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EFFICACY OF HEMOGLOBIN LEVEL IN DIAGNOSING OF BETA THALASSEMIA

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Abstract:
Level of HbA2 more than 4% is the reliable parameter to identify β- thalassemia carrier. However, in some cases the level is not typically increased hence leading to diagnostic dilemma. Thus the objectives of this study were to evaluate the existence of β- thalassemia among borderline HbA2 sample of population of Lahore. Out of 11,790 samples received for thalassemia screening, 405 (3.4%) were found to have borderline HbA2 level. Out of this, 117(28.9%) samples were selected by simple random sampling for PCR. Multiplex ARMS-PCR was used to detect β-globin gene mutation and multiplex gap PCR for α-globin genes deletion. The result revealed 36 (30.8%) had β-globin gene mutations, 8 (6.8%) had α-globin gene deletions and 1 (0.9%) had coexistence of α and β-globin gene defects.

The commonest gene mutation detected were CD 19 (A-G), detected from 17 (45.9%) samples, followed by 9 (24.3%) with IVS 1-1 (G-A) mutation, 5 (13.5%) with Poly A mutation and 1 (2.7%) showed CAP +1 (A-C) mutation. Two samples (5.4%) showed mutations of Poly A and CD19, 2 (5.4%) showed mutation of IVS 1-1 and CD 19 while 1 (2.7%) showed IVS 1-5 and CD 19 mutations. This study showed 10 (27.0%) positive molecular results even though the HbA2 level was at only 3.0%. There was significant number of people with HbA2 between 3.0% and 3.9% and confirmed as thalassemia carriers by PCR. This data may suggest the level of HbA2 need to be revised to ensure the success of thalassemia screening programme in our population.

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INTRODUCTION:

HbA2 determination plays an important role in the screening programs for β-thalassemia in which if the value is more than 4.0%, the person will be presumed as β-thalassemia carrier. Individuals with "grey zone" or borderline HbA2 are difficult to be classified base on hematological parameters alone [1].

The individuals who have borderline HbA2 values are not rare, ranging from 2.2% to 16.7% among population in the countries where thalassemia is commonly seen [2].

If PCR test was not done among borderline HbA2 samples, significant numbers of β-thalassemia carriers will be missed. By neglecting and labeling the samples as normal might cause at risk couple to give birth to an affected offspring, which can give rise to bigger nuisance for the nation with probability of birth of an affected offspring.

In Thailand and other Southeast Asian countries, thalassemia is very common with 20–30% of the populationhaving the α-thalassemia trait, 3–9% having β-thalassemia trait whereas 20–30% having the Hbe trait [3].

In Pakistan, thalassemia is one of the common genetic abnormalities with 4.5% of population are carriers of β-thalassemia [4]. Until November 2016, according to the thalassemia registry, total number of transfusion dependent thalassemia (TDT) patients in pakistan was 6646. Lahore presented with the highest TDT cases which is more than 1600 patients while Lahore, the place where this study was conducted has more than 250 TDT patients [5].

Various factors can contribute for normalization of HbA2 that can interfere with carrier detection such as mild β-thalassemia mutations and coinheritance of other molecular defect such as α- or δ- thalassemia [6].

The aim of this study was to study the significance in diagnosing β- thalassemia carrier among borderline HbA2 samples in Kelantan populations in ensuring a better outcome of our prevention programme in future.

METHODOLOGY:

A total of 11,790 of venous blood samples from various parts of Lahore that had been sent for thalassemia screening in two years period from January 2016 until December 2017 were then analyzed by Capillaries 2 Flex-Piercing System SEBIA, PN 1227.

Every samples with borderline HbA2 (3.0 to 3.9%) were included to determine the proportion of this group.

Samples with borderline HbA2 were randomly selected by systematic random sampling method for molecular study. In this study, 117 samples were sent for Multiplex Amplification Refractory System (MARMS) PCR for β-globin gene mutation and Multiplex Gap PCR for α-thalassemia gene deletion.

Multiplex amplification refractory mutation system (MARMS) are designed to detect 20 types of β-gene mutations which are MARMS-A to –F. The mutations within β-globin gene were detected using 5 different multiplex ARMS-PCR and one single ARMS-PCR reaction.

For MARMS-A, B, C and D, 4 types of mutations in each multiplex system were examined, whereas in MARMS-E, 3 types of mutations were examined followed by 1 mutation in ARMS-F. **Table 1** showed list of mutations of each MARMS-PCR [7].

Multiplex	List of mutation	Amplicon size (base pairs)
MARMS-A	Internal Control CD 41/42 (-TTCT) IVS 1-5 (G>C) Cd 26 (G>A) Cd 17 (A>T)	861 476 319 301 275
MARMS-B	Internal Control Cd 71/72 (A+) IVS 1-1 (G>T) Cd 8/9 (+G) -28 (A>G)	861 569 315 250 145
MARMS-C	Internal control Cd 43 (G>T) Poly A (A>G) IVS 1-1 (G>A) Cd 16 (-c)	861 482 393 315 273
MARMS-D	Internal Control -88 (C>T) Initiation codon Cd 15 (G>A) -29 (A>G)	861 369 248 203 310
MARMS-E	Internal Control -86 (C>G) CAP +1 (A>C) Codon 19 (A>G)	861 367 173 173
ARMS F	Internal control IVS 2-654	493 826

Table 1: List of mutations for each MARMS-PCR

Data were recorded and analyzed by using Statistical Package for the Social Sciences (SPSS) program version 22.0. Descriptive analysis was used to determine the proportion of borderline HbA2 samples. Independent student t-test was also performed to determine the association between different HbA2 levels from 3.0 to 3.9% with the presence of thalassemia gene defect. P-value <0.05 was regarded as statistically significant.

RESULT:

From 11,790 samples, 405 (3.4%) samples were found to have borderline HbA2 between 3.0 and 3.9%. Among 405 with borderline HbA2 samples, 117 samples were tested for β-gene mutation and α-gene deletion study. Out of that, 45 (38.5%) showed positive molecular result in which 36 (30.8%) showed β-globin gene mutations, 8 (6.8%) showed α-globin gene deletions and 1 (0.9%) showed coexistence of α and β-globin gene defects.

Molecular result for β gene mutation was illustrated in **Figure 1**. The commonest β-gene mutation detected were CD 19 (A>G), detected from 17 (45.9%) samples, followed by 9 (24.3%) with IVS 1-1 (G>A) mutation, 5 (13.5%) with Poly A mutation and 1 (2.7%) showed CAP +1 (A>C) mutation. Two samples (5.4%) showed mutations of Poly A and CD19, 2 (5.4%) showed mutation of IVS 1-1 and CD 19 while 1 (2.7%) showed IVS 1-5 and CD 19 mutations.

Figure 1: Frequency of different β-gene mutation among samples with positive molecular result (n=37).

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As summarized in **Figure 2**, β gene mutations were more frequently detected in HbA2 level of 3.5-3.9% (56.8%) compared to 3.0 to 3.4% (43.2%).

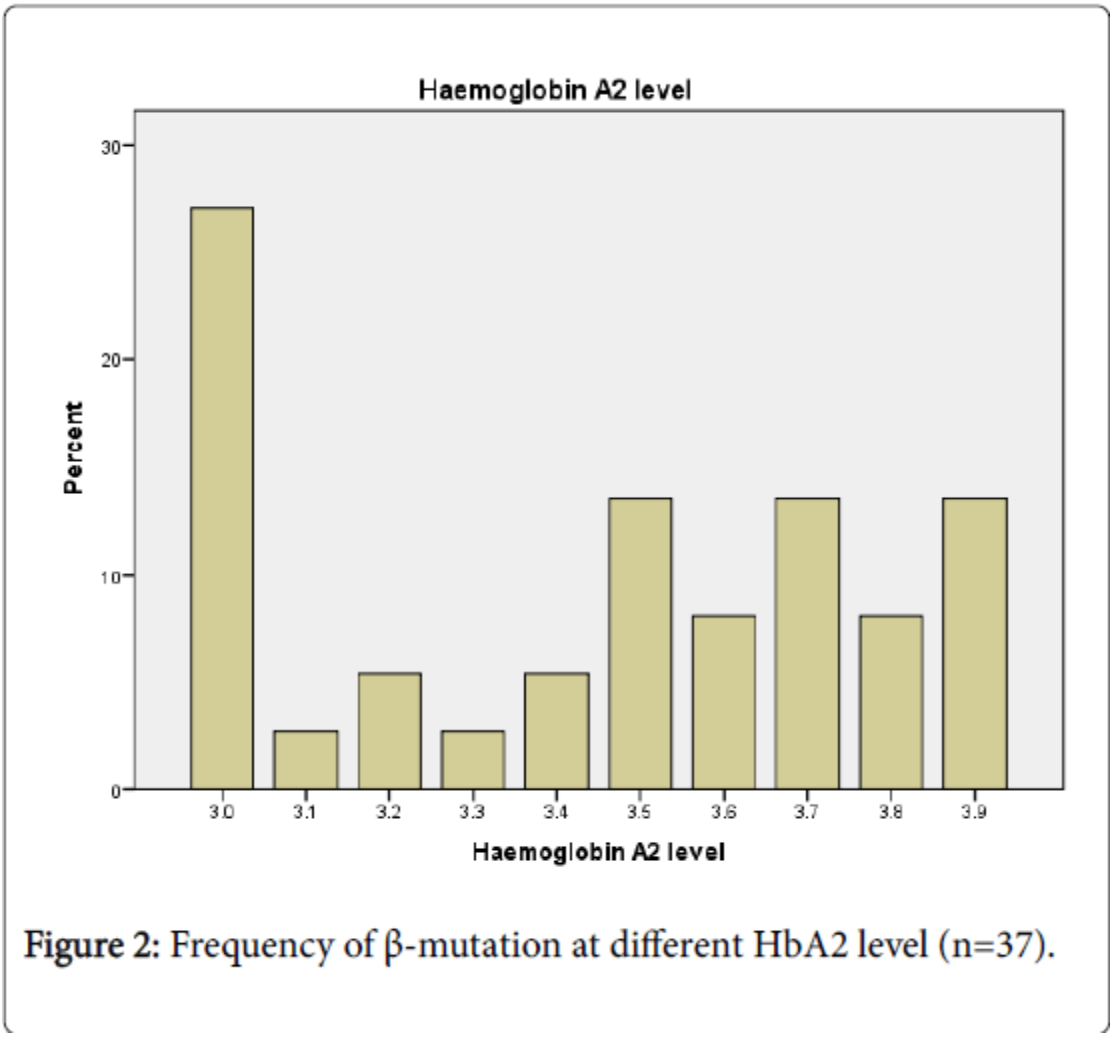

Figure 2: Frequency of β-mutation at different HbA2 level (n=37).

There was statistically significant association between different HbA2 levels and outcomes of molecular result. This study also showed that, 10 (27.0%) positive molecular results were detected at the level HbA2 of only 3%. From 9 samples that were positive for α-thalassemia (including 1 sample with coexistence β-thalassemia), 7 (77.8%) showed -α3.7 type of deletion, while 2 samples (22.2%) showed -α4.2 type of deletion.

DISCUSSION:

A remarkable proportion of carriers of HbA2 level between 3.5% and 4.0% or even lower have been identified. Particular care has to be paid to the interpretation of this borderline HbA2 in which the HbA2 level above the normal reference interval but not in the classical range of β- thalassemia [8].

In this study, 3.4% samples were found to have level of HbA2 between 3.0 and 3.9%. The proportion is comparable to the areas with high endemic for β-thalassemia. Mosca et al. [8] have found that proportion of borderline HbA2 level in Italian population was 2 to 4%.

In their study they had defined borderline HbA2 level as 3.3 to 3.7% [9]. Study done in India among 752 samples found that 3.1% of them had borderline HbA2 values defined between 3.1-3.9% [10].

A study done among Sicily population which is an endemic area for thalassemia involving 23,485 samples from year of 2000 to 2006 found that 16.75% of the samples showed borderline HbA2 level, defined between 3.1- 3.9 by HPLC. Out of that, 410 samples were proceed with molecular test which showed that 22.9% were positive for molecular defect in β, α (or) δ-globin genes [11].

Whereas in our study, among samples that were tested with molecular test 38.5% showed positive molecular result in which 30.8% showed β-globin gene mutations, 6.8% showed α-globin gene deletions and 0.9% showed coexistence of α and β-globin gene defects.

We found the commonest β gene mutation detected among borderline HbA2 were CD 19 (A>G) (45.9%), IVS 1-1 (G>A) (24.3%), Poly A mutation (13.5%) and CAP +1 (A>C) (2.7%). The spectrum was not similar compared to spectrum in classical high HbA2 β- thalassemia carrier in Malaysia.

According to the previous study by Syahzuwan Hassan et al. [12], among the classical β-thalassemia carrier they found a high frequency of carriers with Cd 26 (G>A), IVS1–5 (G>C), IVS 1–1 (G>T), and Cd 41/42 (–TTCT) [12]. This observation strongly suggesting normalization of HbA2 level in β-thalassemia may underlined by the type of mutations.

Study done among borderline HbA2 samples in Italian populations showed that out of 95 positive samples, majority which was 25.3% showed mutation IVS 1 nt6 [11]. Study done among Chinese in Southern Asia showed that out of 165 samples of borderline HbA2 (3.3%- 3.7%), 9.1% were positive for molecular defect in α- or β- globin gene.

Out of that, 4 samples had heterozygous β-mutations with detection of -50 (G>T), -31 (A>C) and Cd 17 (A>T).

No coinheritance of δ-gene detected in all these 4 samples. This study also tested for *KLF1* gene assuming that *KLF1* gene mutation was one of the reasons that cause borderline HbA2. 18 (10.9%) were positive for molecular defect in *KLF1* gene and two samples (1.2%) were positive for α-globin and *KLF1* gene defect [2,5]. The significance of detecting β-thalassemia among borderline level of *HbA2* is prettily obvious.

In this study, β mutations were seen in a significant numbers at the level of 3% of HbA2. To be more comprehensive and informative data, larger scale of study is needed to reveal the factors and its mechanism in lowering down the level of HbA2 in β-thalassemia.

In ensuring the more effective prevention programme in our center, perhaps the value of HbA2 of 4% and above in diagnosing β- thalassemia may need to be revised, with the conjunction with other hematological parameters.

Conflict of Interests:
No potential conflict of interests to declare.

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