MATERIAL AND METHODS
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This study was carried out in the department of Pediatrics in collaboration with the department of Microbiology, M.L.B. Medical College, Jhansi.

The children for study were selected from patients attending the outpatient department, Well-baby clinic and from those admitted to Pediatrics ward of this hospital. These children were grouped as follows.

GROUP A

Twelve healthy children having weight more than 80% of the standard weight expected for their age (standard taken as 50th percentile of NCHS value) were taken as normal healthy controls.

GROUP B

Twenty-seven children having weight less than 71% were selected for study. Children having weight 71% to 80% of the standard weight expected for age were not included in this study as alteration in immune functions are infrequent in mildly under nourished patients.

Children with primary immunodeficiency and those on drugs like - corticosteroids, anti-cancer drug therapy, prostaglandins, catecholamines, histamines, chlorpromazines, acetyl salicylic acid and amphotericin B were not included in any group, as these pharmacologic agents are reported to affect phagocytic functions. Also children
with infections were not included in this study.

Group B children were further subgrouped as:

**Group B₁**

Five children having weight 60-70% of standard
weight expected for age (grade II malnutrition according
to classification of Indian Academy of Pediatrics).

**Group B₂**

Twelve children having weight 50-60% of standard
weight expected for age (grade III malnutrition according
to classification of Indian Academy of Pediatrics).

**Group B₃**

Ten children having weight less than 50% of
standard weight expected for age (grade IV malnutrition
according to classification of Indian Academy of Pediatrics).

**HISTORY**

History was taken regarding the presenting
complaints of the child, along with past history, antenatal,
natal and postnatal history, developmental history, immuni-
sation status and a detailed dietary history which included:

(i) Age up to which breast milk was given.

(ii) Age at which first feed in form of milk were
started, quantum of dilution was also enquired.
(iii) Age at which semisolids and solids were started.
(iv) Present diet in terms of total calories and protein intake per day.

PHYSICAL EXAMINATION

A thorough physical examination was done to observe psychomotor changes, pallor, oedema, skin changes, hair changes, amount of sub-cutaneous tissue and muscle mass.

Eyes were examined for signs of vitamin 'A' deficiency, conjunctival and scleral pigmentation and angular palpebritis.

Skin was examined for xerosis, follicular hyperkeratosis, petechiae, pellagrous dermatosis, flaky paint dermatosis, scrotal and valval dermatosis, mosaic dermatosis, thickening and pigmentation of pressure points and intertriginous lesions.

Lips were examined for angular stomatitis, angular scars, cheilosis and chronic depigmentation of lips.

Tongue was examined for oedema of tongue, atrophic papillitis, for changes of scarlet and raw tongue and magenta tongue.

Teeth were examined for caries, mottled enamel and attrition.

Gums were examined to observe spongy, bleeding gums or their recession.
Thyroid and parotid glands were examined for any enlargement.

Skeletal system was examined for presence of any deformity and signs of vitamin D deficiency such as cranio-tabes, cranial bossing, persistent open anterior fontanelle, costochondral beading and epiphyseal widening.

Thorough examination of cardiovascular system, respiratory system, gastro-intestinal and central nervous system was also done in each case.

**ANTHROPOMETRIC EXAMINATION**

**Weight**

Weight record of children weighing 10 kg or less was done by weighing scale measuring weight to the nearest 0.05 kg and in children weighing more than 10 kg, it was recorded to the nearest 0.25 kg by adult type spring weighing machine.

**Height/Length**

Length was recorded to the nearest 0.1 cm with the patient lying supine on a hard surface. Height was also recorded to the nearest of 0.1 cm.

**Mid Arm Circumference**

Circumference of left upper arm was recorded to the nearest of 0.1 cm at a point midway between the tip of the acromian process of scapula and olecranon process of ulna. While measuring arm was allowed to hang freely.
A flexible steel tape was used to record these measurements.

**Head Circumference**

Circumference of head was measured to the nearest 0.1 cm by placing the tape firmly round the frontal bones just superior to the supra-orbital ridges, passing it round the head at the same level on each side, and laying it over the maximum occipital prominence at the back.

**LABORATORY INVESTIGATIONS**

Routine hemogram - Hb, TLC, DLC, ESR, was done in each case along with following tests:

(i) **Nitro Blue Tetrazolium Reduction Test**

**Preparation**

NBT solution: 0.1% NBT solution was made by dissolving 25 mg of NBT dye (Nitro BT - the Central Drug House P. Ltd.) in powder form in 25 ml of phosphate buffer saline. This solution was stored at 4°C in refrigerator. This was reprepared from time to time but before a period of 6 months.

**The test procedure (Park et al)**

1. Any suitably approachable peripheral vein was punctured with a sterile needle after cleaning the part with spirit swab. The blood was collected in a vial containing EDTA.
2. To a plastic vial, 0.2 ml of blood was taken and 0.2 ml of pooled human plasma and 0.2 ml of 0.1% NBT solution were added to it.

3. This mixture in plastic vial was incubated for 30 minutes at 37°C. The tube was shaken gently at 5 minutes interval to shake the contents of the mixture.

4. After 30 minutes above incubated mixture was centrifuged for 5 minutes, supernatant was then discarded and the deposit was suspended in 0.1 ml of Hank's balanced salt solution.

5. After that smear from above supernatant was made on a glass slide gently taking care not to disrupt the leukocytes. This was then dried and fixed by gentle heating.

6. Counter-staining of this fixed smear was done by dilute carbol-fuchsin for 15 seconds, washed in tap water and dried.

7. Using a 100 x oil immersion objective, 100 neutrophils were counted and percentage of NBT positive cells was determined. Only those neutrophils with a large black deposit (formazan containing cells) were classified as NBT positive.
(ii) **Candida-Cidal Assay**

**Preparation of Candida-albicans**

Test organisms were inoculated into 50 ml of sabouraud 2% dextrose broth and cultured at 33°C; under these conditions, the candida cells grew only in yeast phase.

**Leukocyte Preparation**

Peripheral venous blood collected in a sterile vial containing EDTA was taken in a sterile tube which was kept tilted for 30-45 minutes at room-temperature. At the end of 30-45 minutes leukocyte rich supernatant was removed and washed with Hank's balanced salt solution and finally resuspended in same solution at a final concentration of $10^7$ PMN/ml.

**The Procedure (According to the method of Lehrer & Cline)**

1. Equal volumes (0.25 ml) of leukocyte suspension, AB serum and Hank's balanced salt solution were taken in a sterile plastic vial. All these components were taken in two vials - one of which did not contain leukocyte suspension, sterile technique was used throughout.

2. The vials were incubated for 10 minutes at 37°C.

3. Then 0.25 ml volume of candida albicans at concentration of $10^7$ yeast cells/ml was added and vials were again incubated at 37°C for 60 minutes.
4. After 15 minutes, a drop from above mixture was taken for direct examination and preparation of stained smear to confirm that all added organisms had been ingested.

5. At 60 minutes, 0.25 ml of 2.5% sodium de-oxylcholate (pH 8.7) was added to each vial; at this concentration, de-oxylcholate causes immediate lysis of the blood cells without damage to the candida cells.

6. After that methylene blue 0.01% in distilled water was added to achieve a final volume of 4–5 ml and final dye concentration of about 0.0075%.

7. Then these suspensions were centrifuged for 5 minutes, supernatant was removed and the settled down cell suspension were resuspended in about 0.5 ml of this supernatant fluid.

8. These suspensions were then examined microscopically on a haemocytometer, 100 candida cells were counted in each suspension and percentage of killed candida cells (blue coloured - intensely stained) was scored.

(iii) Estimation of Immunoglobulins (IgG, IgM & IgA)

Procedure

1. 3 ml of venous blood was collected in sterile plain vial for obtaining serum. Serum separated from this blood was kept stored in refrigerator at 4°C.
2. Immunoglobulins were estimated in this serum sample by Radial Immuno-diffusion by Fahey and Mckelvy technique.

3. Commercially available agar-plates, already incorporated with antiserum in it were used having 12 wells in each plate.

4. Three of these wells were filled with standard serum of known concentration. Standard serum was filled in these wells in 3 dilutions (100%, 50%, 25%).

5. Remaining wells were filled with serum samples with the help of a syringe with a fine needle. Care was taken not to under fill or overflow the wells.

6. After filling all the wells, the lid of the plate was replaced and kept aside for 10 minutes.

7. After 10 minutes the plates were left in inverted position for the development of the precipitin rings.

8. The ring diameters were measured in each plate and then standard graph was plotted using the values of the standard serum and their ring diameters.

9. Then the values of the serum samples were found out directly by interpolation on the standard graph.

10. Ring diameters for IgG and IgA were measured after a period of 18 hours diffusion and plates were kept at room temperature for whole period, whereas those of IgM were measured after 48 hours, for 1st 24 hours, the plates were kept at room temperature and thereafter at 4°C in the refrigerator.