MATERIAL AND METHOD
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The present study was conducted in department of paediatrics, in collaboration with department of Pathology.

Forty eight children with measles aged 8 months to 12 years were selected from the Out Patient Department of paediatrics and from those admitted in children ward of this hospital. The nutritional status of all measles patient was assessed according to Harvard weight/age standard. Those having weight above 80% of the 50th percentile of Harvard, were considered as well nourished and those who were below this reference median were taken as malnourished. The malnourished children were further divided into grade I, II, III and grade IV malnutrition group according to classification of Indian Academy of Pediatrics. The study children having 71-80% of expected weight were taken as grade I malnutrition children, similarly those having weight 61-70%, 51-60% and ≤50% of 50th percentile of Harvard standard were classified as grade II, III and IV malnutrition children. The study group children were matched according to age with twenty normal healthy children. They were selected from the well baby clinic.
and from the paediatric Out Patient Department. These children served as controls. With parental consent, 10 ml venous blood was obtained under strict aseptic precautions from measles patient during the first 0-6 days of appearance of rash, for immunological study. Similarly control group children were also investigated. Besides Name, age, sex, address and socioeconomic status of children, the present and past history of illness and family history were questioned in each case.

From parents or other family members detailed history was obtained regarding present illness in chronological order. History of associated complications like diarrhea, dysentery, Whooping cough was asked. Bronchopneumonia and encephalopathy were diagnosed clinically. History of immunisation viz. Polio, DTP, BCG and measles was interrogated. In the past history, history of worm infestations, asthma, pertussis and tuberculosis was interrogated.

In family history, history suggestive of chronic illnesses like tuberculosis and asthma was questioned in parents, siblings and neighbourhood.
Physical Examination

Thorough clinical general examination and the examination of respiratory, Cardiovascular, gastrointestinal and central nervous systems was done in each case.

Weight

Weight was recorded nearest to 0.01 kg by using infant weighing scale for infants 4 kg and adult type weighing machine for children 7 kg.

MATERIAL REQUIRED FOR T & B CELL ESTIMATION

1. Heparin Preservative free.
3. Alsever's Solution
   - Glucose 24.6 gm.
   - Trisodium Citrate dehydrate 9.6 gm.
   - NaCl 90.64 gm.
   - Distilled water 1200 ml.

PH was adjusted to 6.1 with 1% citric acid, sterilized by low pressure autoclaving and stored in refrigerator.

4. Phosphate Buffer Saline (PBS)
   Phosphate Buffer Solution
   (a) 0.12 M = NaH₂PO₄.2H₂O 23.4 gm/litre
   (b) 0.92 M = Na₂HPO₄ 21.3 gm/litre
Normal Saline

NaCl 9.0 gm/litre

Phosphate Buffer Saline

For pH 7.4 - Solution A = 18 ml

and Solution B = 82 ml

and then normal Saline 100 ml was added, solution was sterilized by low pressure autoclaving and stored in refrigerator.

5. Pooled Normal Human Serum - 15 ml Venous blood were drawn aseptically into clean and dry test tube from 4 persons. Then test tube incubated in water bath at 37°C for 30 mins, and then at 40°C for 120 mins. The clot removed gently with glass rod and test tube was centrifuged. The clear serum from each tube was collected and pooled together then stored at -20°C (freezing) in small aliquotes and used once after thawing.

6. Antisheep Haemolysin (Anti Coceptor) (SPAN Diagnostic).

7. Methylene Blue 0.2%

LABORATORY PROCEDURE:

Collection of sample - Ten ml, heparinized peripheral blood sample (2U unit of Heparin/ml of blood) was collected in the sterile tube from each patient for B and T cell studies. Blood was simultaneously collected from these
patient for total and differential leukocyte counts in double exalate vials.

**Total Leukocyte Count (TLC):** One in 20 dilution of blood was made by adding 0.02 ml of blood to 0.38 ml of WBC diluting fluid (Turk’s fluid) in 7.5x10 mm test tube. The suspension was mixed by gentle tilting and rotating by hand for 2 minutes. The Neubaur’s counting chamber was filled using pauster pipett. The preparation was viewed with 5 mm objective under microscope. The number of leukocytes were counted and calculated as below:

\[ TLC = N \times 200/cu \text{ mm}^3 \]

\( N \) is number of leukocytes counted in each mm square area.

**Differential Leukocyte Count (DLC):** A thin and uniformly prepared peripheral blood smear was stained for 8 to 10 minutes with leishman stain, washed with buffered water and dried in air. Leukocytes were counted using oil immersion lens and the percent distribution of different leukocyte was calculated based on the count of 200 cells.

**Absolute Leukocyte Count (ALC):** Absolute lymphocyte count was calculated in every case from the total and differential leukocyte count using following formula.
Estimation of Haemoglobin: Estimation of haemoglobin
was done by Sahli's Method.

Estimation of ESR: Estimation of erythrocyte sedimentation
rate was done by the Wintrobe method, a haematocrit tube
was filled to the 100 ml mark with unsalted blood and
allowed to stand vertically for one hour.

EVALUATION OF T AND B LYMPHOCYTES

Preparation of Lymphocytes rich plasma: The lymphocytes
were separated from the heparinised peripheral blood by
gravity sedimentation method. Ten ml of heparinised blood
(25 unit/ml blood) collected in a sterilised test tube
was kept up right at room temperature for one hour. The
lymphocytes rich plasma was collected and centrifuged at
1000 rpm for 15 minutes, the clear plasma was separated
and the cell button was suspended in minimum essential
medium (MEM). The concentration of lymphocytes was
adjusted to 2-3 \times 10^6 per ml in MEM.

Preparation of Sheep RBC solution: Sheep blood collected
in equal volumes of Alsever's solution was stored in
refrigerator for 3 to 5 days and there after used upto
14 days. Sheep erythrocytes was washed twice with buffer
saline. One volume of packed cell was suspended in 15 volumes
of buffer saline to give a slightly greater concentration than 5% suspension. One ml of this suspension was lysed with exactly 14 ml of distilled water and optical density (OD) was measured at 540 um with distilled water as blank. A lysate with an O.D. of 0.7 represented 5% or 1 x 10⁹ cell/ml. From the O.D. of sample tested and volume of the suspension (V₁), the final volume (V₂) was calculated according to the relationship:

\[ V₂ = \frac{V₁ \times \text{O.D.}}{0.7} \]

Finally suspension was adjusted to make standard solution of sheep RBC.

**Titration of haemolysin**:–

This was first performed so that complement titration was independent of the concentration of haemolysin. 5.0 ml volumes of 2% SNBC are treated with equal volumes of 1 : 30, 1 : 100, 1 : 200, 1 : 400 and 1 : 800 diluted haemolysin in GVBS for 15 minutes at 37°C. The sensitised SNBC is now called Ea.

6.5 ml volumes of 1 : 30, 1 : 100, 1 : 200 and 1 : 400 diluted normal human serum (NHS) are also prepared and then tubes are set up as shown in Table 1.
After incubation at 37°C for 60 min, the tubes were centrifuged and the O.D. measured at 541 nm. Percentage of lysis was calculated by the formula:

$$\text{OD Row A to D} \times \text{OD Row E} \times 100$$

The dilution of antiserum which gave maximum haemolysis with 1 in 100 or 200 diluted human serum was used in subsequent titrations. This was carried out on all new batches of haemolysin.

**Demonstration of T cell by sheep RBC Rosette (E Rosette):**

Sheep RBC were washed three times with MEM and 0.5% suspension was made in phosphate buffer saline. Lymphocytechant was adjusted to 2-3 x 10⁶ per ml in MEM. To 0.5 ml of sheep RBC suspension in test tube, 0.5 ml of lymphocyte suspension was added and mixture was incubated for 15 minutes at 37°C in water bath. After centrifugation for 5 minutes at 500 rps, mixture was incubated at 4°C for over night. Supernatant was removed and pellet was resuspended in remaining fluid (2-3 drops). Finally wet preparations was made and stained with methylene blue. Rosette forming
lymphocytes out of 200 cells were counted under microscope and value expressed as percentage of rosette forming cells.

Three or more SRBC adhering to a lymphocyte were taken as rosette forming cells. The absolute T cell count was calculated as follows:-

\[
\text{Absolute T cell count} = \frac{\text{ALC} \times \text{% T cells}}{100}
\]

Demonstration of B cell by formation of EAC rosette:-
(Sheep RBC coated with anti sheep haemolysin antibody and complement).

To 0.5 ml of 5% SRBC suspension, 0.5 ml of anti sheep haemolysin in appropriate dilution (1:400 assessed earlier) was added and incubated for 15 min. at 37°C. After washing three times with phosphate buffer saline and resuspending in phosphate buffer saline and there after adding 0.5 ml of 1:10 diluted complement (human serum), tubes were incubated for 45 minuts at 37°C. These cells were washed thrice with phosphate buffer saline and then resuspended to make a concentration of 0.5% EAC in phosphate buffer saline.

To 0.5 ml suspension of lymphocytes (2-3 x 10^6/ml), 0.5 ml of EAC suspension in PBS was added and incubated at 37°C for 30 minuts. The solution was resuspended and wet
preparation was prepared and stained with 0.2% methylene blue and rosette forming lymphocytes out of 200 cells were counted.

Three or more SRBC adhered to a lymphocyte were considered to be rosette. Absolute B cell count was calculated as follows:

\[
\text{Absolute B cell count} = \frac{\text{ALC} \times \% \text{ B cells}}{100}
\]

2.4 Dimethyl Chloro Benzen (DMCB) contact skin sensitization test

Stock solution of DMCB in acetone of 1000 μg/0.1 ml and 50 μg/0.1 ml concentrations was made and stored in amber coloured bottles at room temperature, this solution was changed after every three months. Stainless steel ring of 2 cm diameter was placed at the site of application of DMCB so that fixed area was obtained. Sensitizing dose of 1000 μg/0.1 ml was applied on the right upper arm on volar surface slightly towards medial side, simultaneously challenge dose 50 μg/0.1 ml was applied on right forearm on flexor surface on medial side.

After the application of DMCB, these sites were covered and subjects were instructed not to wash the sites for 24 hrs., sites were examined after 48 hrs.
for irritative reaction and at 14th and 21st days for a spontaneous flare, indicated by appearance of erythema induration and vesiculations.

Reaction was graded according to criteria proposed by Sunjeev Rao, P. et al (1981) which are as follows:

+++ Spontaneous flare occurring at both sensitizing dose and challenge dose sites.

++ Spontaneous flare only at sensitizing dose site.

+ Absence of spontaneous flare but reapplication of challenge dose elicits an equivocal delayed hyper-sensitivity reaction.

-ve No reaction. No spontaneous flare occurring even after reapplication of challenge dose.

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