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Evalution of Beta Thalassemia in Young Population with Microcytic Hypochromic Anaemia

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ABSTRACT

Thalassemias are the commonest monogenic disorders in the worldwide.Beta Thalassemia Majorhave severe symptoms and life-threatening anaemia. Beta Thalassemia minor may have mild anaemia. Beta Thalassemia Intermedia have moderately severe anaemia are the major disorders that require lifelong management and are to be considered for prevention. In India, Beta-Thalassemia is prevalent across the country, with an average frequency of carriers being 3-4%.This is a cross sectional study conducted between 2017 oct to 2020 octin Indore in 100 patients as a workup for anaemia and clinically suspected cases of Hemoglobinopathy or beta thalassemia. Blood samples were collected and sent for Haemoglobin Electrophoresis using cellulose alkaline technique.Whole blood sample were analysed for the evaluation of beta thalassaemia in 100 young population who were affected with microcytic hypochromic anaemia. Out of total 12 subjects diagnosed as having three was beta thalassemia major, 02 were males and 01 were females and three was beta thalassemia intermedia, 01 were males and 02 were females. Among the beta thalassemia minor, o4 were males and 02 were females and the remaining 88 were non thalassemia.Most common cause of MHA was 2nd being thalassemia. Differential diagnosis based on complete hemogram and peripheral smear is possible but special tests like serum iron profile and hemoglobin electrophoresis are a must for confirmation of diagnosis.

Keywords: Beta-Thalassaemia, Haemoglobinopathies, Peripheral Blood Smear, Complete Blood Count (CBC),Haemoglobin electrophoresis,.

I.INTRODUCTION

Thalassemia is the most commonest monogenicdisorders in the word.Beta thalassemia is common around the Mediterranean, Indian subcontinent, South East Asia and relatively common in those of African ancestry. It is recognized but rare in the indigenous British population and in other Northern European populations (Weather all and Clegg, 2001; Bain, 2006). The frequency of beta thalassemia ranged between 1 to 17% with overall carrier frequency of 3 to 4 % in India, About 3% of the world's population carries the genes for Beta Thalassemia and it is estimated that every year about 60000 thalassaemic babies are born all over the world.amounting to 30 – 40 million carriers (WHO-TIF, 2007)The frequency of β-thalassemia in India ranges from 3.5% to 15%, in general, population.4 A higher frequency has been observed in certain communities, such as Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas and Gaurs.Limited micromapping has shown an uneven distribution in frequencies of beta thalassemia carriers in different districts in Maharashtra (1-6%) and Gujarat (0- 9.5%) within small geographic regions. The expected annual births of beta thalassemia major babies was also calculated for each district in these 2 states. The rate of homozygosity per 1000 births annually was 0.28 in Maharashtra and 0.39 in Gujarat. The inherited disorders of hemoglobin include the thalassemias and structural variants of hemoglobin. These are the commonest single gene disorders globally with an autosomal

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recessive inheritance and it is estimated that around 300,000 to 400,000 babies with a severe hemoglobin disorder are born each year [1]. Worldwide 56,000 conceptions would have a major thalassemia disorder and among them around 30,000 would have β thalassemia major, the majority of babies being born in middle and low income countries.Thalassemia are a heterogeneous group of genetic disorder of hemoglobin synthesis characterized by a reduction in the synthesis of one or more of the globins chains leads to imbalanced globin- chain synthesis, defective hemoglobin production causing anemia. Beta thalassemiasare a group of inherited blood disorders. They are forms of thalassemia caused by reduced or absent synthesis of the beta chains of hemoglobin that result in variable outcomes ranging from severe anaemia to clinically asymptomatic individuals. Global annual incidence is estimated at one in 100,000.[4]. Beta thalassemias occur due to malfunctions in the hemoglobinsubuesuffererdnit beta. These defects are numerous and include deletional or non-deletionalmutations .

Non - deletion forms: These defects, in general, involve a single base substitution or small insertions near or upstream of the β globin gene. Most often, mutations occur in the promoter regions preceding the beta-globin genes. Less often, abnormal splice variants are believed to contribute to the disease.

Deletion forms: Deletions of different sizes involving the β globin gene produce different syndromes such as (β^o) or hereditary persistence of fetalhemoglobin syndromes. Alleles without a mutation that reduces function are characterized as (β). Mutations are characterized as (βo) if they prevent any formation of β chains, mutations are characterized as (β+) if they allow some β chain formation to occur. Clinically thalassaemias are divided into alpha, beta, delta beta, or gamma delta beta thalassaemias according to the type of chains affected basically, there are many types of thalassemias that are based according to the types of globin chains. The most clinically relevant types are alpha and beta thalassaemia. .Beta thalassemia minor (Beta thalassaemia trait) carriers are generally asymptomatic, but may be suffered from anemia during physiological conditions such as childhood, pregnancy and stress. Beta thalassaemia major which is also known as cooley'sanemia is a fatal condition that is associated with severs haemolytic anemia, jaundice, organomegaly, bone deformities and requires lifelong regular blood transfusion. While beta thalassaemiaintermedia are a clinical condition which is intermediate between beta thalassemia major and beta thalassaemia trait [9]. Although nutritional anemia are still very common but thalassaemia is the most common inherited disorder in Pakistan resulting in considerable morbidity and mortality. Pakistan has a population 160 million people. The annual rate of population is below 15 years of age due to strong cultural preference for consanguineous marriage in Pakistan it is estimated that there is relatively high prevalence of inherited disorders [10,11.] The carrier frequency of beta thalassaemic gene is estimated to be around 6% in Pakistani population [12] .Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis.The condition was first described by Cooley and Lee in 1925. More than a decade later, Wintrobe and colleagues described milder form of Cooley's anemia in both parents of the children with classic Cooley's anemia. The word thalassemia is described from Greek word "thalas" meaning sea and "haimas" meaning blood because all early cases were reported in children of Mediterranean origin.

II.MATERIALS & METHODS

A cross sectional study was conducted for a period from 2017 oct to 2020 octin a hospital in a rural area in Indore district of Madhya Pradesh on indoor patients in departments of internal medicine.A total number of hundred sample were selected in this study. All Patientsattending Medicine OPD or admitted in Medicine ward with microcytic hypochromic anemia (MCV<80fl ,MCH<27pg)and a clinically suspicion of hemoglobinopathy or beta thalassaemiawere included in the present study.Patients with recent blood transfusion (<3 months) were excluded. This study was carried out to assess the evaluation of beta thalassaemia in young population with microcytic hypochromic anemia in the population of Madhya Pradesh.Whole blood sample was collected by venepuncture technique and following tests were performed such as complete blood count, morphological examination of blood smear, cellulose acetate Haemoglobin Electrophoresis.Complete blood count (CBC) Blood cell indices were measured using Sysmex

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XS- 800i fully automated blood cell counter &Cellenium 19 which was calibrated with commercially available control.

Inclusion criteria

All young Population patients who presented with pallor and were detected to have microcytic hypochromic anemia on peripheral examination.

Exclusion criteria

1. Those who are not willing.
2. Peripheral picture other than microcytic hypochromic anemia.
3. Confirmed cases of iron deficiency anemia.

Sample Collection

Whole blood sample(10ml) was collected by venepuncturefrom all subject recruited in the study. For complete blood count and peripheral smear (7ml) of blood was transferred to a tube containing EDTA anticoagulant and another (3ml) was transferred to another EDTA containing tube for the determination of Cellulose Acetate Haemoglobin Electrophoresis.

The following investigation were done to diagnose microcytic hypochromic anemia using complete hemogram by counter and peripheral blood smear examination .

1. Hb%
2. Hematocrit
3. MCV
4. MCH
5. MCHC
6. RDW
7. TC
8. Platelet count

Haemoglobin electrophoresis was performed using Inter Lab Genio S electrophoresis apparatus and commercially available Interlab Master Kit at PH 8.6

Haemoglobin electrophoresis was performed to identify variant and abnormal haemoglobin including haemoglobin A1 (HbA1), haemoglobin A2 (HbA2), haemoglobin S (HbS), haemoglobin F (HbF), and haemoglobin C (HbC).

The following references range was taken.

- HbA1 : 95%-98%
- HbA2 : 1.5%-3.5%
- HbF: < 2% (age-dependent)
- HbC: Absent
- HbS: Absent

III. RESULTS & DISCUSSION

A total of 100 cases were included for evaluation of microcytic hypochromic anaemia in the studyOut of which 54 were males and 46 were females. The age group ranged from 1 to 25 years.88% were non thalassemiaand 12% cases had shown beta thalassemiabands onHemoglobinelectrophoresis.Out of total 12 subjects diagnosed as having three was beta thalassemia major, 02 were males and 01 were females and three was beta thalassemia intermedia, 01 were males and 02 were females. Among the beta thalassemia minor, 04 were males and 02 were females and the remaining 88 were non thalassemia.

1. Age distribution of patients :

Age distribution of the patients studied is shown in Table 1

Age in years	Number of patients	Percentage
1 years	15	15%
1-12 years	40	40%

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12-25 years	45	45%
Total	100	100.0

Table 1: Age distribution of patients

2. Gender distribution of patients

Gender distribution of the patients studied is shown in Table 2

Gender	Number of patients	Percentage
Male	**54**	**54.0**
Female	**46**	**46.0**
Total	**100**	**100.0**

Table 2 : Gender distribution of patients

3. Diagnosis based on electrophoresis

Diagnosis based on electrophoresis studied is shown in Table 3

Diagnosis	Number of patients	Percentage
Non thalassemia	88	88.0
Beta thalassemia	12	12.0
Total	100	100.0

Table 3: Diagnosis based on electrophoresis

Haematological Diagnosis:

RBC indices show microcytic anaemia. In the present study different haematological parameters were carried of different patterns of beta thalassemia.RBC normal value Men – 4.7 to 6.1 (millions/µL), women – 4.2 to 5.4(millions/µL). In case of beta thalassemia minor - Red Blood Cell Count 5.84(millions/µL) and beta thalassemia major - Red Blood Cell Count 1.99 (millions/µL), beta thalassemia intermedia - Red Blood Cell Count 1.47 (millions/µL), Thalassemia major is characterized by reduced Hb level ((50 < 70 fl and MCH > 12< 20 pg). Thalassemia intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCVand MCH, with increased Hb A2 level.

Red Blood Cell Index	Normal	β-Thal Major	β-ThalIntermedia	β-Thal Minor
[MCV fl]	80-100	<50<70	50-80	<79
[MCH pg]	27-31	12-20	16-24	<27
[MCHC g/dL]	31-35	<29	Reduced	<30
Hemoglobin [Hb g/dl]	Males: 13.8 -18.0 Females: 12.1- 15.1	<7	7-10	Males: 11.5-15.3 Females: 9.1-14

Hematological profiles of Normal and Beta-Thalassemia patients

Out of total 12 subjects diagnosed as having three was beta thalassemia major, 02 were males and 01 were females and three was beta thalassemia intermedia, 01 were males and 02 were

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females. Among the beta thalassemia minor, 04 were males and 02 were females and the remaining 88 were non thalassemia. Table 4

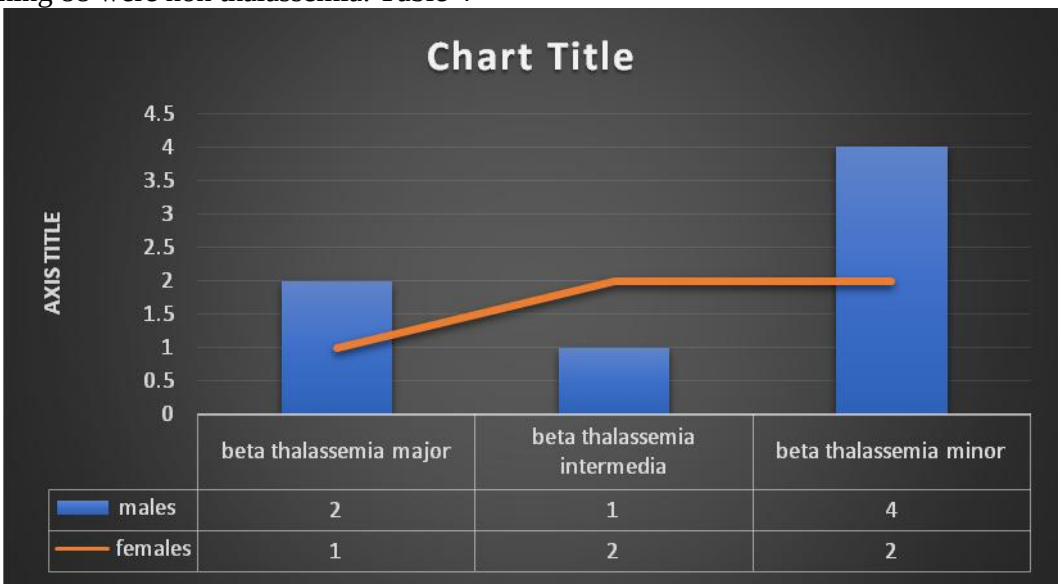

Table 4: Diagnosis based on electrophoresis

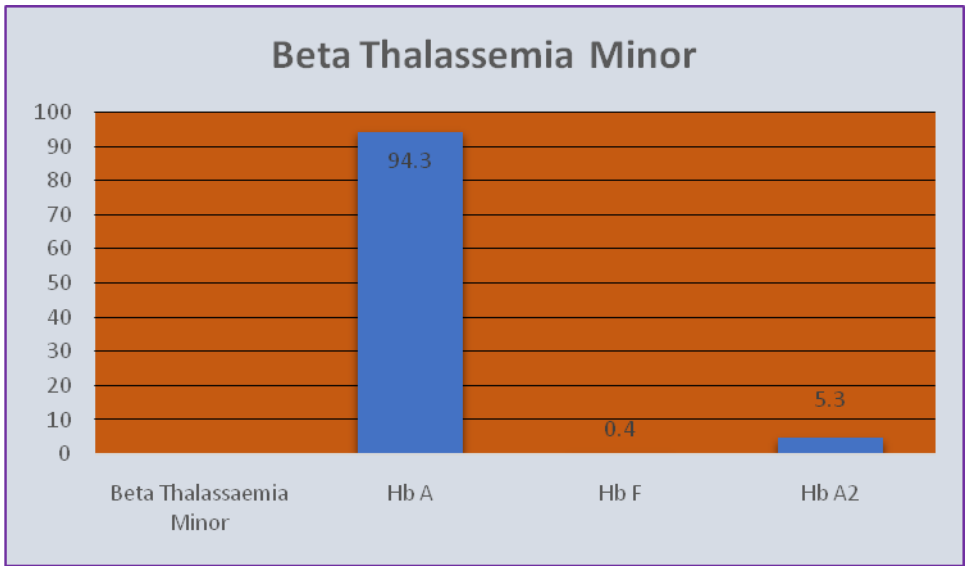

Figure No: 1 Frequency of different haemoglobin types in beta thalassemia minor

Similarly In case of beta thalassemia minor haemoglobin electrophoresis showed Haemoglobin A 94.3, Haemoglobin F 0.4, Haemoglobin A_2 5.3 as shown in Figure 1

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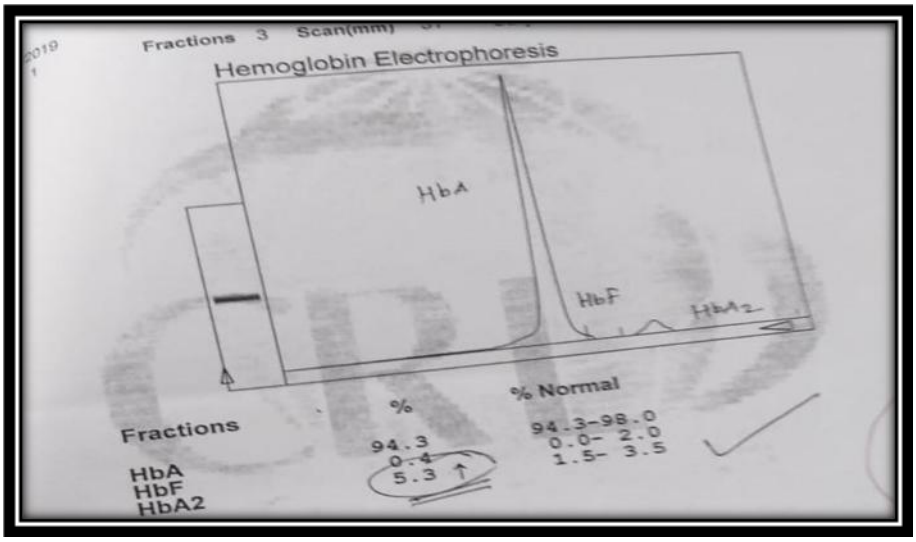

Hb electrophoresis of Beta thalassemia minor

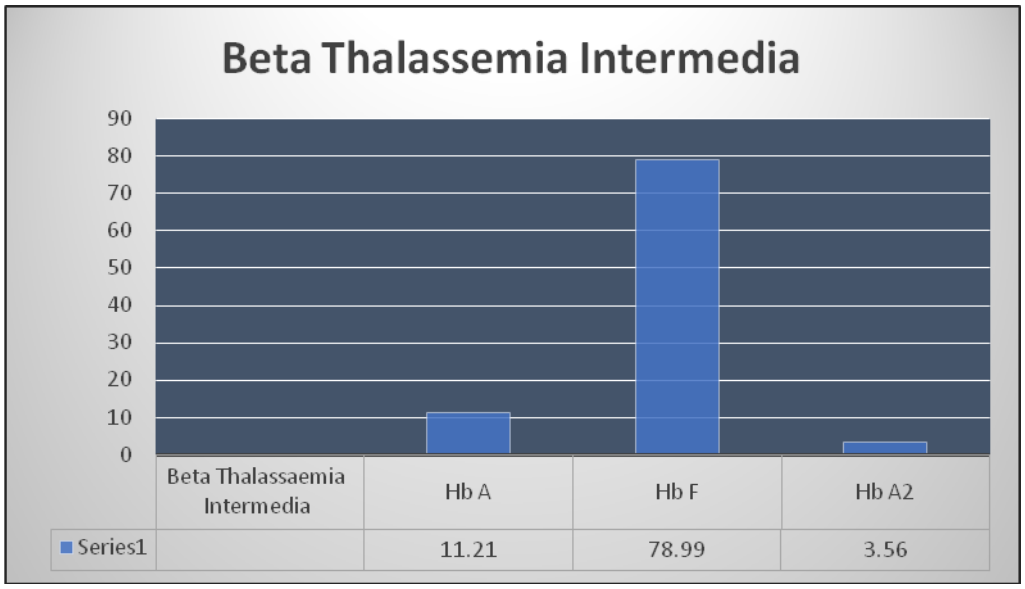

Figure No: 2 Frequency of different haemoglobin types in beta thalassemia minor

In case of beta thalassemia intermedia haemoglobin electrophoresis showed Haemoglobin A 11.21, Haemoglobin F 78.99, Haemoglobin A_2 3.56 as shown in Figure 2

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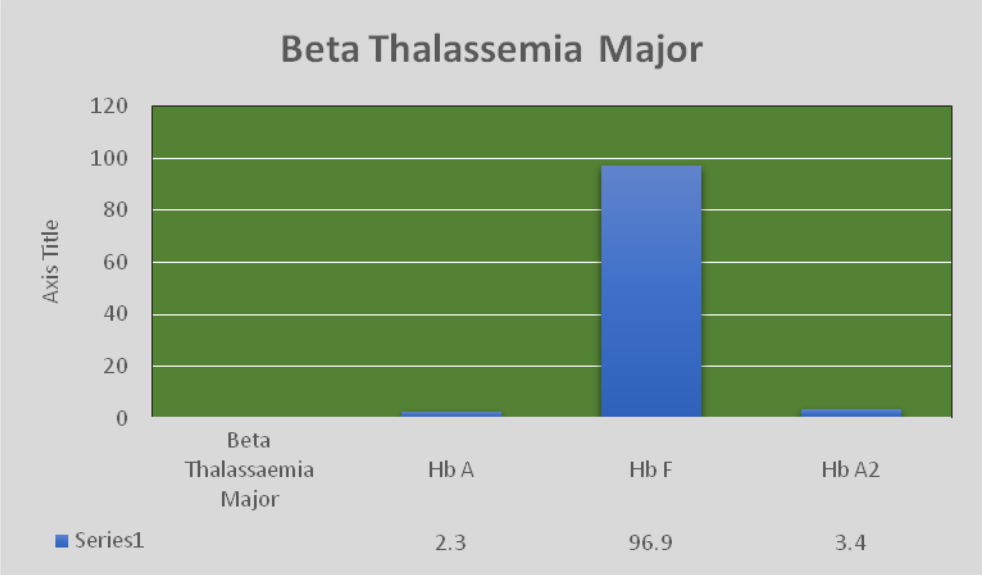

Figure No: 3 Frequency of different haemoglobin types in beta thalassemia minor

In case of beta thalassemia major haemoglobin electrophoresis showed Haemoglobin A 2.3, Haemoglobin F 96.9, Haemoglobin A_2 3.4 as shown in Figure 3

DISCUSSION

Beta thalassemia is an inherited blood disorders and are present in general population in varying prevalence. Some populations/communities have higher prevalence of these disorders due to preference to consanguineous marriages.Beta thalassemia syndromes are a group of hereditary disorders resulting from genetic deficiency in the synthesis of beta-globin chains. In the homozygous state (i.e., thalassemia major), it causes severe, transfusiondependent anemia, whereas the heterozygous state (trait or thalassemia minor), causes mild to moderate microcytic anemia. Those presenting with clinical severity lying between that of thalassemia major and minor are said to have thalassemia intermedia. Patients with thalassemia minor generally don't require specific therapy whereas those with thalassemia major are transfusion dependent and need iron chelation therapy. Splenectomy, allogenic stem cell transplantation and supportive measures are also required.

There are numerous haemoglobin variants in the Indian population many of which remain undetected due to lack of available infrastructure. Depending on the area of distribution, different hemoglobinopathies have been detected. Beta-Thalassemia minor is the commonest haemoglobin abnormality in the Indian subcontinent which is a similar finding in our study. In beta-thalassemia minor, the Hemoglobin A2 (HbA2) values range between 3.5 to 9%. Low Hemoglobin, reduced MCV, MCH and raised RBC count suggest Beta thalassemia trait.

For diagnosing Beta–thalassemia major the HbF values of equal to or more than 90% of the total Hb are considered.

Individuals with beta thalassemia major require regular blood transfusions, as frequently as every 2-4 weeks in severe cases. Individuals with beta thalassemia intermedia occasionally require blood transfusions such as when suffering from an illness or infection or when planning to undergo surgery.Individuals with beta thalassemia minor usually do not develop symptoms and do not require treatment. It is important that individuals with beta thalassemia minor be correctly diagnosed.

Beta thalassemia is an inherited blood disorder characterized by reduced levels of functional hemoglobin. Hemoglobin is found in red blood cells; it is the red, iron-rich, oxygen-carrying pigment of the blood. A main function of red blood cells is to deliver oxygen throughout the body. Beta thalassemia has three main forms – minor, intermedia and major, which indicate the

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severity of the disease. Individuals with beta thalassemia minor usually do not have any symptoms (asymptomatic) and individuals often are unaware that they have the condition. Some individuals do experience a very mild anaemia. Individuals with beta thalassemia major have a severe expression of the disorder; they often require regular blood transfusions and lifelong, ongoing medical care. The symptoms of beta thalassemia intermedia are widely variable and severity falls in the broad range between the two extremes of the major and minor forms. The characteristic finding of beta thalassemia is anaemia, which is caused because red blood cells are abnormally small (microcytic), are not produced at the normal amounts, and do not contain enough functional hemoglobin. Consequently, affected individuals do not receive enough oxygen-rich blood (microcytic anaemia) throughout the body. Affected individuals may experience classic signs of anaemia including fatigue, weakness, shortness of breath, dizziness or headaches. Severe anaemia can cause serious, even life-threatening complications if left untreated. Affected individuals are treated by regular blood transfusions. Because of repeated blood transfusions individuals with beta thalassemia major and intermedia may develop excess levels of iron in the body (iron overload). Iron overload can cause a variety of symptoms affecting multiple systems of the body, but can be treated with medications. Beta thalassemia is caused by mutations in the hemoglobin beta (*HBB*) gene. Individuals with beta thalassemia minor have a mutation in one *HBB* gene, while individuals with the intermediate and major forms have mutations in both *HBB* genes.

Thalassemia has become a worldwide clinical problem due to increased immigration of ethnic groups with high prevalence of thalassemia[13] . Pakistan is one of the country include in the thalassaemic belt with highest prevalence of thalassemia [14]. Married couples consisting of two carriers have 25 % chances that any child they have will be affected. The estimated rate of birth of affected infants is 1.3 per 1000 live births and 5000-9000 children with beta thalassemia major born per year, although no scientific literature is documented till now in Pakistan [16,17.] They also reported that the prevalence of the beta thalassemia trait in the population of Punjab is 5.5% .another study was reported that also showed highest frequency of beta thalassemia trait in Gujrat followed by Sindh Punjab, Tamil Nadu, South India and Maharashtra [20].

IV. CONCLUSION&FUTURE PROSPECTS

Microcytic Hypochromic Anaemia is a very common problem in clinical practice, early detection of which helps in the correct treatment. Most common cause of Microcytic Hypochromic Anaemia in this study was 2nd being thalassemia. Diagnosis of the Microcytic Hypochromic Anaemia was achieved easily with the help of complete hemogram and peripheral blood picture. Differential diagnosis based on complete hemogram and peripheral smear is possible but special tests like serum iron profile and hemoglobin electrophoresis are a must for confirmation of diagnosis. Therefore, steps are needed to be taken for proper diagnosis and management of patients with anaemia especially in rural areas in order to reduce burden of the disease as well as cost of treatment and general outcome of the patient.Hemoglobin electrophoresis is a must in the diagnosis of thalassemiaEarly diagnosis of which helps to start transfusion therapy and yield better prognosis. Iron overload being a major complication in thalassemia showed increased serum iron levels.

The future as a genetic disease, thalassemia remains an ideal target for gene therapy. Several clinical trials are under way, and several patients have undergone treatment with some preliminary signs of success. There is also an increasing interest in raising fetalhemoglobin levels to ameliorate anaemia in patients with β-thalassemia major and especially those with thalassemia intermedia. Manipulation of the hepcidin pathway holds great promise for treating anaemia of inflammation. Although tremendous progress has been made, much remains to be elucidated about iron metabolism, including the receptor for absorption of heme iron. Finally, the role of new markers — such as polymorphisms in a key iron-sensing protein, transmembrane protease serine 6 (TMPRSS6), which may increase the risk of iron deficiency — remains to be explored

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