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# Diagnosing Beta Thalassemia trait in a developing country

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ABSTRACT

**Background:** Beta Thalassemia trait (BTT) is diagnosed by detecting hemoglobin A2 ($HbA_2$) >3.8% on either High Performance Liquid Chromatography (HPLC) or cellulose acetate electrophoresis (CAE). HPLC is an accurate and reproducible but costly alternative to more conventional CAE which is labor intensive but easy to interpret and inexpensive. **Objective:** To determine the sensitivity of CAE and HPLC keeping PCR as gold standard for the diagnosis of BTT. **Study Design:** Cross sectional. **Place and Duration of Study:** Armed Forces Institute of Pathology Rawalpindi. May 2014 to January 2015. **Patient and Methods:** Five ml EDTA anti-coagulated blood was collected from 100 PCR proven cases of BTT. $HbA_2$ levels were measured by running samples directly on HPLC. But for CAE, first a hemolysate was prepared which was then applied to cellulose acetate membrane at an alkaline pH (7.9). After elution of $HbA_2$ band in Tris EDTA borate buffer (pH of 8.9), $HbA_2$ concentration was calculated by measuring its absorbance in a photometer at a wavelength of 416 nm. **Results:** Mean age of the patients was $28.8 \pm 8.1$ year. The most common mutation was Fr 8–9 (35%) followed by IVS1-5 (25%) mutation. Mean $HbA_2$ levels by CAE and HPLC were $4.97 \pm 0.42$ and $5.54 \pm 0.59$ respectively. All the patients had $HbA_2 > 4\%$ on both CAE and HPLC. None of our patients had false negative result either on CAE or HPLC. **Conclusion:** CAE has comparable sensitivity with HPLC for detection of Beta Thalassemia Trait.



## Introduction

Beta Thalassemia (BT) is one of the most prevalent inherited hemoglobin disorders in the world [1], with a carrier rate of 5% among the Pakistani population [2]. It is estimated that with a 5% carrier rate, over 5000 infants with Beta Thalassemia Major (BTM) are born every year in Pakistan [3–5]. Screening for Beta Thalassemia trait (BTT) and identification of its compound heterozygotes with variant hemoglobins is essential for diagnosis and genetic counseling that holds the key for prevention and control of BTM.

BTT is suspected on finding hypochromic and microcytic red cell indices with near normal hemoglobin levels and

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slightly raised red blood cell count on routine complete blood count [1]. Laboratory confirmation of the BTT depends primarily on detecting elevated levels of hemoglobin $A_2$ ($HbA_2 > 3.8\%$) in the patient's blood sample [6]. The $HbA_2$ levels can be quantified by many methods but the two most commonly used are Cellulose Acetate Electrophoresis (CAE) followed by elution and the automated method of High Performance Liquid Chromatography (HPLC) [7]. CAE is the conventional and commonly used technique for diagnosis of BTT. It is reproducible, inexpensive and relatively easy to interpret as shown in Fig. 1. Additionally this procedure can be performed in smaller and resource constrained laboratories. The only drawback is that it is labor intensive [8].

The cation exchange HPLC is a rapid, accurate, reproducible and less labor intensive alternative method for detection of many hemoglobinopathies including BTT [9]. HPLC offers the distinct advantage over conventional CAE as it can identify and quantify $HbA_2$, fetal hemoglobin (HbF) and other hemoglobin variants more accurately. It is also very useful for pediatric group of patients, as only 5 μl of blood is sufficient for analysis. Its utility is more in diagnostic centers where there is increased workload. However major drawbacks include cost considerations, heavy processing equipment and expertise to interpret results. Additionally it has been proven to overestimate $HbA_2$ percentage especially in the presence of sickle hemoglobin (HbS) [10]; which is a variant of Beta globin found in a significant percentage in different ethnicities of Pakistani population [11].

Polymerase chain reaction (PCR) is a highly sensitive and specific method for diagnosis of BTT which can clearly identify the type of mutation affecting Beta globin gene [12]. However, it requires expensive molecular equipment and high degree of technical skill due to which it is not carried out routinely during the workup of BT patients. It is therefore reserved for cases which present either with atypical red cell indices and/or are not detected on CAE or HPLC [13, 14].

Previously no local study has done comparison between CAE and HPLC based on $HbA_2$ levels in PCR confirmed BTT patients. The aim of this study was to compare the sensitivity of CAE against HPLC to establish that CAE can still be used as an effective diagnostic tool not only in District Hospital laboratories (Level B) but also in Central/Regional hospital laboratories of under resourced countries like Pakistan [15]. Pakistan despite having a high prevalence of BT has inadequate resources to cater for BT screening and diagnosis at mass level. Additionally fragmented and substandard transfusion system in Pakistan is inept to cater for the transfusion needs of BTM patients in the country [16]. So inexpensive but effective diagnostic modalities like CAE are required to screen the masses for BTT in Pakistan.

## Objective

To determine the sensitivity of CAE and HPLC keeping PCR as gold standard for the diagnosis of BTT.

## Material and methods

It was a cross-sectional study carried out from May 2014 to January 2015 in the hematology department of Armed Forces Institute of Pathology (AFIP) Rawalpindi. A total of 100 patients were included in this study.

### *Sample selection*

All those cases reporting for extended family screening and for prenatal diagnosis and found to be positive for one of the Beta globin gene mutations on PCR were included in this study. For control group, PCR negative cases for BT mutations were tested. While all cases having silent mutations

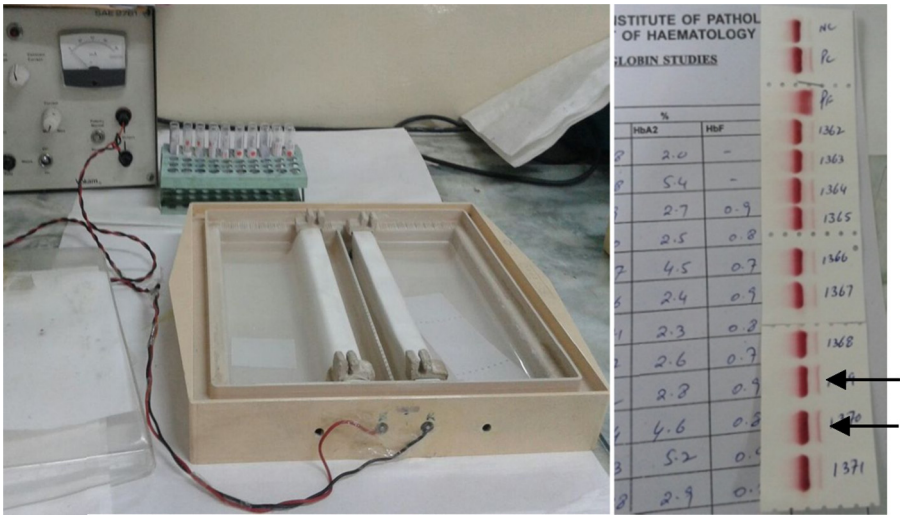


**Fig. 1 – Conventional Cellulose Acetate Electrophoresis tank with buffer and electrical supply and cellulose acetate strip showing normal control (NC), positive control (PC) and Positive for HbF (PF). Arrows indicating Raised $HbA_2$ levels on strip. PC and PF on the strip showing BTT and BTM respectively**

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