# Lab Diagnosis of Thalassaemia

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Hypochromia and microcytosis are the hallmark of thalassaemia. Once iron deficiency is excluded thalassaemia is the next most common cause of hypochromia and microcytosis in Pakistan. Since thalassaemia is an inherited disorder it uniformly affects all red cells whereas in iron deficiency the red cells of varying ages are affected differently. Another important distinguishing feature of thalassaemia is basophilic stippling that lacks in iron deficiency.

**Typical β-thalassaemia trait**

Most people with typical $\beta^o$-thalassaemia trait have haemoglobin in the low normal range, raised TRBC, low MCV (usually $\leq$ 75 fl) and low MCH (usually $\leq$ 25 pg) (Table 1). RDW is lower in thalassaemia (usually < 41fl) than in iron deficiency (usually > 41fl). Diagnosis of β-thalassaemia trait is confirmed by Hb-$A_2$ level that typically ranges between 3.5-7.0%. Hb-A2 levels between 3.0 and 3.4.0% are considered borderline and may be seen in normal people or β-thalassaemia carriers with coexisting iron deficiency or α-thalassaemia trait.

**Atypical β-thalassaemia trait**

People who have β-thalassaemia mutation but whose phenotype is masked by a variety of mechanisms are categorized as atypical β-thalassaemia carriers. Their identification is often difficult. It is important to identify them because their marriage to an individual with β-thalassaemia trait can produce a child with thalassaemia major.

<u>$\beta^+$-thalassaemia</u>

Mild $\beta^+$-thalassaemia trait is relatively uncommon in Pakistan. Most people are due to Cap+1 (A-C) mutation that is seen in about 2.5% of β-thalassaemia carriers. They have almost normal haematological parameters including Hb-$A_2$ level (Table 1). Most Cap+1 carriers have MCH in the lower normal range (around 26 pg). Because of the silent nature the carrier status of Cap+1 carriers is usually discovered when they give birth to a child with thalassaemia major. The confirmation of Cap+1 mutation is possible only by PCR.

<u>Co-existing iron deficiency</u>

Since iron deficiency is very common in Pakistan it is also common to see it co-exist with β-thalassaemia trait. Such individuals are more anaemic and have low serum ferritin but their Hb-$A_2$ levels are usually high. Approximately 10% of people with β-thalassaemia trait and co-existing iron deficiency have normal Hb-$A_2$ levels. In such patients Hb-electrophoresis may be repeated after correction of iron deficiency or PCR may be done to resolve the ambiguity.

Co-existing α-thalassaemia and β-thalassaemia trait

Co-inheritance of α-thalassaemia and β-thalassaemia trait is not uncommon in Pakistan. It results in masking of the haematological features of typical thalassaemia trait. One gene deletion α-thalassaemia (-α/αα) does not affect the features of typical β-thalassaemia. People with co-existing β-thalassaemia trait and two gene deletion α-thalassaemia (-α/-α or --/αα) tend to have "normalized" red cell indices (usually around the lower normal range). However, their Hb-$A_2$ level is usually >4.0%. In doubtful cases PCR may be required to show the co-existence of β- and α-thalassaemia mutations.

Hb-D/$\beta^o$-thalassaemia

It is not uncommon to see individuals having co-inheritance of $\beta^o$-thalassaemia and Hb-D trait. They have hypochromic microcytic red cell indices. Hb electrophoresis shows raised Hb-$A_2$ and single band in the region of Hb-D. They do not have Hb-A because of the presence of Hb-D mutation on one chromosome and $\beta^o$-thalassaemia mutation on the other. Since no Hb-A is formed they show only Hb-D and Hb-$A_2$. The diagnosis may be confirmed by parent's study (one having β-thalassaemia trait and the other Hb-D trait) or by PCR. It is important to distinguish Hb-D/$\beta^o$-thalassaemia from homozygous Hb-D because the former when married to a $\beta^o$-thalassaemia carrier can result in the birth of a child with thalassaemia major.

Table 1. Comparison of haematological parameters in β-thalassaemia trait and normal individuals from Pakistan (Ahmed 1998).

| **Parameter** | **Normal** | **$\beta^o$-thalassaemia trait** | **$\beta^+$-thalassaemia trait** |
|---|---|---|---|
| Hb | | | |
| Male | 13.6 ± 1.7 | 12.6 ± 1.2 | 12.1 ± 1.8 |
| Female | 11.6 ± 1.7 | 10.5 ± 1.1 | |
| Female (Pregnant) | 11.3 ± 1.5 | 9.6 ± 1.5 | |
| TRBC | | | |
| Male | 5.34 ± 0.80 | 6.23 ± 0.62 | 4.46 ± 0.69 |
| Female | 4.81 ± 0.66 | 5.39 ± 0.62 | |
| Female (Pregnant) | 4.05 ± 0.41 | 4.81 ± 0.70 | |
| MCV | 84.7 ± 7.8 | 63.0 ± 5.6 | 82.4 ± 3.8 |
| MCH | 28.1 ± 3.3 | 19.9 ± 1.6 | 26.0 ± 1.92 |
| Hb-$A_2$ | 2.5-3.5% | 4.0-7.0% | 2.8-3.2% |

**β-thalassaemia major**

The blood picture of typical β-thalassaemia major shows moderate to severe hypochromic, microcytic anaemia, marked aniso-poikilocytosis, and numerous nucleated red cells (Fig. 1). The white cell count is usually raised due to the presence of numerous nucleated red cells that are counted as white cells by the electronic counters. The actual

white cell count may be obtained by correcting for the number of nucleated red cells seen on the blood smear.

The diagnosis of β-thalassaemia major is confirmed by finding markedly raised fetal haemoglobin (Hb-F) that ranges from 30% to over 95% (Table 2). In an un-transfused patient the presence of Hb-A indicates $\beta^{+}$-thalassaemia whereas its absence indicates $\beta^{o}$-thalassaemia.

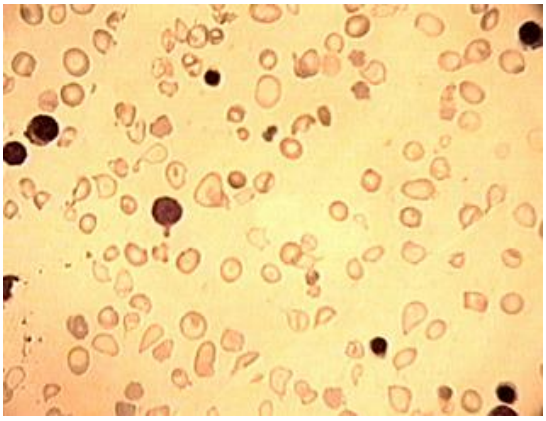

Fig. 1. Peripheral blood picture in β-thalassaemia major showing marked aniso-poikilocytosis, hypochromia, microcytosis, and many nucleated red cells.

**Diagnosis of β-thalassaemia major in previously transfused patients**

Recent blood transfusion(s) can create considerable confusion in the diagnosis β-thalassaemia major. The typical picture is modified. Depending on the amount and the frequency of blood transfusions the red cell morphology becomes dimorphic (Fig 2) while all of the red cell parameters are also normalized. The Hb-F is reduced due to an immediate effect of haemodilution and a late acting suppression of endogenous erythropoiesis. The Hb-F may fall to <1% in patients who are on chronic blood transfusions. Table 2 gives a comparison of the haematological parameters in patients of β-thalassaemia major with and without blood transfusions.

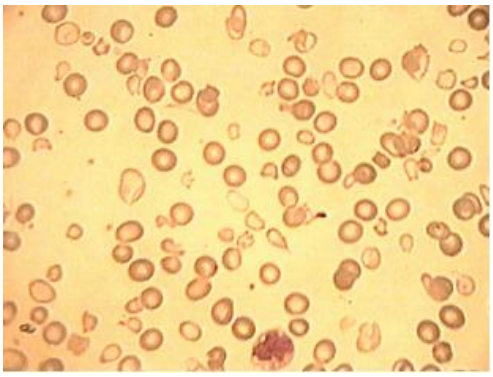

Fig. 2. Peripheral blood film in patient of β-thalassaemia major who has received recent blood transfusions. A mixture of hypochrmic microcytic red cells and the recently transfused normochromic normocytic red cells is seen.

It is common practice to withhold blood transfusions in the multiply transfused patients of β-thalassaemia major in the hope that the haematological picture would become clearer. This may be useful in patients who have received only few blood transfusions. But it is of no use in patients who have received multiple blood transfusions because erythropoiesis in such patients usually remains suppressed for a long period. The diagnosis in the multiply transfused patients can be established by demonstrating β-thalassaemia trait in the parents or by PCR for the β-thalassaemia mutations.

Table 2. Comparison of the haematological parameters in patients of β-thalassaemia major with and without blood transfusions (Ahmed et al, 2003).

| **Haematological Parameters** | **Untransfused (n=171)** | | **Transfused (n=109)** | | ***p* value** |
|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | |
| Hb (g/dl) | 6.3 | 1.9-9.0 | 6.2 | 2.3-11.2 | 0.20 |
| MCV (fl) | 70 | 57-83 | 74 | 58-98 | 0.014 |
| MCH (pg) | 21 | 15-29 | 24 | 16-31 | 0.015 |
| Hb-F (%) | 95 | 30-97 | 31 | 0.5-97 | <0.001 |

**References:**


1. Suhaib Ahmed (1998) Approach for prevention of thalassaemia in Pakistan. PhD Thesis, University of London.

2. Suhaib Ahmed; Zahur ur Rehman; Karamat A. Karamat (2003) Diagnosis of β-thalassaemia major in previously transfused patients. JCPSP 13: 19-20.