SUMMARY AND CONCLUSION
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Anaemia is an inevitable complication of leukaemia occurring either earlier or later, during the course of the disease. It usually appears early and progress rapidly in acute leukaemia. In chronic leukaemia, in the earlier stage it is usually not so marked. The factors causing anaemia in leukaemia have been classified as follows - (1) "Crowding out" of erythroblasts, (2) haemolysis, (3) haemorrhage, (4) cytotoxic drugs, (5) metabolic competition and (6) other miscellaneous conditions like infection, sepsis, hypersplenism etc. The role of haemolysis has been considered by many investigators since the early report in 1906 (Hirschfield). From many indirect evidences like persistent reticulocytosis, increase in serum bilirubin concentration, increased faecal urobilinogen, increased autohaemolysis, failure to raise haemoglobin even with frequent and successive blood transfusion, it was considered that haemolysis occurred in leukaemia, resulting in anaemia and later on this was confirmed by the direct measurement of the life span of red cells first by means of the Ashby technique and later by radio-isotopic method. Defective erythropoiesis either qualitative or quantitative was observed in the present series of patients. Anaemia associated with low reticulocyte count was more marked in acute leukaemia than in chronic cases. In many patients with acute leukaemia the haemoglobin, PCV and reticulocytes remained significantly low. In these patients the bone marrow had significantly diminished number of erythroid
cells precursors. This proved that in those patients depressed erythrocytic activity was the main cause or one of the causes of anaemia. In those cases from the reticulocyte count it was very difficult to assess the degree of haemolysis. In chronic leukaemia patients the Hb and PCV remained comparatively higher along with the higher reticulocyte count but the increased reticulocyte count had no definite correlation with the PCV. Patients with higher reticulocyte count were usually less anaemic and they had better erythroid activity in bone marrow. In remission it was clearly noted that a significant number of patients had normalisation of the reticulocyte count along with the improvement of anaemia as evidenced by the rise in Hb and PCV of erythrocytes. This erythropoietic response clearly indicated that the disease process interfered with the erythropoiesis and the disturbed erythropoiesis improved in some patients after the control of the disease. Abnormal erythropoiesis evidenced by the presence of megaloblastoid cells in all the patients with Ery.L., 2 in CML, 3 in ANL, 1 in ALL was present. During remission there was normalisation of the erythropoiesis except in erythroleukaemia where the megaloblastoid cells were present, but in lesser number. The morphological changes like poikilocytosis, hypochromia, presence of microcytes and ovalocytes noted during the initial phase of the disease, became almost normal in some patients during the period of remission.
From the relevant study in the pattern of haemoglobin and the amount of foetal haemoglobin it was found that 20 out of 147 patients had raised value of foetal haemoglobin in the initial stage. Elevation of foetal Hb was noted in both granulocytic and lymphoid leukaemia of acute and chronic type. In general, the level of foetal haemoglobin was not found to have any definite correlation with the degree of anaemia. During remission 12 out of those 20 patients showed normalisation of the levels of foetal haemoglobin. From this it is evident that due to the activity of the disease process the foetal haemoglobin level was increased and after treatment, during remission the raised level of foetal haemoglobin returned to its original normal value.

The role of haemolysis as a cause of anaemia was evaluated from assessment of absolute reticulocyte count, mechanical fragility, pre and post incubation osmotic fragility, acid serum haemolysis, rate of autohaemolysis at 24 and 48 hours, level of serum bilirubin, level of plasma haemoglobin, the status of Coombs' test, iso- and auto-agglutinin in serum, urobilinogen excretion in faeces, Heinz bodies in peripheral blood and sucrose lysis test. Significant abnormalities like increased reticulocyte count in chronic leukaemia, increased rate of incubation auto-haemolysis in all the types of leukaemia specially more in ANOL, CLL and AML, positive Coombs' test, increase of plasma haemoglobin, specially in AML, CML and M.MY. patients were noted.
The increased reticulocyte count was observed more frequently in chronic leukaemia which consisted of 28 out of 58 patients with CML and 7 out of 20 CLL. The increase in the rate of incubation autohaemolysis at 24 hours as observed in 105 out of 135 patients was significant at 1% level in CML, ALL, AML, CLL, AMOL and Ery.L. and at 5% level in M.my. patients. The highest number of patients with increased value of incubation autohaemolysis at 24 hours were in patients with CLL and it was 90%. In CML, AML and ALL the percentage of number of patients with increased auto-haemolysis were 72.9%, 73.3% and 80% respectively. In Multiple myeloma 2 out of 3, in Ery.L. 3 out of 4 and all the 5 AMOL had increased values of incubation autohaemolysis. At 48 hours incubation, the increase in the rate of autohaemolysis was significantly abnormal in many patients in all types. In CML, 44 out of 47 (98.6%), in AML 27 out of 30 (90%), in ALL 24 out of 25 (96%), and all the 20 CLL patients had abnormally increased value of auto-haemolysis at 48 hours. All the 5 AMOL, 4 Ery.L. and 1 out of 3 M.my. had increased value of incubation autohaemolysis at 48 hours.

The level of plasma haemoglobin remained increased in 13 out of 147 patients and this was more in AML (8 out of 32 AML). The direct Coombs' test was positive in 36 out of 146 (24.6%) patients. Of 146 patients, 23 (15.7%) had both increased rate of incubation autohaemolysis and positive Coombs' test, 86 had only increased autohaemolysis and 13 had only Coombs' test positive.
In those 122 patients with increased incubation auto-haemolysis and/or positive direct Coombs' test, anaemia of varying degree was present in majority of the patient and 41 had increased reticulocyte count.

Increased osmotic fragility of red cells was present in 52 out of 146 patients and increased incubation fragility at 24 hours was present in 68 out of 146 patients with different types of leukaemia. Total serum bilirubin remained increased in 10 out of 147 patients in the present series. Abnormally increased agglutinin titre was in 3 out of 87 patients and 1 had increased foecal urobilinogen out of 47 cases. In 77 patients, 7 had positive sucrose lysis test. Hanri test was positive only in 1 out of 92 patients.

On follow up study it was observed that during remission when the disease remained controlled there was improvement of Hb level and the abnormality in haemolytic tests also improved significantly towards normal level. The 35 patients with increased osmotic fragility in the initial stage were studied later and 25 showed marked improvement to normal range during remission.

Out of 30 patients with increased auto-haemolysis in initial stages, 24 became normal and 6 had some improvement of the abnormality of the auto-haemolytic tests during remission period.
The data presented in part 1 and 2 showed that two factors were actively involved in the causation of anaemia in leukaemia. One factor was the disordered erythropoiesis, either quantitative or qualitative, during the active phase of the disease. During remission period the defective erythropoiesis assumed a more or less normal form. The other factor, haemolysis, either occult or overt was demonstrated clearly. Due to gross abnormality in the erythropoiesis the overt form could not be manifested in all cases, especially in the acute leukaemia. However, the occult form was present in majority of the patients as evident by the abnormal results of the tests for detection of haemolysis. Haemolysis and abnormal erythropoiesis varied from one patient to other. There were some patients where the abnormality of erythropoiesis was predominant, and in other the haemolysis was more pronounced to cause anaemia.

In some patients there were decreased reticulocyte count along with the low level of Hb and low percentage of erythroid cells in the bone marrow specially in acute leukaemia whereas in other group there were increased reticulocyte count along with increased erythroid cells in the bone marrow but the level of Hb was low. The disturbed erythropoiesis in the former and increased haemolysis in the later were the cause of anaemia. The abnormality of the autohaemolytic test was present in both groups, from which it became very difficult to say that in acute leukaemia whether dis-erythropoiesis alone or in combination with
haemolysis was the cause of so severe anaemia. However, during remission, there was marked improvement of both the factors and it was very likely that the disease process itself was responsible for anaemia. The knowledge regarding the aetiology of leukaemia is still incomplete and in the absence of any definite etiologic agent, the cause is definitely not known regarding the factor for this dis-erythropoesis and haemolysis.

From the data in the part III it appeared that there were 7 patients with G-6-PD deficiency out of 147 investigated patients with different types of leukaemia. These 7 patients had abnormally low level of preincubated erythrocytic GSH and abnormally lowered values of post incubated erythrocytic GSH. The deficiency of the enzyme G-6-PD and GSH and its unstability was not present in majority of the patients. The small number of patients who had low values for the enzymes in the initial stage of the disease, improved to normal level in remission excepting one. This showed clearly that the activity of the enzyme was depressed due to leukaemia. As yet there is no adequate explanation for the reduction of enzyme value in these 7 patients during the active phase of the disease. Probably there was somewhat rapid utilization of the enzyme during that period. During remission period, presence of comparatively healthy cells produced by normal clone of the stem cells, in peripheral blood, showed normal values for the enzymes. Whether this deficiency was effect of leukaemia or not is very
difficult to explain but it can be said that the enzyme
deficiency was not the only cause of early red cell destruction
as in the absence of any deficiency of the enzymes the red cells
of other leukaemic patients showed evidences of early haemolysis
or prehaemolytic changes. Similarly the activity of GR remained
normal in 147 patients excepting one and from this it appeared
that in H.M.P. shunt the enzymes had no role for the early
destruction of red cells. In presence of normal G-6-PD, GR, GSH
and its stability in majority of the patients the other remaining
enzymes like glutathione synthetase and glutathione peroxidase in
the hexosemonophosphate pathway are not expected to be disturbed.
From this it may be concluded that in the pathogenetic mechanism
of early haemolysis, in leukaemia the enzymes in the hexose
monophosphate pathway are not depressed to such an extent as to
cause haemolysis. However, it may be an additional factor in some
cases. The other enzyme PK in the Embden-Meyerhof pathway was found
to be low in 23 out of 69 (33.33%) patients with different types
of leukaemia. Of those 23 patients, 21 belonged to the group of
acute leukaemia. In those 23 patients increase in reticulocytes
were noted only in 4 but increased autohaemolysis was recorded
in 16 patients. The decrease in the PK value was highly
significant at 1% level in AMOL and Ery.L. and at 5% level in AML.
The majority of the patients with PK deficiency had increased
value of incubation autohaemolysis at 24 hours and that clearly
demonstrated the presence of pathological change in erythrocytes.
There was correlation between the incubation-autohaemolysis and PK activity which was significant at 1% level in CML, M.my. and ALL.

In the active phase of the disease, probably the anaerobic (EM) pathway was abnormally utilised by red cells arising out of leukaemic clone, causing a depletion of the PK. Alternatively, these leukaemic red cells might have started with a low level of enzyme. During remission, increased number of red cells produced by normal clone of precursor were in circulation and showed normal PK value.