MATERIAL AND METHOD
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The study was conducted on a total of 18 patients suffering from malaria attending the out patient department and those admitted to the M.L.B. Medical College Hospital, Jhansi. This study included only those patients suffering from malaria which were easy to follow for second blood sample when the malarial infection subsides.

Six age and sex matched healthy individuals of similar socio-economic and geographical background served as control for this study.

All patients in which suspected to have malarial fever were subjected to following :-

1- The clinical history and findings of physical examination were recorded on a predesigned proforma.

2- 2.5 ml of blood was aseptically collected from antecubital vein of each patient of malaria in a clean dry test tube.

3- The sera were separated by centrifugation at 3000 RPM for 10 minutes and were stored in deep freezer.
4- Haemoglobin was estimated by cyanmeth method and was expressed in gram/dl.

5- Thick and thin blood smear were prepared for each and subjected to Field (1941) and Leishman's (1865) staining procedures respectively.

FIELD STAINING PROCEDURE (DACIE, 1984):

1- The prepared thick smear allow to dry then dip into Field's stain A for 1-2 seconds.

2- Rinse in buffered water (pH 6.8 - 7.0) until stain ceases to flow from the smear (5-10 seconds).

3- Then the slide dip into Field stain B for 1 second.

4- Rinse rapidly in buffered water for 10 seconds Shake off excess water and leave the slide upright to dry.

5- Dried smear examined in oil immersion.

LEISHMAN'S STAINING PROCEDURE (DACIE, 1984):

1- Keep the air dried blood slide on a rack with smear surface upward.

2- Flood of blood smear with Leishman's stain and leave it for 2 minutes.
3- After 2 minutes add double the volume of buffered water and mixed with stain and leave if for 10-12 minutes.

4- Then wash it in stream of water until it has aquired a pinkish tinge (upto 2 minutes).

5- Dried smear examined in oil immersion.

**ESTIMATION OF SERUM IMMUNOGLOBULINS:**

Serum immunoglobulins level was estimated by single radial immunodiffusion technique of Mancini and Carbenara (1965) using non-partigen plates of Ig G, Ig M and Ig A, provided by commercial sources (Behring Pharmaceuticals).

**PRINCIPLE:** (Mancini et al, 1965):

The principle of this technique is "Antigen diffuses radially from the point of application into an antibody containing gel and circular precipitate or ring forms at the zone of equivalence, keeping antibody concentration and gel thickness constant the area covered by precipitin ring is proportional to the concentration of antigen".
METHOD:

The cover of Aluminium container was pulled off using a tab provided for this purpose and plastic container was removed and allow the open plate to stand for about 5 minutes at room temperature for evaporation of any condensed water which may have penetrated into the wells.

Then the walls were filled with 5 microlitre of undiluted patients sera with the help of microlitre pipette. After filling of wells with serum allow the plate to stand tightly closed at room temperature.

EVALUATION:

After expiration of diffusion period 2 days for Ig G and Ig A and 5 days for Ig M serum immunoglobulin the diameters of the precipitates were measured by scale provided by firm under oblique illumination.