DISCUSSION
One hundred and five beta thalassemia trait (BTT) cases were detected. In the Clinic group of consecutive 100 anemia cases, BTT was 12. The incidence detected in hospital population by Pillay et al\textsuperscript{20} was 0.6%, Jain et al\textsuperscript{21} 1.75% and Chatterjea et al\textsuperscript{19} was 3.7%. The present incidence was higher. This may be due to this clinic being a referral hematology Unit. Eighteen individuals were detected as BTT from 201 individuals of Lohana community (8.9%). The incidence of BTT is high which is reported by other workers. Sharma et al\textsuperscript{22} reported 13.6% in Lohana. Bhatia et al\textsuperscript{24} reported 10.7% in Cutbhi Lohana, 17.2% in Halari Lohana, 6-8% in Sindhi Lohana and 5.2% in Punjabi Khatri. The present study and other worker's report indicate the incidence of BTT is high in Lohana community.

Beta thalassemia has a high incidence in a broad tropical belt extending from the Mediterranean basin through the Middle East and Far East. There are very few accurate figures for the incidence of the beta thalassemia genes. Although attempts have been made to obtain relatively accurate gene frequencies in a few population, it has not always been possible to apply techniques which would satisfy the full diagnostic criteria for the different forms of beta thalassemia. Many series of study are based on small samples often with a hospital bias.\textsuperscript{1}
OCCURRENCE OF BTT IN DIFFERENT AGE GROUP & SEX.

Highest incidence was 12.38% in 3rd decade (20-30 yrs) in both sexes. In male 12.38% (13) cases were found in 1st decade (0-10 years). In female highest incidence was in 3rd decade i.e. 19.04%. The findings are similar to those of Agarwal et al (1982).42

SYMPTOMS OF BTT.

It is now clear that the true heterozygous states for the common forms of beta thalassemia are usually symptom free and associated with a mild degree of anemia. The mild degree of anemia which accompanies the condition brings them to the notice of the physician. Literature on Symptomatology of BTT is difficult to evaluate. In 123 cases of BTT, Gardikas (1968)129 found 12 cases (9.75%) with complain of fatigue and lassitude, 5 cases (4.06%) of mild pyrexia without any cause and 22 cases (17.8%) related to gall stone. Mazza et al (1976)130 noted 40% of their patients complained of weakness. Symptoms of this kind was not reported by Pootrakul et al (1973)131 and Knox-Macaulay et al (1973)132 in large series of Thai and British subjects. Since the hematological findings were similar in these studies, the difference in the associated symptomatology is difficult to
It is possible of course that British and Thai subjects are more stoical than their Mediterranean counterparts. Whatever the cause it is quite evident that the vast majority of patients of BTT are symptomless. The only common clinical presentation of BTT is mild refractory anemia. Callender et al (1961) found patients with BTT have symptoms of pain in abdomen (in left upper quadrant) which may be due to perisplenitis. In the present study out of 87 BTT cases 37 cases were symptomatic. Pallor was found in 9 cases (24.32%), fever in 8 cases (21.62%), cough and cold in 7 cases (18.91%), jaundice in 3 cases (8.1%), abdominal pain in 1 case (2.7%) and fatiguability was found in 5 cases (13.51%) (Table No.1). Similar findings have been reported by Gardikas (1968) and Mazza et al (1976).

SIGNS OF BTT.

The physical findings in BTT seem to vary between races. In the majority of individuals there are no abnormal physical signs. In the present study the signs found were pallor in 32.18% cases, splenomegaly in 9.18% cases and hepatomegaly in 10.34% of cases. Jaundice, palpable lymphnodes and petechie were found in one case each. The incidence of palpable spleen was varied enormously in reports from Mediterranean region. Fessa (1959) found splenomegaly in 50% cases in Greece. While Gardikas (1968)
observed splenomegaly in 14 out of 123 BTT cases of the same racial group. Mazza et al (1976) observed enlargement of liver in 10% cases and splenomegaly in 19% cases respectively. Splenomegaly is not a feature of BTT. In a suspected case of BTT with splenomegaly, Weatherall et al (1981) had made a practice to look for another cause. Agarwal et al (1982) found 32 cases of splenomegaly, hepatomegaly in 30 cases, jaundice in 9 cases and hemolytic facies in 10 cases from a series of 143 cases of BTT.

INDIVIDUAL PARAMETERS.

Hemoglobin (Hb):

In beta thalassemia trait there is ineffective erythropoiesis which results in decreased hemoglobin synthesis. Carrier of BTT can have varying degree of anemia. In the present study of 105 cases of BTT, Hb was significantly low (\( P < 0.001 \)) than that found in control cases (Table No.4). Castaldi et al (1974) found significant degree of anemia in 86 out of 87 male BTT and in 61 out of 70 female BTT. In the present study there was no significant difference in Hb level between 10 males and 10 females BTT with ID. Further there was statistical significant difference (\( P < 0.01 \)) in Hb level between nonthal without iron deficiency cases (Mean \( 11.96 \pm 2.8 \text{ gm} \)) and nonthal iron deficient anemia (Mean Hb \( 6.73 \pm 2.5 \text{ gm} \)) table No.8. Agarwal et al (1982) found
mean Hb level was significantly lower in symptomatic HTT
9.66 ± 2.2 gm% than in asymptomatic HTT 11.3 ± 1.8 gm%
(P < 0.25). The mean Hb was lower in cases with spleno-
megaly (8.92 ± 2.23 gm%) than in cases with non palpable
spleen 9.9 ± 2.13 gm% (P < 0.5).

RED BLOOD CELL COUNT (RBC).

The RBC count is slightly raised in BTT. There
was no significant difference in RBC count of BTT table No.4
(Mean is 5.22 x10¹² ± 0.7) and that of normal (Mean 5.23x10¹²
± 0.6/dl) (P > 0.1). There was no significant difference
in RBC count of male and female BTT with and without iron
deficiency table No.6. The mean RBC count (Mean 3.57 x10¹² ±
0.8/dl) in nonthal ID was lower than nonthal without iron
deficiency anemia (Mean 4.74x10¹² ±0.68) (P < 0.01) (Table No.8).
This corresponds with the statement of Weatherall and Clegg¹.
Hammond et al (1964)⁶⁸ reported 50% cases of BTT had higher
values of RBC count than control. Weatherall (1964b)¹³⁶
observed that the degree of anemia in African Negroes with
BTT was less than anemia in Mediterranean BTT cases. This
finding has been confirmed in more recent series by Charache

RBC MORPHOLOGY:

In almost all 87 cases in the clinic group,
morphological abnormalities of RBC was marked. Microcytosis,
hypochromia, anisocytosis, target cells, schistocytes were
found. Basophillic stippling was found in smears of four cases. Hypochromia is due to rapid cell division. Basophillic stippling is due to ineffective erythropoiesis and precipitation of alpha chain. The findings of peripheral smear corresponds with the observation of Weatherall et al (1964b)\(^{136}\). Over 350 beta thalassemia heterozygotes, they had not seen a case without any abnormality in the red cell morphology. Jain et al (1984)\(^{25}\) had observed microcytosis, hypochromia, anisocytosis, target cells, polychromasia and occasional nucleated erythrocytes.

**MEAN CORPOSULAR VOLUME (MCV).**

Hemoglobin level is low in thalassemia and it takes a long time for intra-cellular concentrations of Hb to reach a critical level to initiate cessation of nuclear activity. Cell division continues till this stage. Size of RBCs is reduced due to repeated cell division\(^{14}\). The MCV of 105 BTT was significantly lower than that of control cases (Table No.4). The MCV of BTT was significantly (\(P < 0.01\)) lower than that of nonthalassemic without iron deficiency anemia (Table No.7). There was significant difference in MCV values of nonthal without ID and nonthal with ID\((P < 0.01)\) (Table No.8). There was no significant difference between MCV of BTT with and without ID (\(P > 0.1\)) Table No.6. The MCV is reduced in both BTT and IDA. In IDA there is decrease in the synthesis of alpha chain Ban Basset et al (1974)\(^{98}\).
The observations are comparable with that of Hammand et al\textsuperscript{68} and Mehta (1982)\textsuperscript{77}. Pearson et al (1974)\textsuperscript{139} recommended MCV to be a reliable screening test for thalassemia trait when the RBC counts and indices are done in electronic counter. False positive results will come from IDA cases. In Negro population MCV may not be decreased in all cases of ETT. Pootrakul et al (1973)\textsuperscript{131} found a wide scatter of both MCV and MCH values.

In the present study with (cut off point) antimode of MCV at $\leq 67.5$ fl (Fig.27) 12 cases of ETT were false negative. But if the 80 fl is taken as the antimode as recommended by Weatherall et al (1981)\textsuperscript{1} then all 105 would be positive (Table No.21).

**MEAN CORPUSCULAR HEMOGLOBIN (MCH):**

The parameter MCH follows changes like MCV. In ETT it is reduced. In the present study the difference in MCH of ETT and control was significant (P $\leq 0.01$) table No.4. There was no significant difference in MCH of male and female ETT without ID table No.5 (P $\geq 0.1$) or with ID table No.6 (P $\geq 0.1$). In non-thalassemia cases there was significant difference in MCH of those with ID and without ID table No.8 (P $\leq 0.01$). The present findings of MCH is comparable with those of Pootrakul et al (1973)\textsuperscript{131} (Mean 20.0 ± 3.0) and Mazza et al (1976)\textsuperscript{130} (Mean 23.5 ± 1.02).
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC).

MCHC is usually normal. In the present study the MCHC of BTT cases and control group were not significantly different table No.4 (P > 0.1). There was no significant difference in MCHC values of BTT in male and female cases without iron deficiency anemia table No.5 (P > 0.1). Table No.6 shows significant difference (P < 0.02) between male and female BTT with ID. In non-thalassemia cases MCHC has significantly lower in IDA compared to non-ID table No.8 (P < 0.01). This shows effect of iron on synthesis of hemoglobin.

OSMOTIC FRAGILITY.

There is no agreement found in literature about the relationship between a change in osmotic fragility and the age of the cell. Different techniques have been used to obtain red cell functions of different cell age. Using serial osmotic hemolysis Simon and Topper (1957)\textsuperscript{140} Marks & Johnson (1958)\textsuperscript{141}, Marks et al (1958 a,b)\textsuperscript{142,143} Lavy et al (1959)\textsuperscript{144}, Sass et al (1963)\textsuperscript{145} concluded that young red cells were osmotically less fragile than older ones. On the other hand Cruz et al (1941)\textsuperscript{146} showed that new red cells formed after repeated bleeding were less resistant to hemolysis by hypotonic solution than the older cells. The red cell survival in
heterozygous thalassemia is normal by the Ashby technique Kaplan and Zudzer (1950). But Cr\textsuperscript{51} half life was just below the normal range in three reported series of Pearson et al (1960), Malamos et al (1961), Gallo et al (1975). Evidence for a small red cell population with a significantly shortened survival was reported by Honetz et al (1968). In the present study there was significant difference (P < 0.01, Table No.4) in osmotic fragility of red cell of BTT (Mean 46.5 ± 10.7) and that of the control cases (Mean 82.9 ± 21.4). There was no significant difference in the RBC osmotic fragility in male and female with BTT with or without IDA (P > 0.1, Table No.5). There was no significant difference in the RBC osmotic fragility in non-thal IDA and nonthal non-ID (P > 0.1, Table No.8). Bianco et al (1952) reported increased osmotic resistance in 417 BTT cases. Mehta et al (1972), Agarwal et al (1982) and Jain et al (1984) found osmotic resistance to be increased in BTT cases.

Katamis et al (1981) developed the naked eye single tube red cell osmotic fragility test (NESTROFT) and recommended its use for mass screening test for BTT. In the present study all 46 cases of BTT were NESTROFT positive (100%). As NESTROFT was included later in study in 46 cases of BTT NESTROFT was done. One case of thalassemia major with HbA2 >3.5%, 2 cases of thal intermedia and 4 cases of sickle-thal were NESTROFT positive. 16 cases of non-thalassemia with HbA2 <3.5% gave false positive result (Table No.12). In the survey
of Lohana community out of 201 cases, NESTROFT was positive in 18 cases of BTT (100%) and other 9 cases giving false positive results in 4.4% table No.13. In beta thalassemia there was no false negative. Only 1 case (0.65%) of non-beta thal trait was positive whom HbA2 > 3.5 was considered, but it was a case of thal major. When only NESTROFT was considered 11.5% was false positive from all the cases. In beta thalassemia trait no false negative was detected (Table No.14). NESTROFT was highly sensitive but not specific for BTT cases. In Katamis et al's (1981)124 work all 72 BTT except one was NESTROFT positive. 33 normal samples were negative for NESTROFT.

HbA2.

Whatever method may be used i.e. electrophoresis or chromatographic techniques, it appears that the upper limit of normal for HbA2 values is somewhere in the region of 3.3% and that in heterozygous beta thalassemia the range is from 3.5% to 7% with a mean of approximately 5%.

In heterozygous beta thalassemia this results in an alternation of the HbA/A2 ratio from the normal figure of 40 to a value in the region of 20. It is important to determine whether such an alternation reflects a relative or absolute increase of HbA2 to twice its normal percentage. There would have to be a decrease in hemoglobin A synthesis of a greater magnitude than that which usually occurs in heterozygous beta
SCATTERDIAGRAM SHOWING CORRELATION BETWEEN Hb A\text{a} AND M.C.H. IN BT

No. OF CASES 105

SECOND FREQUENCY

\[ r = 0.45 \]

\[ p < 0.01 \]
thalassemia. But several series have shown that there is no relationship between the level of HbA2 and hemoglobin level. Assuming that HbA2 is homogenously distributed among the red cells the average amount of HbA2 per cell as derived from the MCH and % of HbA2 can be computed as approximately 0.7 Pg. In an individual with an MCH of 20 Pg and HbA2 level of 5%, this value increase to a level of approximately 1.0 Pg. Weatherall et al (1964b) pointed out that there is a reasonable correlation between the percentage of HbA2 and MCH. For any given HbA2 the absolute amount of HbA2 is increased by about 33% to the range of 1 Pg. In the present study there is correlation coefficient (r= -0.45) Fig.No.29 between MCH & HbA2 ( P ≤0.01 ).

Increased HbA2 synthesis results from increased activity at both CIS and trans delta chain loci. Individuals who are heterozygous for both beta thalassemia and the delta chain variant hemoglobin A2 have increased levels of both normal (α2δ2) and abnormal minor hemoglobin component Cappelline (1959a), Weatherall et al (1976b). The increased output of delta chain is not the product of the delta chain locus adjacent to the beta chain locus carrying the thalassemia mutation but due to increased output from both delta chain loci: Quoted from Weatherall's thalassemia syndrome 1981 pp.232.
Early studies indicated that there is no correlation between HbA2 and Hb level, PCV, red cell morphology, HbF or any other hematological findings except possibly the MCH (Fessas (1959))\textsuperscript{134}, Weatherall (1964b)\textsuperscript{136}, Poottrakul et al (1973).\textsuperscript{131} Thus analysis of this type gives no clue as to the possible mechanism for the elevation of HbA2 in this disorder. A major difficulty in speculation about the possible mechanisms involved in setting the level of HbA2 in different conditions is a lack of basic information about the factors which mainly cause the red cells to contain approximately 40 times as many beta chains as delta chains. Wood et al (1978)\textsuperscript{152} reviewed the regulation of delta chain synthesis and concluded that a major factor is the relative instability of delta as compared with beta chain mRNA. It is apparent from an examination of the absolute delta chain values in the various forms of beta and delta beta thalassemia that there is imbalanced globin chain synthesis with excess alpha chain production. How this might bring about an absolute increase in delta chain synthesis is far from clear, if this is needed to be the mechanism. It seems unlikely that it is mediated at the transcriptional level (de Sandre and Veltore, 1972)\textsuperscript{153} and one is left with the intriguing possibility that some how excess alpha chains stabilise the relatively unstable delta chain mRNA allowing the synthesis of more delta chains than normal or increase the likelihood of the formation of hemoglobin A2 tetramers.
SERUM IRON (SI), TOTAL IRON BINDING CAPACITY (TIBC)
TRANSFERRIN SATURATION (TS) :

The serum iron saturation levels in heterozygous beta thalassemic cases show considerable variability. The mean values have not shown significant difference from normal (Katamis et al, 1972a, Pootrakul et al, 1973). The total iron binding capacity or serum ferritin level have also no abnormality. In the present study, there was no significant difference in SI, TIBC between BTT and normal control, Table No.4 ($P > 0.1$). Transferrin saturation of BTT and that of control case was significant ($P < 0.05$) table No.4. In non-thalassemia cases there was significant difference in SI, TIBC, TS between iron deficient and noniron deficient individuals table No.8 ($P < 0.01$). In male and female BTT cases without iron deficiency there was no difference in TS ($P > 0.01$) Table No.5. But in BTT with ID there was difference in total iron binding capability in both sexes ($P > 0.1$) Table No.6. The mean serum iron in male BTT without ID (153.80 ± 49.5) was more than mean SI in female BTT without ID (126.3±46.43) and it was statistically significant ($P < 0.05$) table No.5. The transferrin saturation in male BTT without ID (Mean 35.78±10.5%) was more than female BTT without ID (Mean 28.24±10.4) with $P < 0.01$ (Table No.5. But for serum ferritin level, there was no difference ($P > 0.01$) in both sexes i.e. in male (Mean 30.14 ± 4.8 μg/dl) and in female mean(29.11±10.4) table No.5. All the present findings also indicate regarding
the variability of iron status. In cases of BTT without ID there was low serum iron in female than male. The cause may be due to their menstrual blood loss. Out of 88 cases of BTT 68 cases both male and female had no iron deficiency and 20 cases of BTT had ID. Iron status is different in different BTT cases. The findings are similar to observation of Fajgion et al (1982). Iron absorption varies according to degree of ineffective erythropoiesis. Ineffective erythropoiesis increases iron absorption.

HbA2 AND ITS RELATION WITH IRON STATUS:

Various workers have studied the iron status and HbA2 level in BTT cases. Mehta (1982) studied the HbA2 levels in 102 subjects of BTT with and without ID and concluded that HbA2 level is not influenced by iron deficiency. Josephson et al (1958) found HbA2 to be reduced in BTT with IDA. Ban Basset et al (1974) reported decreased synthesis of alpha chain in IDA. This may explain the decrease level of HbA2 in IDA cases. In the present study there was no significant difference in HbA2 level in cases of beta thalassemia, trait with and without ID (Table No.7) t(de)=1.008(P >0.1). The scatterdiagram(Fig.No.20) shows that there is no decrease in HbA2 in beta thalassemia with ID. This observation is similar to that of Mehta (1982) and Saraya et al (1984) who concluded that HbA2 was not reduced in BTT with ID and it does not affect for diagnosis of BTT.
The HbA2 level between BTT and nonthal non ID cases was significant $t(ac)=22.8\ (P \leq 0.001)$ (Table No.7). This corroborates the observation of Ben-Basset et al (1974)\textsuperscript{98}. There was no significant difference in the HbA2 level in nonthal ID (Mean 1.74 ± 0.6) and nonthal without iron deficient cases (Mean 1.9 ± 0.7) ($P > 0.1$) (Table No.8).

Table No.7 shows the statistical significance between HbA2 level of nonthal ID and thal trait with ID $t(de)=18.98\ (P \leq 0.01)$. Similarly HbA2 value was significant between nonthal and thalassemia trait with ID $t(kl)=4.19$, ($P \leq 0.01$). This corroborates that in iron deficiency alpha chain synthesis is lowered. Iron status in 88 cases of BTT where iron status could be studied, 20 were ID and 68 BTT without ID.

**COMPARISON OF HbA2 CONTENT ESTIMATION BY TWO DIFFERENT METHODS:**

Out of the available methods electrophoresis with elution and microchromatography are accepted methods for estimation of HbA2. Cellulose acetate electrophoresis with elution is best. But cost factor is important. HbA2 estimation by paper electrophoresis and elution has given reliable results as reported by Mehta et al (1982)\textsuperscript{79}, Krishnan et al (1986)\textsuperscript{110}. In the present study a comparative study of HbA2 content estimation was done between paper electrophoresis with elution and microchromatography using DEAE cellulose. HbA2 levels in both the methods showed positive correlation in
3 groups (P<0.001) table No.15; r value in group I (BTT) 0.35 (Fig.20) 0.61 in group II (Non-thal IDA-Fig.No.21) and 0.22 in group III (Non-thal without iron deficiency)(Fig.No.22). The correlation of 2 methods is similar to that by Krishnan et al (1986). Ghosh et al (1985) had compared the paper electrophoresis with HbA2 estimation by densitometry and Microchromatography in normal subjects and BTT cases. In microchromatography they found HbA2 level to be clearly separated between the normal cases (Mean 1.75 ± 0.6) and BTT (Mean 5.03± 1.22). Densitometry of paper electrophoresis could not separate the normal cases (Mean 4.2 ± 2.51, Range 0.44 to 8.30) and BTT (8.09 ± 3.94, range 0.76-16.2 ). There was a great degree of overlapping between the values obtained in normal subjects and beta thalassemia trait. In the present study there was statistical significance in the value of HbA2 of both beta thal trait and IDA table No.16 and thalassemia trait and other nonthalassemia without iron deficiency(P<0.001). In case of IDA and other miscellaneous non-thal without iron deficient cases there was statistical significance of HbA2 in microchromatography (P<0.01) table No.16. In beta thalassemia trait cases there was no effect of iron deficiency in both the methods, scatterdiagram (Fig.No.20). Saraya et al (1984) also found the microchromatography method suitable to differentiate BTT without ID (4.9 ± 0.9 ) or with ID (4.5 ± 0.9) from normal 2.3±0.3 and concluded that iron
deficiency does not seem to have any significant effect on HbA2 in case of BTT.

Cut off point for HbA2 level to separate BTT from the other cases as determined by frequency distribution was 3.25 with paper electrophoresis and 5.25 with microchromatography (Fig. No. 23 and 28). With the cut off (antimode) value all the cases of BTT were detected with paper electrophoresis method whereas 30% cases were missed with microchromatography method (Table No. 17 & Fig. No. 24). The present study indicates that paper electrophoresis with elution is a more sensitive method for detecting beta thalassemia trait compared to microchromatography method. Specificity of both methods is high as only one non carrier of beta thalassemia fβ1 in the range of beta thalassemia by paper electrophoresis whereas there was no overlap with microchromatography method.

pH of developing buffer - The pH of the developing must be accurate. HbA2 is eluted at pH 8.3. Accordingly the pH of developing buffer must be between 8.3 and 8.35. Trace amount of HbA is eluted with HbA2 at pH 8.25 and lower, Efremov et al (1974)\textsuperscript{106}. The sensitivity of pH meter is variable and accurate digital reading of pH is difficult to maintain. If accurate pH is not maintained, then there is chance of no elution of HbA2 or traces of HbA may be eluted along with HbA2.
Quality of DEAE cellulose - Adjustment of the slurry to the right pH depends on the batch of DEAE cellulose (Moors et al, 1979). Cost factor is important to purchase the better quality of DEAE cellulose. Inferior quality DEAE cellulose may not give satisfactory results. This has been observed in the present study on comparison of a better quality and another cheaper quality of DEAE cellulose which did not give satisfactory result. It has been reported by Efremov et al (1974) that HbA2 estimation can be done from whole blood or blood collected on filter paper. More number of samples can be accepted and reports can be available on the same day. This method may be convenient for mass population survey for detection of thalassemia carriers.

HbA2 estimation by paper electrophoresis with elution is economic. One can see the different Hb bands in electrophoretic strip and can run control samples. In India paper electrophoresis with elution still continues as accept method. The present study and other workers Mehta et al (1982) and Krishnan et al (1986) have reliable results. HbF

Majority of beta thalassemia heterozygotes have either no elevation of HbF or only slight increase in the range of 1.3%. There is always a second genetic abnormality in conditions with high values of HbF. Most common of this is heterocellular HPFH.
Knox-Macaulay et al. (1973) suggested that some beta thalassemia heterozygotes with unusually high HbF% might also be heterozygous for Swiss type of hereditary persistence of fetal hemoglobin (HPFH). Wood et al. (1976b, 1977b), have published two series of interaction of heterocellular (HPFH) with beta thalassemia and found that hematological findings were the same as those of other beta thalassemia heterozygotes who have levels of HbF in excess of 4-5% are compound heterozygotes for beta+ or beta0 thalassemia and heterocellular HPFH. In the present study there was no statistical significance in HbF (P > 0.1) in BTT and normal control cases (Table No.4). Only one case of BTT had HbF 13%. This may be a compound heterozygotes for beta+ or beta0 thalassemia & heterocellular HPFH. The HbF% of the male and female BTT without IDA (Table No.5) had no statistical significance (P > 0.1). Similarly the HbF value had no statistical significance (P > 0.1) in cases between male and female BTT with IDA (Table No.6). In nonthalassemia cases also there is no significant difference in HbF% (Table No.8). This corroborates findings of Katamis et al. (1972). In all cases of BTT except two in clinic group HbF is not raised beyond 6%. This findings are similar to those of Beaven et al. (1961), Fessas (1959) and Weatherall (1964b).
DISCRIMINANT FACTORS (DF):

Iron deficiency anemia and thalassemia usually have the similar picture in peripheral blood smear i.e. hypochromic and microcytic red cells. In thalassemia the Hb is 7-10 gm% whereas in IDA it is less. Clinically they may be differentiated. Serum iron, transferrin saturation or serum ferritin and HbA2 estimation can differentiate the two conditions. But it is not possible in all centres or in mass population survey. Where these facilities are not available the discriminant factors are used. In the present study (Table No.18) 33.3% of BTT cases were positive for DF1 and 97.8% cases were positive (sensitive) in iron deficiency cases. DF1 was more sensitive for iron deficiency anemia than beta thalassemia trait cases. Table No.19 shows DF2 to be more sensitive (89.1%) positive) in IDA cases than BTT where 58.1% cases were positive. 55.23% of BTT cases were positive for DF3 whereas in IDA 93.47% of cases were positive. In the present study it was found that the discriminant factors are more sensitive to IDA cases than BTT. Mentzer et al (1973)\textsuperscript{30} reported 87 cases out of 103 patients to be correctly classified by DF2 and 86 of 103 by DF1. They had excluded IDA cases with low Hb gm%, for comparison. In the present series all IDA cases have been included. If the IDA cases with low Hb are excluded the sensitivity of DF1 and DF3 will rise. England & Frazer (1973)\textsuperscript{79} found thalassemia
trait patients were erroneously classified as IDA when other causes of anemia, such as hemolysis, bleeding or pregnancy were present. The coexisting disease which influence the red cell count invalidates both MCV/RBC, DF2 and DF1 as diagnostic indices in iron deficiency and thalassemia trait. In population survey for cases of iron deficiency anemia with low Hb, discriminant factors may not be helpful.

DIAGNOSTIC STATUS OF THE HEMATOLOGICAL PARAMETERS:

The diagnosis of beta thalassemia trait in individual cases is usually based on several hematological parameters which have different degree of sensitivity and specificity (Wasi et al, 1975). The commonest essential parameters are Hb gm%, MCV, HbA2, RBC morphology, Osmotic fragility and NESTROFT. Taking the antimode into consideration in 105 cases of BTT (Table No.21) i.e. Hb \( \geq 10 \) gm%, HbA2 \( \geq 3.2\% \), MCV \( \leq 67.5 \) fl, osmotic fragility \( \leq 57.5\% \) hemolysis and abnormal RBC morphology, 65.71% cases were sensitive to Hb \( \geq 10 \) gm%, 100% cases to HbA2, 60% cases to MCV \( \leq 67.5\% \), 100% cases RBC morphology, 81.6% cases to osmotic resistance and 100% to NESTROFT respectively. When these parameters are compared with other workers' antimode which have been recommended, there is similarity with the present work. Hb \( \geq 10 \) gm%, and HbA2 \( \geq 3.5\% \) have 100% sensitivity. MCV was less sensitive (40%) than the recommended value of \( \leq 80 \) fl.
where it was 100% sensitive. Wasi et al (1975) have recommended \( \mu \text{80 fl} \) where they have included alpha thalassemia also. But in the present study only BTT have been included keeping the diagnostic criteria of \( \text{HbA2} \geq 3.5\% \), osmotic fragility at \( \leq 57.5\% \) hemolysis was more sensitive 81.6\% than the recommended \( \approx 55\% \) of Dacie and Lewis having 78.10\% of cases. It was almost similar. There is 100\% sensitivity of NESTROFT in the present study and that of Katamis et al.

In the clinic group (Table No.22) the cases were divided into 3 groups e.g. Group-I with all 5 parameters abnormal, Group-II \((1-v)\) one parameter normal and other 4 parameters abnormal and Group III \((1-IV)\) with 2 parameters normal and 3 abnormal. Accordingly 26 cases were in group-I. In group II, 21 cases with normal MCV, 12 with normal Hb and 6 cases were with normal osmotic fragility. In group-III, 7 cases with normal MCV and osmotic fragility, 4 cases with normal Hb & osmotic fragility, 11 cases with normal Hb & MCV. As per the recommended values of the parameters, 45 cases were in group-I, 24 cases in group-II due to increased MCV + 15 cases due to decrease fragility. In group-III, 3 cases were with 2 abnormality.

In the present study globin chain synthesis or DNA analysis was not possible. So \( \text{HbA2} \geq 3.5\% \) has been taken as the diagnostic criterion as mentioned earlier. Cases with
HbA2 ≥3.5% have not been included as BIT. The following cases** have altered values in the hematological parameters which requires globin synthesis or DNA analysis to exclude the suspicion of BIT.

Silver Stroni et al (1978) 178 have reported BIT cases with normal HbA2 after studying the globin chain synthesis.

1. James : MCV-58 fl, MCH-19.4 Pg, MCHC 33.4 g/dl, DF2-19.4, DF3-1.4, screening test +ve Fragility at 0.4% buffer saline 21.6%, HbA2 3.3%.

2. Anmital : MCV-74.4 fl, MCH-24 Pg, MCHC 32.1 g/dl, DF1-0.4, DF2-13.5, DF3-0.94, Screening test +ve, Osmotic fragility at 0.4% -48.6% hemolysis, HbA2-3.3%.

3. Dimple Rajesh : MCV 77 fl, MCH 32.4 pg, MCHC 32.8 g/dl, DF1-0.85, DF2-14.1, DF3-0.97, osmotic fragility at 0.4% buffer 58.9%, HbA2-3.3%.

4. Dilip : MCV-67.3 fl, MCH-26.7 pg, MCHC-39.6 g/dl, DF1 -2.2, DF2-14.6, DF3- 0.84, HbA2-3.0%.

5. Bharati : MCV-57.6 fl, MCH 18.4 pg, MCHC 32 g/dl osmotic fragility 37.9%, HbA2-3.4%.

6. Mani : MCV 65.2 fl, MCH 20.8 pg, MCHC-32.0 g/dl, DF2-14.2, Osmotic fragility at 0% buffer saline-41%, HbA2-2.3%.

7. Chandra Sekhar. : MCV-62.29 fl, MCH 18.8 pg, MCHC-30.4 g/dl, DF2-10.2, DF3-0.93, osmotic fragility at 0.4% buffered saline 4.9%, HbA2-3.4%.

POPULATION SURVEY PROCEDURE :

It is necessary to identify BIT cases for several reason i.e. (i) to assess the prevalence of BIT in a population (ii) to prevent unnecessary prolonged hematinic therapy (iii) to identify the couples who can be made aware of the risk of birth
of a child with thalassemia major and offer them facilities of prenatal diagnosis if available.

Diagnosis of BTT is based on HbA2 \(7/3.5\%\). Initially there should be screening test for the parameter which is highly sensitive and can commonly be used for diagnosis of BTT. Any screening test in order to be useful for population survey should have the following characteristics:

(i) It should be simple to perform.
(ii) It should not require sophisticated and expensive equipment.
(iii) It should be possible to perform on a capillary blood sample.
(iv) It should be cheap.

When electronic counter is available, the screening test for BTT based on MCV \(75\) fl is ideal than NESTROFT Wasi et al (1975)\(^{158}\). In the absence of electronic counter NESTROFT satisfies all the criteria mentioned. It is simple and highly sensitive. The cost of the test works out to be Rs.1/- per test compared to Rs.17/- for HbA2 by paper electrophoresis and elution (Table No.24). Thus if one has to screen one thousand individuals, presuming the incidence of BTT as 3% in India and false positive NESTROFT as 10% the cost of survey will be Rs.2300=00 using NESTROFT as a screening test and doing HbA2 only in positive cases. The cost of screening
1000 individuals by doing HbA2 will be Rs.10,000=00. Besides screening can be completed in 2 weeks time using NESTROFT (100 test/day) whereas with HbA2 it will take 20 weeks (10 test/day).

**Table No.24.**

Showing comparison of cost of NESTROFT and HbA2 estimation by paper electrophoresis and elution.

<table>
<thead>
<tr>
<th>Component</th>
<th>NESTROFT</th>
<th>HbA2 Paper Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technician time</td>
<td>20*</td>
<td>136 **</td>
</tr>
<tr>
<td>Needle</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Syringe</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Reagent glass ware</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Equipment</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>173</td>
</tr>
<tr>
<td>Pre test</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

* Salary 1200/- P.M. 2 hours to do 50 tests by a semiskilled Technician. NESTROFT being a very simple test, it does not require a skilled technician.

** Salary Rs.2000/- P.M. 4 hours to do 10 tests by a skilled technician.
In fact it takes just 2 hours to do 50 NESTROFT tests and the technician's time cost had been theoretically worked out to be ₹200 for 50 tests. On the other hand HbA2 by paper electrophoresis required the services of a skilled technician taking 4 hours to do 10 tests and the technician's time cost worked out to be ₹136/-

1st flow chart is recommended for screening population.

All samples

NESTROFT.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
</table>

- MCV, Transferrin Saturation (TS)
- Hb paper electrophoresis

- BTT HbA2 (7.3%)  
  - ETT/Alpha thal
  - Normal HbA2
  - Low MCV, Normal TS

- Sickle cell disease, Iron deficiency
- E thal, E trait anemia
- Thal major low TS. (Hb Band on electrophoresis)

2nd flow chart for NESTROFT:

NESTROFT.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
</table>

- Iron therapy for 3 months and repeat NESTROFT.

NESTROFT Positive

- HbA2

NESTROFT Negative

- HbA2 had IDA.

HbA2 BTT

- Alpha thalassemia trait
- BTT with normal A2.
3rd Flow chart scheme suggested for mass screening for BTT when MCV determined on Coulter counter

MCV < 75 fl

HbA2 determination

HbA2 > 3.5%

BTT

Normal + decreased fragility

Reduced Serum Iron

Beta/Alpha thalassemia.

Genetic Counselling:

Genetic counselling is a communication process which deals with the human problems associated with the occurrence, or the risk of recurrence of a genetic disorder in a family. It involves helping families comprehend the medical facts, appreciate the way heredity contributes to the disorder, understand the alternatives and choose a course of action which seems appropriate to them. Murphy et al (1975) quoted by Pai (1986) 159.

To prevent the birth of beta thalassemia major cases the prospective couples are to be counselled. In premarital stage if both are BTT, marriage should be discouraged as they are expected to have 25% beta thalassemia major. In a
population, where different types of thalassemias are prevalent, it is relatively common to counsel couples in which the spouse is a carrier of the classical high HbA2 beta thalassemia and the other of a thalassemia like condition with normal HbA2 and HbF. This phenotype could be due either to environmental causes, such as iron deficiency or to one of several isolated or combined thalassemia mutations. The best defined thalassemia disorders associated with thalassemia like hematological phenotype and normal HbA2 and HbF levels are heterozygous alpha-thalassemia, heterozygous $\gamma\delta\beta$ and beta thalassemia, the double heterozygous state for delta and beta thalassemia including both beta$^+$ and beta$^0$ variants and, at least in some instances, heterozygous beta thalassemia associated with iron deficiency anemia (Silverstroni et al, 1978, Weatherall & Clegg, 1981, Pirastu et al, 1983) quoted by Paglietti et al (1985).

Prenatal diagnosis of thalassemia:

Prenatal diagnosis of beta thalassemia major was accomplished for the first time in 1975 by analysis of fetal blood obtained through placental aspiration at an advanced gestational age of 18 weeks. Due to continuous improvements of methodology in DNA analysis and in the introduction of new methods for fetal tissue sampling (Model, 1985) prenatal diagnosis of thalassemia can be accomplished now within the first few weeks of gestation.
Fetal blood analysis:

Fetal blood sampling is carried out at 18 weeks gestation either with fetoscopy or placental aspiration and is associated with relatively high fetal mortality 5.4%. This approach should be limited to those couples at risk for beta thalassemia in whom DNA analysis is not practicable. Fractionation of invitro synthesized globin chain on Corboxy Methyl cellulose column remains the most commonly used method. There is overlap in $\beta/\gamma$ ratio between beta thalassemia heterozygotes and beta$^+$ thalassemia homozygotes and is the cause of misdiagnosis occurring in approximately 0.8% of cases tested.

DNA analysis:

Fetal DNA may be obtained either from amniotic fluid cells or from chorionic villi. Amniocentesis is associated with a very low risk (0.3%) of fetal loss while chorionic villi biopsy carries a risk of fetal loss around 4%. Chorionic villi tissue can be collected as early as 9-10 weeks of gestation. Diagnosis within the 1st trimester is obviously more acceptable to many couples who might object for both psychological and ethical reasons to termination of pregnancy in the 2nd trimester.
Thalassemia that are caused by relatively gross structural changes in DNA such as deletion or inversion may be detected directly by restricted endonuclease analysis and by hybridization with appropriate globin gene probes\textsuperscript{166}.

Majority of beta thalassemia are caused by single nucleotide substitutions, deletion or addition of a few nucleotides. A few of these mutations alter a recognition site for restriction enzyme and can be detected directly by southern blot analysis\textsuperscript{167}. Contamination of chronic villous samples with maternal DNA may cause misdiagnosis. DNA finger printing with a minisatellite probe\textsuperscript{168} a technique which produces a DNA pattern completely specific for an individual, may eventually be used to detect decidual contamination and avoid this pitfall.

DNA analysis may replace the fetal blood analysis for prenatal diagnosis. Fetal blood will be needed for those couples: (i) in whom the mutation has not been defined (ii) those presenting too late for a molecular characterisation of the defect, (iii) in those in whom linkage analysis with polymorphic site is not applicable.

The characterisation of pattern of polymorphic restriction sites (haplo type) in beta thalassemic chromosomes from several populations and direct oligonucleotide hybridization
or sequence analysis of cloned beta globin genes from each
haplo type has led to defining the population. In
each population a few mutations account for the majority
of cases of beta thalassemia. Methods to screen beta thala-
semia heterozygote simultaneously for two mutations have
been devised and this approach may be used for prenatal
diagnosis in cases at risk for a genetic compound.

Impact of prenatal diagnosis at community level:
The prevention programmes aimed at the control
of thalassemia major based on voluntary heterozygote screen-
ing and prospective prenatal diagnosis have been introduced
recently in several Mediterranean countries namely Cyprus,
Greece, Sardinia and the Ferrara province in Italy. The
programme has been largely effective resulting in a consistent
decline in the incidence of thalassemia major. W.H.O. working
group (1983) found two main reasons for residual thalassemia
major births in those communities. Main reason is complete
lack of any information about thalassemia in both parents.
Second reason might be nonacceptance of prenatal diagnosis after
genetic counselling because of ethical reason or of late
gestational stage. It indicates appropriate education of the
community and introduction of chorion villous sampling for
early detection of thal major will result in a further reduction
in the incidence of thalassemia major in these Mediterranean
communities. On the other hand prevention programme based on carrier screening and genetic counselling in the absence of prenatal diagnosis (Carried out several years ago) produced no consistent effect on birth rate of thalassemia\textsuperscript{172}.

A study observed that in Hindu, Sikhs & Pakistani community, a minority of couples do not accept fetal diagnosis and termination of pregnancy at least within the 2nd trimester\textsuperscript{171,173}. Cao et al (1986)\textsuperscript{174} suggest alternative method of controlling beta thalassemia in those populations will have to be explored.

**Future prospect:**

Further progress in the technology of DNA analysis will probably take place. Non-radioactive probes with a larger Shelf-life and wider availability than radioactive ones will be available\textsuperscript{175}. This may allow the introduction of prenatal diagnosis based on DNA analysis in less developed countries where thalassemia is prevalent. Primer-mediated enzymatic amplification of specific beta globin gene sequences in genomic DNA may increase the speed and sensitivity of the test and make it possible to analyse successfully very small samples ($\sim 20$ mg) of genomic DNA\textsuperscript{176}. The techniques which detect single base pair mismatches in RNA/DNA or DNA/DNA hybrids may be applicable in the near future to the study of genomic DNA\textsuperscript{177}. These methods
may make prenatal diagnosis easily accessible by DNA analysis even when the underlying mutation has not yet been identified. Even with the continuous improvement in the methodology of prenatal diagnosis, the homozygous state will not be completely prevented because in several population the interruption of pregnancy as a method of controlling the birth of affected children is not commonly accepted.

Thalassemia major:

Twelve cases were found to be thalassemia major. Their ages were 5 months to 6 years (Mean 27.8 ± 22.8 months). Amongst them 5 were male and seven females. Katamis et al (1975) reported a series of thalassemia major with mean presentation age 13.1 months. In the present study the mean age of presentation was eight months. Hb, PCV, TRBC, MCV, MCH were low (table No.9). Reticulocyte had not significantly raised. RBC morphology was microcytic hypochromic and anisocytosis in all cases. Poikilocytes (5 cases) target cells (9 cases) and basophilic stippling was found in 3 cases. All the findings were due to ineffective erythropoiesis. Iron status was found to be variable. All had normal transferrin saturation (TS) except one case had iron deficiency. In this case it could have been due to wrong laboratory technique. Iron absorption studies show variable results. Larizza et al (1958) found values at lower limit of
normal whereas Smith et al (1957b) & Erland et al (1962) found increased absorption. The level of iron absorption is related to the degree of ineffective erythropoiesis and erythroid hyperplasia as the iron absorption is reduced after transfusion, Weatherall (1981)\(^1\).

HbF was found to be variable. In 9 cases HbF was raised and in 3 cases it was normal. HbF production is a major feature of both beta\(^0\) or beta\(^+\) thalassemia. Transfusion dependent homozygous beta\(^+\) thalassemia usually have about 50% of total hemoglobin at presentation. Once they have started on regular transfusion, the level of HbF is usually extremely low in the peripheral blood and the diagnosis of beta\(^0\) and beta\(^+\) thalassemia becomes impossible without hemoglobin synthesis study\(^1\). The 3 cases in the present study with low level of HbF had received blood transfusions. Description of low level of HbF in homozygous beta thalassemia who have received transfusion may give totally erroneous indications of their real output of HbF.

On repeated transfusions the endogenous production of HbF is reduced variably. This is same for HbA2 also. In patients who have already received transfusion, the HbA2 is variable. In patients with rich production of HbF, HbA2 is negatively correlated\(^1\).

In the present study the HbF and HbA2 had also almost negative correlation \(r = -0.0072\) Fig. No. 30. Estimation of HbF & HbA2 in beta thalassemia cases will not be conclusive
in patients who have received blood transfusion prior to estimation of the said two parameters.

Thalassemia intermedia:

The heterogeneity of the beta thalassemia are well known. Although the genetic mechanisms responsible for producing many of intermediate forms of thalassemia are still not well understood. It is quite clear that this clinical syndrome can result from many different inherited disorder of hemoglobin synthesis. The major defect in all the thalassemias is imbalanced globin chain synthesis and the majority of clinical features can be ascribed to the deleterious effects as erythropoiesis caused by globin chains which are produced in excess. This can be studied by (i) complete family study (ii) measurement of the relative rate of alpha/beta chain synthesis using radioactive amino acid in corporation technique (iii) DNA restriction endonuclease mapping. As the later two procedures was not possible, genetic study was based on family study.

In the present study 3 cases of beta thalassemia intermedia were detected in the clinic group. HbA2 was normal in all 3 cases, HbF was increased in 3 cases.

The 3 cases were thalassemia intermedia with normal HbA2 and high HbF. Aksoy et al (1982) and Weatherall and Clegg (1972) have reported the presence of some beta thalassemia with normal HbA2.
Sickle cell beta thalassemia:

Sickle cell beta thalassemia is a double heterozygous state for HbS & beta thalassemia. Clinically it is less severe than sickle cell anemia, Powel et al (1975) and Silverstroni et al (1945). Hb-beta° is associated with more severe disease than HbS beta+ thalassemia(Sergeant et al,1973). The relative amount of HbF also may influence the severity ( Shaeffor et al,1976), quoted from Wintrobe's Clinical Haematology, 8th Edition Lea & Febiger. Sickle cell thalassemia gives the blood picture of both thalassemia trait i.e., microcytic hypochromia and sickling test positive.

Diagnosis can be done by family study. One of the parent is a thalassemia trait with raised HbA2 i.e, 7/3.5% and the other is sickle cell trait/disease. This can be done by family study and Hb electrophoresis. In the present study(Table No.11) 5 cases of sickle cell thalassemia were detected. In all cases either the father or mother was having HbS & the other raised HbA2 7/3.5%. The parameters like Hb, MCV, MCH and osmotic fragility were low. NESTROFT was positive in all 4 cases where the test was done. None were iron deficient. HbF was raised in all cases. HbA2 was raised in 1 case and the rest had normal HbA2. There are reports of raised HbA2 in sickle cell thalassemia cases. An elevated level of HbA2 is not always found in sickle cell beta thalassemia. The exception to the rule seems to occur in individuals with unusually high levels of HbF in particular, those who have also inherited the Swiss HFEH gene. As the number of case is small it is not possible to comment on the HbA2 levels.