar atmosphere.

In 1955, Walsh showed that this phenomenon of atomic absorption could serve as a spectrochemical basis for quantitative determination of metals. He demonstrated that this measurement of metals by Atomic Absorption Spectrometry are more sensitive than measurements by flame emission spectrophotometry and less subject to interference from other elements. The subject has been comprehensively reviewed by various workers (Allan, 1962; David, 1960; Allan, 1962; Fuwa and Vallee, 1963; Maret and Henkin, 1971; Sunderman, Jr. 1973; Buttrimovitz and Purdy, 1977 and Smith Jr et al, 1979).

Principle: Aspiration of the serum into burner produces thermal molecular dissociation and dispersion of metal atoms throughout the flame. Small proportions of these atoms become excited to emit light but the overwhelming majorities of the atoms remain in the ground state and are capable of absorbing discrete wave length of incident light. These specific wave
lengths are provided by a lamp with a hollow cathode. Beam of the light is passed through in flame several times, and then is focussed upon the enterance slit of a diffract action grating monochrometer. The absorptions of light at the specific wave lengths are proportional to the concentration of that particular metal in the sample.

The abundance of atoms in the ground state in comparison with those in the excited state accounts for the greater sensitivity of atomic absorption spectrophotometry relative to flame emission spectrophotometry.

Light emitted from the flame constitutes a potential source of error in AAS, as a light energy which strikes the photomultiplier tube represents the net balance of emission and absorption. This source of error is avoided by modulating the incident light beam with a mechanical chopper, and tuning this photomultiplier detector circuit to the same frequency of modulation, under these conditions, the detector circuit responds only to the pulsed signal from the light beam and does not respond to the continuous signal produced by light emission from the flame. The alternating current from photomultiplier detector circuit is amplified and recorded.
Procedure:

Parker and Colleagues (1967) described two procedures viz simple dilution and removal of protein using trichloroacetic acid prior to aspiration for determination of copper and zinc by AAS. In the present study the simple procedure of direct aspiration was employed. The AAS adjusted to the operating conditions as listed in the table given earlier. Standard solutions for copper, zinc were prepared in required strengths copper-1, 2 and 4 part per million; zinc 0.5, 1 and 2 part per million. With these standard solutions of each metal absorption for each solution strength were measured separately and standard curves for each metal were established separately.

After establishment of standard curved serum copper, and zinc were estimated as follows:

Serum samples were aspirated without dilution directly into the flame for copper and zinc. Estimation of absorbance for each metal was done at a time. Duplicate measurements of the absorbance were made for each sample. After every sample measurement distilled water was aspirated into the burner until null meter returned to null point. Prior to determination of values in the unknown samples,
aliquotes of standard solutions of copper, and zinc were also measured in order to check the precision of the procedure. Serum copper and zinc concentrations were calculated with the help of calibration curves.