Association of XmnI Polymorphism with Fetal Hemoglobin Level in Sudanese Patients with Sickle Cell Disease

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Abstract

Background: Sickle cell disease (SCD) is an inherited blood disorder that affects red blood cells. The study of various modulating factors, and genetic factors affecting the clinical severity of the SCD is an interesting research focus especially in communities with a distinct genetic background. The XmnI polymorphism is a common genetic variation that was reported in previous studies to increase fetal hemoglobin (HbF) level. This was a descriptive cross-sectional study, conducted in El-Obeid city in Northern Kordofan state, western Sudan, during the period from August to November 2016. The Xmn1 polymorphic site was determined by polymerase chain reaction. Data was analyzed using SPSS software program version 20. P-value of 0.05 and below was considered of significance. In present study HbF level among normal individuals AA , shown significant difference ($p<0.05$) between presence of Xmnl +/+ and absence of Xmnl -/- site. in patients with SS , the HbF level was higher in those who had one or two Xmnl sites as compared to those with the site absent . In patients with sickle cell trait AS and AA, only the presence of the one and two Xmnl site (+/+) compared to the absence of the site (-/-) was associated with significant increase in the HbF level. There is a close link between the Xmn1 polymorphism site and HbF level. A wide range of HbF level was obtained both in the present and absence of this site. Further studies with a large sample size as well as analysis BS haplotypes among the patient with sickle cell anemia population are needed for better understand of possible association .

Keywords: *XmnI Polymorphism, SCD, Sickle cell disease, Fetal hemoglobin, HbF*

Introduction

Sickle cell disease (SCD) is an inherited autosomal recessive disorder with presence of Hb S in blood. This disease affects millions of peoples globally which results in serious complications due to vasoocclusive phenomenon and hemolysis .Sickle hemoglobin (Hb S) is a structural variant of normal adult hemoglobin (Hb A) caused by a mutation in the HBB gene that leads to the substitution of valine for glutamic acid at position 6 of the β-globin's subunit (βS) of the hemoglobin molecule[1]. The pathological process in sickle cell disease is caused by the sickling phenomenon[2].The basis of sickling in patients homozygous for the disorder, called sickle cell anemia or Hb SS, is polymerization of deoxy-Hb S resulting in the formation of multistranded fibers that create a gel and change the shape of RBCs from biconcave discs to elongated crescents. The polymerization/sickling reaction is reversible following reoxygenation of the hemoglobin. Thus, an RBC can undergo repeated cycles of sickling and un sickling[3].The C-T substitution at position – 158 of the Y^G globin gene referred to astheXmn1-y polymorphism is reported to be a common sequence variant inall population groups, present at a frequency of 0.32 to 0.35. [4].Clinical studies have shown that under conditions of hematopoietic stress, for example in homozygous B-thalassemia and sicklecell

disease the presenceof the Xmn1 – Y^G site favors a higher Hb F response This could explain why the same mutations on different B chromosomal backgrounds are associated with disease of different clinical severity[5]. The y^G – 158(C – T) polymorphism plays important function in the disease severity of Sickle cell anemia. The Xmn1 restriction site at – 158 position of the y^G – gene isassociated with increased expression of the y^G – goblin gene and higher production of HbF [6].In Sudan several studies were conducted among patient with sickle cell disease [7,8,9], but there are no studies to assess association between Xmn1 polymorphism and HbF level in sickle cell disease patient the prevalence in different area in Sudan. The aim of present study to association of XmnI polymorphism with fetal hemoglobin level in Sudanese patients with sickle cell disease.

Material and Methods

This was a descriptive cross-sectional study, conducted in El-Obeid city in Northern Kordofan state, western Sudan, during the period from August to November 2016. Known patients with sickle cell anemia attending Elobied children specialized hospital and Elkowity Hospital. Permission was taken from the committee of Kordofan university , El-Obeid Teaching Hospital and Elkowity Hospital, also consent was taking from patients and co–patients in Hospital.100 patient volunteer was selected using simple random technique. 2.5 ml of venous blood was collected from each participant under complete antiseptic condition. Hemoglobin F was measured by modified Betke Method. The Xmn1 polymorphic site was determined by polymerase chain reaction. Data was analyzed using SPSS software program version 20. P-value of 0.05 and below was considered of significance.The sickle cell mutation was confirmed by amplifying the 5' region of the B. Globin genes followed by restriction digestion with Ddel A 650- bp fragment 5'to the YG sense was amplified using the Primer5'AACTGTTFCTTTATAGGATTTT-3 and 5'AGGAGCTTATTGATAACCTCAGAC-3. The amplification condition were initial denaturation 94c for 5 min followed by 30 cycles of 94c for 1 min and 55c for 1 min 72c / min. with a final extension of 5min at 72c the PCR product was digested with three unit of Xmn1 restriction enzyme and separated by electrophoresis on 3% agarose gel.

Result

Significantly different comparing Xmnl +/+ with Xmnl +/- ($p<0.05$) or comparing Xmnl +/+ with -/- ($p<0.05$) in sickle cell patients (SS), the HbF level was significantly higher in those who had two Xmnl sites ($p<0.05$) compared to those with only one Xmnl site and with absent site ($p<0.05$) in patients with SS who had one Xmnl site and Xmnl -/- site there is no difference in HbF level(Table I). .In AS patient's presence of two Xmnl +/+ site compared with one Xmnl site ($p<0.05$) and one Xmnl site compared with Xmnl -/- site ($p<0.05$) had significant higher level of HbF(Table II). Significantly different comparing Xmnl +/- with Xmnl -/- ($p<0.05$) or comparing Xmnl +/+ with +/- ($p<0.05$) in normal control (AA), (Table III).

Table 1: The HbF percentage and Xmnl in SS group.

Xmnl	SS(n=60)	HbF(mean ± SD)	P.value
+/+	16(27%)	81.97 ± 3.30	p<0.05
+/-	20(33%)	21.84 ± 13.83	p<0.05
-/-	24(40%)	20.28 ± 10.86	p<0.05

Table II: The association betweenHbF percentage and Xmnl in AS group.

Xmnl	AS(n=30)	HbF(mean ± SD)	P.value
+/+	4(13%)	17.45 ± 0.58	p<0.05
+/-	10(34%)	0.8 ± 0.57	p<0.05
-/-	16(53%)	0.04 ± 0.11	p<0.05

Table III: The association between HbF percentage and Xmnl in AA group.

Xmnl	AA(n=10)	HbF(mean ± SD)	P.value
+/+	2(20%)	0.95 ± 0.63	p<0.05
+/-	5(50%)	0.37 ± 0.22	p<0.05
-/-	3(30%)	0.02 ± 0.08	p<0.05

Discussion

The XmnI polymorphism is a common genetic variation that was reported in previous studies to increase HbF level and therefore ameliorate the severity of the sickle cell disease.Our study shows that a polymorphism of the XmnI was found to be associated with higher expression of HbF in sickle cell and sickle cell trait patients. Several studies confirmed the association between XmnI and fetal hemoglobin [10,11].Appositive association was observed between the HbF level and the presence of Xmn1 site in SS and sickle thalassemia groups Recently, other genetic association studies shown that several single nucleotide polymorphisms, associated with variation in the expression of HbF in sickle cell disease[12].The XmnI polymorphism is known to influence the γ^G gene expression in sickle cell anemia and to increased HbF concentrations when they are under conditions of erythropoietic stress[13]. Study conducted by FarizKahhaleh et.al to assist the Association of Xmn1 polymorphism and consanguineous marriage with fetal hemoglobin in Syrian patients with sickle cell disease they concluded that a strong evidence on the importance of Xmn1 polymorphism and consanguineous marriage, among other factors, in the prediction of clinical severity and hydroxyurea response in SCD patients[14]. In sickle cell disease and sickle cell trait patients of Elobied, the presence of this polymorphism is associated with high HbF level. This is the first report of the frequency of the -158 XmnI γ^G-globin polymorphism in patients with SS and AS in Elobied.

Conclusion

There is a close link between the Xmn1 polymorphism site and HbF level. A wide range of HbF level was obtained both in the present and absence of this site. Further studies with a large sample size as well as analysis BS haplotypes among the patient with sickle cell anemia population are needed for better understand of possible association.

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