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# CLINICAL AND HAEMATOLOGICAL PRESENTATIONS OF HAEMOGLOBIN E DISORDERS FROM NORTHERN PAKISTAN

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## ABSTRACT

**Objective:** To evaluate clinical and haematological findings in any form of HbE disorder in Pathan families from Northern Pakistan.

**Material and Methods:** It was a hospital based, prospective observational study, carried out in thalassemic patients, at Haematology Department, Kuwait Teaching Hospital and Fatimid Foundation, Peshawar, from July 2010 to December 2011.

**Results:** During the study period, a total of 13 cases of HbE were detected in 3650 registered thalassemics with an incidence of 0.35%. 6/13 (46%) cases of compound heterozygous HbE/ β thalassemia were found, with four of them behaving as β Thalassemia Major (BTM) and two as β Thalassemia Intermedia (TI). 7/13 (54%) cases were heterozygous HbE, all asymptomatic. No case of homozygous HbEE was detected.

**Conclusion:** Haemoglobin E disorders are not uncommon in Pathan families from Northern Pakistan and may behave as BTM or TI.

**Key Words:** HbE, HbEE, HbE/ β Thalassemia, Pathans, Northern Pakistan.

## INTRODUCTION

Inherited haemoglobin (Hb) disorders fall into *Quantitative* hemoglobin disorders, with reduced globin chains e.g. α/ β-thalassemias; and Qualitative/ structural haemoglobin variants, resulting from amino-acid substitutions in α/ β chains[1]. Thalassemias are the commonest genetic disorders in the world[2]. In Pakistan, the carrier rate for β thalassemia gene is 4.5-7%, highest in Khyber Pakhtunkhwa (KPK) region, probably because of strict inter-familial marriages in Pathan families[3,4].

There are >700 Qualitative Hb variants, most of them are harmless, but *HbS, C, D and HbE* are clinically important. Occasionally one may inherit thalassemia gene from one parent, and structural Hb gene from the other, resulting in compound heterozygote e.g. HbS/ β Thal, HbE/ β Thal or HbS/ E[5].

Haemoglobin E results from substitution of Lysine for Glutamic acid at codon 26 of β globin chain. It is believed to be the commonest β-chain variant in the world[6]. Prevalence is very high in Southeast Asia, especially in Cambodia, Laos and Thailand, with a carrier rate up to 88%[7]. HbE is also common in Vietnam, Malaysia, North-Eastern India and Bangladesh[8,9].

*Heterozygous Hb E (HbE)* is asymptomatic with nearly normal Hb level[10]. *Homozygous Haemoglobin E (HbEE)* individuals are also asymptomatic but with a mild anemia[11]. In contrast, clinical picture of *HbE/ β thalassemia* is variable[12] with some of them having splenomegaly, growth retardation, bone deformities and leuko-erythroblastic blood picture just like BTM[13].

On Hb electrophoresis at alkaline pH, Hb E migrates slowly with Hb $A_2$. On High-Pressure Liquid Chromatography (HPLC), HbE has similar retention time as $HbA_2$, but $HbA_2$ levels rarely exceed 9% in β thalassemia trait where as the concentrations of HbE may range from 30%-90% in HbE disorders[14]. No HbA may be due to homozygous HbEE or HbE/ β-thal, which can be distinguished by parents or DNA studies[15].

For correct management, distinction among different HbE types is necessary. In contrast to BTM, in HbE/ β thal cases, transfusions may be curtailed to maintain mean pre-transfusion Hb around 7 g/dl rather than 10 gm/dl. In HbE/ β thalassemia patients, judicious use of transfusions, Iron chelating therapy and Folic acid is the main stay of treatment. Patients with HbE disorders are excellent candidates for Hb F inducers, as even a small increase in Hb may lead to transfusion independence[16]. The present study was aimed to detect and characterize the clinical and

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hematological parameters of HbE disorders in KPK area of Northern Pakistan.

## MATERIAL AND METHODS

It was a hospital based, prospective observational study, carried out from July 2010 to December 2011, at Haematology Department, Kuwait Teaching Hospital and Fatimid Foundation, Peshawar, Inclusion criteria were any Hb disorders, diagnosed with HbE in any form on Hb Electrophoresis. Exclusion criteria were any other Hb disorder or where splenectomy was already done.

During this period, 3650 cases of BTM, registered with Fatimid Foundation, Peshawar were reviewed clinically and Hb electrophoresis was performed where indicated. 03 ml venous blood sample was collected in commercially available EDTA bottles. CBC haemogram was obtained using Sysmex KX-21 after running controls. Smear was made and stained with Giesmsa for morphology. Samples were then run on Cellu-gel Electrophoresis System- Malta Italy. Controls for HbA, $HbA_2$, HbF and HbS were included in each batch. Initially, if HbE was found in $HbA_2$ region on this method, case was labeled as either HbE trait or compound heterozygous (HbE/ β -thal) depending upon presence or absence of HbF. Later on the results were confirmed from AKUH Lab Karachi, where analysis was done on Bio-Rad Variant II HPLC system.

Once an index case of HbE was diagnosed, targeted screening was done in close relatives for the possible detection of asymptomatic HbE trait carriers. A performa was used to record clinical and laboratory data in each case. All the results were entered on Microsoft Office Excel 2007, and statistical analysis done.

## RESULTS

A total of 13 cases of Hemoglobin E were detected in 3650 registered cases of BTM with an incidence of 0.35%. Out of these, 46% were compound heterozygotes (HbE/ β thal), distributed mainly in four families with 4 males and 2 females. Epidemiologically 2 cases were each from Peshawar and Mardan districts, and one each from Tirah and Bara areas of FATA. Clinically 2 cases presented with milder disease with age of presentation around 9 years and labeled as Thalassemia Intermedia (TI) and 4 cases were transfusion dependent since < 1 year of age and were having typical features of BTM, including splenomegaly. Hematological features of these HbE/ β thal cases are shown in Table 1. 7/13 (54%) cases were diagnosed as having HbE trait. 5 were males and 2 females. 3 cases were from Peshawar district and one each from Mardan, Bara, Tirah and Nowshehra. Clinically all these HbE trait cases were asymptomatic, none having splenomegaly or history of blood transfusion. Hematological features of HbE trait cases are presented in Table 2. No case of HbEE was detected in this study.

**Table 1: Hematological parameters in Hb E/ β Thal cases (n=6)**

| Parameter | Mean ± SD |
|---|---|
| Hb (g/dl) | 7.7 ± 1.6 |
| Hct (%) | 26.0 ± 3.0 |
| TRBC (X $10^9$/L) | 3.5 ± 0.55 |
| MCV (fl) | 71 ± 3.7 |
| MCH (pg) | 21.8 ± 1.7 |
| MCHC (g/dl) | 30.4 ± 1.7 |
| Morphology | Aniso-poikilocytosis, Microcytosis & hypochromia with leukoerythroblastosis in all cases |
| HbA Level | 18.83% ± 21 |
| HbE (+A2) Level | 39% ± 5.5 |
| HbF Level | 42% ± 10 |

**Table 2: Hematological Parameters in Hb E trait cases (n=7)**

| Parameter | Mean ± SD |
|---|---|
| Hb (g/dl) | 12.3 ± 1.8 |
| Hct (%) | 37 ± 7.0 |
| TRBC (X $10^9$/L) | 5.25 ± 0.7 |
| MCV (fl) | 73 ± 6.5 |
| MCH (pg) | 23.8 ± 1.7 |
| MCHC (g/dl) | 32.7 ± 1.0 |
| RDW (CV) | 38.2 ± 1.8 |
| Morphology | RBC's were microcytic in 5 cases, cases, and normocytic in 2 cases |
| HbA Level | 65.4 (+/- 6.5) |
| HbE (+A2) level | 34 (+/- 6) |
| HbF level | 0.6 (+/- 0.7) |

## DISCUSSION

On literature search for local studies, the earliest case report of 2 cases of HbE// β-thalassemia in 1966 by Khaleque was from a Muslim family from Dacca, East Pakistan[18]. In 1987, Imran et al reported a frequency of 0.5% for an abnormal Hb variant at alkaline pH, reported as HbC/ E, in children from NWFP Pakistan[19]. The most recent study was published a couple of months back from AKUH Karachi where HbE

was detected in 41 cases out of 11404 with an incidence of 0.36%. HbE trait was seen in almost 50% cases, HbE / β Thal in 34% and HbEE in 15% cases. HbE/ β Thal cases were the most severely affected[20].

A WHO study in 1989 showed that HbE/ β Thal cases have extremely variable parameters[22]. Mean Hb was 7.0 g/dl, comparable to our study, mean MCV was around 71 fl in both studies. In WHO study HbE was around 50% as compared to 40% in our study.

In 1997, Weatherall and colleagues initiated a long-term HbE/ β-thalassemia study. Transfusion therapy was stopped to better define baseline clinical and lab data[22]. In our study, some of the cases were already being treated with regular blood transfusions so they were having variable amount of HbA/ HbF. The other possibility of raised Hb A in our cases may have been HbE/ $\beta^+$ Thal rather than HbE/ $\beta^0$ Thal.

A study in 2000, by Fucharoen from Thailand showed that patients with HbE/ β-thalassemia had average Hb of 7.7 g/dl, almost the same as our study. 50% cases presented as BTM, and the other 50% behaved as TI[23]. Although a small study, our results showed that 4/6 (66%) behaved as BTM, and 2/6 (33%) as TI. The cause of the striking variability in individuals with HbE/ β-thalassemia even within same family remains largely unknown[24]. Raised HbF level and $\beta^+$ mutation lowers the clinical severity[25].

## CONCLUSION

Awareness, better diagnostic facilities, carrier rate for β thalassemia genes in Pathans, a high rate of detection of HbE/ β Thal can be predicted for KPK region of Pakistan to reduce the complicatins.

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