CHAPTER 10

INVESTIGATIONAL MODALITIES

Each patient having undergone different clinical and non-clinical examinations, was then subjected to various investigatory procedures in order to study the initial haematological picture and other findings. For this purpose, a frame-work of the various investigations to be carried out was prepared.

INVESTIGATIONS

1. Haemoglobin (Hb)
2. Red Cell Count (R.B.C.)
3. Colour Index (C.I.)
4. Packed Cell Volume (P.C.V)
5. Mean Corpuscular Volume (M.C.V.)

6. Mean Corpuscular Haemoglobin (M.C.H.)

7. Mean Corpuscular Haemoglobin Concentration (M.C.H.C.)

8. Peripheral ~~xxxx~~ Blood Smear for :

   (a) Size and shape of R.B.Cs. and filling of Hb.
   (b) Abnormal cells
   (c) Malarial Parasites (M.P.)

9. Reticulocylocyte count :

   (a) 1st day
   (b) 5th day
   (c) 9th day
   (d) 11th day
   (e) 13th day
   (f) 15th day
   (g) 18th day
   (h) 21st day

10. Platelet count :

11. Leucocyte Count (W.B.C. Count) :

   (a) Total
   (b) Differential - Polymorphs - (P)
   Lymphocyte - (L)
   Eosinophil - (E)
   Monocyte - (M)
   Basophil - (B)

12. Serum Proteins :

   (a) Total
   (b) Albumin
   (c) Globulin
   (d) A/G Ratio

13. Serum Iron Estimation on First day of Treatment :

   (a) 0 Hours (Fasting)
   (b) 4 Hours after drug
   (c) 8 Hours after drug

14. Serum Iron Estimation on Last Day of Treatment :

   (a) 0 Hours (Fasting)
   (b) 4 Hours After drug
   (c) 8 Hours After drug

15. Marrow Biopsy (Sternal puncture)

16. Urine examination :

    (a) Albumin
    (b) Sugar
    (c) Microscopic

17. Stool Examination :

    (a) Occult blood
    (b) Ova
    (c) Larva
    (d) Vegetative
    (e) Cystic

18. Screening of Chest

19. Electrocardiogram (E.C.G.)

20. In Bleeding Cases :

    (a) Bleeding Time (B.T.)
    (b) Clotting time (C.T.)
    (c) Prothrombin Time
    (d) Platelet count

DETAILS OF INVESTIGATIONS

Out of the above mentioned investigations, No.1 to 8 were carried out initially (0 week) and at the end of I, II and III week of treatment. Reticulocyte count was repeated on the days already mentioned in the list. Platelet count, W.B. C. count, and Serum Protein Estimations were carried out initially only (i.e. before starting the iron therapy). Serum Protein Estimations were carried out soon after the admission of the patient, to avoid the benefidial effect of diet, if any. Serum iron estimations were carried out on first and last day

(21st day) of treatment.

A new and separate set of instruments was kept for the purpose. The syringes and needles were either autoclaved or boiled for 15 minutes and dried completely. The sternal puncture needle was always autoclaved.

The blood samples were collected both initially and subsequently, in the early morning between 6-00 a.m. and 7-00 a.m. with the patients in the fasting stage. The blood withdrawn from the cubital vein without statis was collected in plain bulbs as well as in Wintrobe's bulb (containing Wintrobe's Oxalate Mixture). Smears and wet preparations for reticulocyte and platelet counts were prepared directly from the syringe blood.

Reticulocyte counts and platelet counts were done immediately. Rest of all the haematological investigations were completed within 6 hours of collection of blood samples. Serum proteins estimations were carried out after the separation of serum. The blood samples for serum iron estimation were kept at room temperature for about 1 to 1½ hours to allow the serum to separate. Then the samples were stored in refrigerator till serum iron estimations were carried out on the next day.

MODES OF INVESTIGATIONS

1. Haemoglobin Estimation :

Haemoglobin estimation was carried out by Sahli's acid-haematin method. The same Hellige's haemoglobinometer was used all throughout the study. The readings were taken in day light each time. The readings were taken 10 minutes after the mixture of blood and the acid and the same time interval was observed on each occasion.[137] The results were expressed in gram per 100 ml. of blood. In all cases having haemoglobin less than 6 gm. per 100 ml. of blood, twice the volume of blood was taken for Haemoglobin Estimation and the results were halved for the sake of greater accuracy.

2. Erythrocyte Count (R.B.C. Count):

Red cell count was done from the wintrobe's bulbs. In each case the red cell count was carried out thrice and the mean of the three readings was taken. (The dilution of blood in R.B.C.Pipette was carried out Separately for each of the three counts).

3. Leucocyte Count (W.B.C.Count) :

White cell count was also done from the Wintrobe's bulbs and was carried out once only in each case.

4. Packed Cell Volume (P.C.V):

The blood from the Wintrobe's bulb was used for this. The blood was centrifuged in Wintrobe's tube in a Clay-Adams centrifuge at 3000 r.p.m. for 30 minutes and then for every five minutes till complete packing i.e. till "Constant Volume" was obtained.

5. Reticulocyte Count :

Reticulocyte counts were carried out on fixed alcoholic brilliant cresyl blue preparations made on clean slides. The reticulocytes were counted for thousand erythrocytes and reported as the per cent of erythrocytes.

6. Platelet Count :

Platelet count was also carried out from the same above preparation. The platelets were counted for thousand erythrocytes. Then, knowing the actual erythrocyte count, the platelets were calculated and reported in lacs per c.m.m.

7. Peripheral Blood Smear :

The smear was prepared as usual and was stained with Leishman's stain. Then the smear was examined to assess the size and shape of red cells, filling of heamoglobin, any abnormal cell and also for malarial parasites. Accordingly it was reported whether the cells were microcytic and hypochromic or otherwise and whether anisocytes, poikilocytes or

target cells were present or absent. If anisocytes or poikilocytes were present, the degree of anisocytosis and poikilocytosis was reported.

8. Differential Count (D.C.):

A differential count of 100 W.B.Cs. was carried out on the same smear used above. A thin portion - tail end - of the smear showing the even distribution of cells (Cells neither overlapping nor too far apart) was selected for the purpose. The report was checked by the impression of the smear gained by a general survey.

9. Marrow Reports :

The marrow smears were stained by a combined leishman and Giemsa technique. Marrow reports were expressed as qualitative assessments based on surveys of many fields of the stained smears. The marrow smears were also subjected to Prussion blue reaction to study the state of iron stores.

10. Serum Proteins :

Total serum proteins were estimated by Van Slyke's specific gravity method. Albumin and globumin were estimated by Biuret method.

11. Serum Iron Estimation :

The serum iron estimation was carried out by the method[78]

described by Marrack (1956) after due modifications. Three samples of blood (0 hours, 4 hours and 8 hours after drug) from each patient were collected on the first day of treatment. Similarly, three such samples were collected on 21st day of treatment.

12. Urine Examination :

Urine (a catheter specimen, if necessary) was examined for the presence of albumin, sugar and microscopic abnormalities. A special care was exercised not to miss microscopic haematuria.

13. Stool Examination :

The stoold was examined for the presence of parasites and occult blood. The simple method and concentration method for stool examination as also the Benzidine test for occult blood are fully described in the preceding Chapter.

14. Screening of Chest :

Screening of the chest was done to assess the cardiac size, besides the clinical assessment. A special care was taken not to miss pulmonary tuberculosis or any other abnormality.

15. Electrocardiogram :

Each patient was subjected to electrocardiographic study in order to find out the electrocardiographic changes

in iron deficiency anaemia. As already mentioned in Chapter 9, all the cases were carefully selected and anaemia cases of varying aetiology, but WITHOUT other associated diseases likely to give rise to electrocardiographic changes, were included.

In each case, twelve lead electrocardiogram was taken— three bipolar limb leads, three unipolar limb leads and six unipolar chest leads.

TRIAL CASES

In order to establish the accuracy of the serum iron estimation technique and also to find out the hours of peak serum iron levels after administration of Ferrous Sulphate and Ferrous Fumarate, first 20 cases were taken up as trial cases and these cases were EXCLUDED from the next 40 cases of the study proper.

Our of these 20 cases, 10 cases were subjected to the administration of Ferrous Sulphate (180 mg. of E.I.) and 10 cases were subjected to the administration of Ferrous Fumarate (180 mg. of E.I.) and blood samples were collected at the end of 3 hours, 4 hours and 5 hours after the administration of drug. The Serum Iron levels were estimated and it was found that the peak level reaches 4 hours after the administration of Ferrous Sulphate as well as Ferrous Fumarate.

Somewhere in the early part of the study of these

trial cases, the tendency on the part of some of the patients to spare and preserve the tablets from the given dose of totally three tablets per day was observed and action was taken to prevent this as mentioned earlier.