Association of Low Serum Iron with Alpha Globin Gene Deletions and High Level of HbF with Xmn-1 Polymorphism in Sickle Cell Traits

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Abstract Usually sickle cell traits are asymptomatic but co-existence of various factors may alter the clinical as well as biochemical levels. In India sickle cell traits are neglected condition. Here we are presenting the alpha deletion in association with low serum iron and increased HbF level with Xmn-1 carriers in sickle cell traits. Sickle traits with alpha deletions had significantly low level of serum iron (P -value <0.05) with low level of reticulocytes and red cell indices while Xmn-1 polymorphism associated with increased HbF level. Study concludes low serum iron associated with alpha deletions and high level of HbF associated with Xmn-1 polymorphism in sickle cell traits.

Keywords Sickle traits · Gap-PCR · Hemoglobinopathies

Introduction

Heterozygous condition of sickle gene (Sickle trait) is asymptomatic condition and mostly people have no clinical complications. People at high risk for having sickle cell traits are those whose ancestors come from Africa, South or Central America, Caribbean, Mediterranean countries, India and Saudi Arabia. Point sickle trait has high risk in athlete and cause medical emergency due to polymerization of S hemoglobin and prevent blood circulations [1].

Sickle cell trait occurs in ~300 million people worldwide, with the highest prevalence of ~30–40% in sub-Saharan Africa. Long considered a benign carrier state with relative protection against severe malaria. Sickle cell trait occasionally can be associated with significant morbidity and mortality [2]. Clinically Important feature of alpha thalassemia is its interaction with sickle gene. It has been shown that co-inheritance of alpha thalassemia improve the hematological parameters of heterozygous beta thalassemia and result in a relatively mild clinical picture of homozygous β thalassemia [3, 4]. The interaction of alpha deletions and effect on features of sickle traits has not been characterized in India. The (C-T) variation at position-158 upstream of the γ globin gene, which is detectable by the restriction enzyme Xmn-1, known to affect HbF production [5]. The effect of -158 C > T mutation on expression of γ globin gene has been the subject of considerable interest. Increased HbF play role an ameliorating factor in patients who were mildly affected despite being homozygotes or compound heterozygotes for β^0 or β^+ thalassaemia [6]. However, the none of the researcher reported the possible association of HbF with Xmn-1 polymorphism in sickle cell traits. In India researcher are less focused on sickle traits where the carrier ignored to their complexity and laboratory investigations. Thus, our aim was to investigate the co-existence of alpha thalassemia and Xmn-1 polymorphism in sickle cell traits and their association with serum iron and HbF level.

Material and Method

Study subjects were sickle cell traits; who were attending the hematology department for hemoglobinopathies screening in AIIMS, New Delhi. Duration of sample collection was

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3 years and study was approved by institutional ethical committee. About 5 ml venous blood drawn after taking signed consent of the patient. Complete blood count was measured by automated cell analyzer (SYSMEX K-4500, Kobe Japan). A cation exchange high performance liquid chromatography (HPLC-Bio-Rad-Variant™ Bio Rad, CA, USA) used for quantitative assessment of hemoglobin variant HbA, HbF, HbA2 and HbS and diagnosis of sickle cell traits. Isolation of DNA done by phenol–chloroform method and DNA quantification done by nano drop spectrophotometer. Molecular confirmation of sickle traits done according to Waterfall et al. (2001) [7] with few modifications. Identification of alpha deletions and triplication done by Gap-PCR. Alpha 3.7, 4.2 kb deletions and triplication $\alpha\alpha\alpha$ (anti 3.7 kb) done according to Baysal et al. (1994) [8] and Smetanina et al. (1996) [9]. Identification of South African deletions done according to Shaji et al. (2003) [10] while South East Asian deletion identification done according to Chang et al. (1991) [11]. Xmn1 polymorphism analysis done by PCR-RFLP according to Sutton et al. (1989) [12]. Serum iron study done by standard laboratory method. Mean values and standard deviation used to evaluate the hematological data. *t* test used to compares the mean of groups on GraphPad (version-3.06) software. *P*-value <0.05 was considered statistically significant. Sickle trait and Xmn-1 polymorphism agarose gel picture are shown in Figs. 1, 2, 3, respectively.

Result and Discussion

A total of 90 sickle cell trait patient's were characterized (52 male and 38 female with mean age 22.27 ± 4.11). 80% study subject were the parents of the patients who were diagnosed as SCD patients. Subject was from entire part of India. Fifteen sickle cell traits from Orissa, 12 from Bihar, nine from Jharkhand, six from Madhya Pradesh, and eight from Karnataka, nine from Maharashtra, eight from

Fig. 1 Agarose gel picture of PCR amplified products of sickle cell traits

Fig. 2 Check gel for Xmn-1 polymorphism

Fig. 3 Restriction digestion with Xmn-1 enzyme (lane 1,4,5,8,9,10,11 heterozygous and lane 2,3,6,12 homozygous while lane 7 normal for Xmn-1 polymorphism)

Rajasthan, seven from Gujarat, while five from Delhi, seven from Uttar Pradesh, and four from Hariyana. Seven (7.78%) sickle cell traits (5 male and 2 female with mean age 21.2 ± 3.1) out of 90 were heterozygous for alpha 3.7 kb deletions while 83 patients (50 male and 33 female with mean age 22.3 ± 3.6) were negative for alpha deletions. None of the patient were identified with 4.2 kb, alpha triplication and double (–SA, –SEA) deletions. Reticulocytes and red cell indices were significantly low in sickle traits with alpha 3.7 kb deletions in association with low serum iron (*P* value <0.05). Sickle traits with alpha

Table 1 Hematological parameters of sickle traits with alpha deletions and without alpha deletion in association with low serum iron

Mean $\pm$ SD			
Parameters	SA with alpha del. (N = 7)	SA without alpha del. (N = 83)	P-value
WBC (ths/ μ l)	8.6 $\pm$ 2.4	8.1 $\pm$ 2.3	0.58
RBC (millions/ μ l)	3.4 $\pm$ 0.4	4.5 $\pm$ 0.7	<0.001
HGB (g/dl)	12.8 $\pm$ 1.7	13.5 $\pm$ 1.3	0.18
HCT (%)	29.1 $\pm$ 4.5	34.3 $\pm$ 6.1	0.03
MCV (fl)	73.3 $\pm$ 10.8	83.3 $\pm$ 5.8	<0.001
MCH (pg)	20.8 $\pm$ 5.6	25.7 $\pm$ 4.8	0.012
MCHC (g/dl)	26.9 $\pm$ 3.2	30.4 $\pm$ 2.8	0.03
PLT (ths/ μ l)	246.7 $\pm$ 62.8	236.8 $\pm$ 57.8	0.66
Serum iron (μ g/dl)	83.4 $\pm$ 21.7	109.3 $\pm$ 24.6	0.008
TIBC (μ g/dl)	319.2 $\pm$ 13.5	298.4 $\pm$ 23.6	0.024
Transferrin saturation (%)	24.6 $\pm$ 4.8	30.4 $\pm$ 6.8	0.030

deletions; mean serum iron was 83.4 ± 21.7 μ g/dl while without alpha deletion was 30.4 ± 6.8 μ g/dl. Serum iron and transferrin saturation were significantly low while TIBC was significantly higher in sickle traits with alpha deletions (P -value <0.05). Details of hematological and iron parameters are given in Table 1. Iron metabolism in sickle cell disease, largely differs as compared to thalassemia [13, 14]. There is no evidence of iron overload in non-transfused SCD patients, and iron deficiency may be even develop, possibly related to intravascular hemolysis and the resulting excessive urinary losses of iron [15]. Due to the different physiopathology of anemia in thalassemia and sickle cell disease, there are significant differences in the physiopathology of iron overload and iron-related complications in these disorders [16]. Among the sickle trait; 25 were heterozygous ($\pm$), and eight were homozygous (+/+) carrier for Xmn-1 polymorphism. HbF level was $1.2 \pm 0.6\%$ in sickle traits with normal Xmn-1 polymorphism and $6.4 \pm 2.1\%$ in traits with heterozygous ($\pm$), while $9.2 \pm 3.6\%$ in homozygous (+/+) carrier for Xmn-1 polymorphism. Hb, reticulocytes and red cell indices were within the normal range amongst the group. There is insufficient evidence to suggest an independent association with retinopathy, cholelithiasis, priapism, leg ulcers, liver necrosis, avascular necrosis of the femoral head, and stroke. Despite these associations, the average life span of individuals with sickle cell trait is similar to that of the general population. Nonetheless, given the large number of people with sickle cell trait, it is important that physicians be aware of these associations [2]. Bauer and Fisher (1943) [17] first suggested clinical manifestation of sickle-cell trait. A Mexican study also found the of α -globin gene mutations in sickle trait as well as normal individual [18].

The exact molecular basis of the interaction of α -thalassemia with sickle trait in India has not yet been described. Few studies state the sickle cell patient with alpha thalassemia have higher total hemoglobin levels and changes compatible with less rapid hemolysis [19–21]. However, the clinical significance of the interaction of alpha thalassemia with sickle cell disease still controversial. The strong association of Xmn-1 site with the Arab Indian haplotype is thought to be associated with high fetal hemoglobin concentration and confer a benign course of the disease. However, the clinical presentation of sickle cell disease in different regions in our country is highly variable [22]. This heterogeneity is likely to be due to the presence of different β -thalassemia alleles or interaction with modulating genetic factors like associated α -thalassemia and a gene for raised HbF production (Xmn I polymorphism) [23]. Heterozygosity for presence of Xmn I site polymorphism is also likely to influence phenotype [24] and Xmn-1 polymorphism absence reduction is associated with acquired HbF elevation [25]. In India none of the literature available in association of iron with alpha deletions in sickle cell traits. Observation of the study conclude the alpha deletions significantly associated with low level of serum iron while increased HbF level associated with Xmn-1 polymorphism in sickle cell traits.

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