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## The association of the -158 XmnI $\gamma$ G globin polymorphism with HbF level in sickle cell anemia Sudanese Patients

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### Abstract

**Background:** Sickle cell hemoglobinopathy is a genetic disorder caused by the presence of hemoglobin S (HbS),  $\gamma$ G-158 (C $\rightarrow$ T) polymorphism plays an important function in the disease severity of sickle cell anemia, The XmnI restriction site at -158 position of the  $\gamma$ G-gene is associated with increased expression of the  $\gamma$ G-globin gene and higher production of HbF, Previous studies have suggested that a variety of genetic determents influence different clinical phenotypes. The genetic variants that modulate HbF levels have a very strong impact on ameliorating the clinical phenotype. **Aim:** This study aims to associate between XmnI (... $\gamma$ G-158 C $\rightarrow$ T ...) polymorphism and fetal hemoglobin level among Sudanese patients with SCA. **Materials and methods:** In this descriptive cross-sectional study 60 blood samples from diagnostic cases were analyzed using a Hematology analyzer (Sysmex KX21N), capillary electrophoresis (MINICAP), using "G-spin™ Total DNA Extraction Kit", PCR-RFLP techniques. **Results:** Patients with SCA were analyzed for XmnI polymorphism and association between this polymorphism and severity of SCA was evaluated, the presence of one XmnI (+/-) site CT 2% in SS patients compared with XmnI/- site CC98% had shown difference regarding HbF level, thus the Polymorphic association was founded. **Conclusion:** In our descriptive cross-sectional study we concluded that the effect of the polymorphism on the Hb F level was established.

**Keywords:** SCA: Sickle Cell Anemia,  $\gamma$ G-158 C $\rightarrow$ T, HbF: Fetal Hemoglobin

### Introduction:

Sickle cell hemoglobinopathy is an autosomal recessive genetic disorder caused by the presence of hemoglobin S (HbS) (Clarke and Higgins 2000). HbS is a variant of normal hemoglobin (HbA) and is due to a mutation in the  $\beta$ -globin gene, where thymidine replaced adenine resulting in the substitution of glutamic acid by valine at position 6 of the  $\beta$ -globin

chain (Flint *et al.*, 1998). The term 'sickle-cell disease' (SCA) includes all clinically manifested genotypes of sickle cell hemoglobinopathy (proportion of HbS >50%). Sickle cell anemia (SCA) affects millions throughout the world. It is particularly common among people whose ancestors are from sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and Mediterranean countries, such as Turkey,

Greece, and Italy (WHO Media Centre 2011). The lack of widely available newborn screening or early access to comprehensive preventative care causes the early mortality of SCA in Africa, which is strikingly high. Various published reports estimate mortality rates between 50 and 90% before the age of five years (Williams et al. 2009; Grosse *et al.*, 2011)

There are many reported pathological consequences of high levels of HbS in SCA, among which are vaso-occlusive disease and increased hemolysis. Patients suffering from SCA show increased susceptibility to infections, disturbances of growth and development, and chronic end-organ damage (Makani *et al.*, 2007). SCA represents a heterogeneous entity with extremely variable clinical severity. Interindividual variations in HbF levels are one of the main contributors to the clinical heterogeneity observed in SCA patients. Higher expression of HbF in adulthood is reported to ameliorate morbidity and mortality in SCA possibly through interfering with the HbS sickling process (Powars et al. 1984; Platt *et al.*, 1994; Steinberg 2005, 2009; Thein and Menzel 2009).

DNA sequence variations within the  $\beta$ -globin gene cluster, in particular, the (C→T) variation at position -158 upstream of the  $\gamma$  globin gene is a common genetic alteration, which is reported in several clinical studies to predispose carriers to increased HbF production, particularly when exposed to erythropoietic stress, as in SCA. This could explain why carriers of a certain  $\beta$ -globin gene mutation may have a disease of different clinical severity. The aforementioned genetic variation, also known as rs7482144, is detectable by the Xmn1 restriction enzyme (Pandey *et al.*, 2012; Sharma and Mahanta 2013).

To the best of our knowledge the condition of the (-158 Xmn I  $\gamma$ G globin polymorphism has not been reported in sickle cell anemia patients from Sudan. The present

study was aimed mainly to investigate the association of the -158 XmnI  $\gamma$ Gglobin polymorphism with HbF level in Sudanese sickle cell anemia patients.

#### Patients and methods:

We studied 60 sickle homozygous patients, 26 of them were females, while 34 were males. Patients under Hydroxyurea treatment and blood transfusion cases were excluded. About 5 ml blood sample was collected from sickle cell anemia patients attending Ahmed Gasim Children's Hospital, Khartoum, Sudan after taking their consent from their parents. The study was approved by Al Neelain University ethical review board and Blood samples were collected after approval of patients' parents. Clinical evaluation was done during physical examination (visually appeared) as well as laboratory evaluation (the presence of anemia was evaluated through hemogram analysis). Complete blood count and red cell indices were measured by an automated cell analyzer (SYSMEX KX21N). Quantitative assessment of hemoglobin Hb F was measured by capillary electrophoresis (MINICAP).

Genomic DNA extraction was done using "G-spin™ Total DNA Extraction Kit INtRON Biotechnology, Korea". Polymerase chain reaction (PCR) was done, 3 $\mu$ l of DNA will be amplified in a total volume of 20 $\mu$ l PCR mixture containing 1 $\mu$ l of each primer and 15 $\mu$ l distilled water. A 650-bp fragment to the  $\gamma$ G gene was amplified using the primer 5' - AACTGTTGCTTTATAGGATTTT-3 and 5' -AGGA GCTTATTGATAACCTCAGAC-3. The amplification conditions were as follows: initial denaturation at 94°C for 5 minutes, 30 cycles each consist of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and final extension at 72°C for 10 minutes (PACINT MOEZ *et al.*, 2018).

Then PCR product was digested with 1  $\mu$ l of Xmn1 restriction enzyme "PCR-RFLP method" presence of T allele, one fragment of 450 -bp was produced. The

presence of the normal allele C loses cleavage site for XmnI and thus an intact 650-bp fragment was produced and separated by electrophoresis using 3% agarose gel. **Statistical Analysis:**

All data were analyzed using the statistical package for social sciences (SPSS) version 20.0 (IBM Inc, Chicago, IL, USA). Normality of the data assessed by the Shapiro-Wilk. Data are expressed as mean  $\pm$  standard deviation (SD) the statistical differences in allele frequency and genotype distributions were determined by calculating the odds ratio.

### Results:

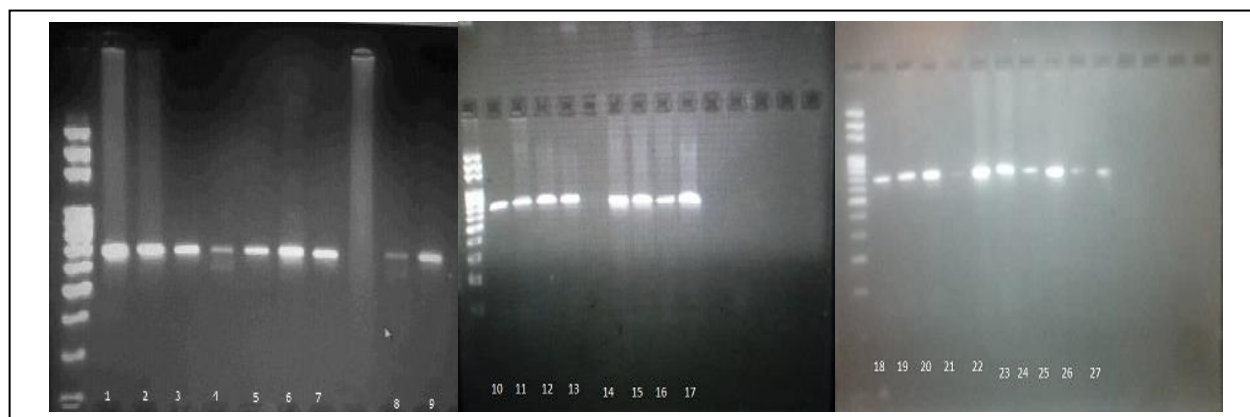
In our study, complete blood count was conducted for sickle cell anemic patients which showed no significant difference in HbF concentration regarding the severity of the disease, RBCs count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean

corpuscular hemoglobin concentration (MCHC), Hct% and HbF are presented in table 1.

60 cases of sickle cell anemic patients were enrolled, distribution of patients according to gender was 26 (43.3%) females and 34 (56.7%) males}, age {4-10 years old was 17 (28%) and 43(72%) was 10-16 years}, and according to the severity of disease 33 (55%) were sever cases and 27 (45%) were moderate. Our results according to gene type of patients showed that genotype frequencies of the XmnI  $\gamma$ G globin polymorphism (-158C>T) (rs7482144) among sickle cell anemic patients were 3% heterozygote (2 cases) (CT) and 97% (58 cases) were homozygous for the wild-type allele (CC).

Also, our result shows no significant association between polymorphism of gene and sex of the patient's *P-value*: 0.683, age *P-value* 0.490, and severity of the disease *P-value* 0.702.

**Fig (1): 3% agarose gel electrophoresis pattern:**



Shows electrophoresis pattern of RFLP product of XmnI site. First lane\* of all three gel shows the molecular weight marker with 100 bp DNA ladder. Lane (4, 8) shows RFLP product with XmnI (+/-) site. And other Lanes (1,2,3,5,6,7,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27) show product with XmnI (-/-) sites.

**Table (1): The comparison of hematological parameters across the gene type:**

Parameters	CT (Mean±SD)	CC (Mean±SD)	P-value
Hb-F %	4.00±2.26	12.9±5.75	0.035
Hb (g/dl)	6.50±0.42	6.40±1.06	0.901
HCT %	19.7±2.76	19.0±3.23	0.782
RBC/μL	2.58±0.46	2.43±0.56	0.710
MCV (fL)	76.1±3.68	83.5±5.62	0.022
MCH (g)	25.4±3.18	28.2±2.73	0.160
MCHC (g/dl)	33.3±2.47	33.2±2.09	0.948

Shows hematological, HbF% and genotypic characteristics of the sickle cell anemic patients in which the significant association between polymorphism of the XmnI gene and HbF was with *p-value*: 0.035

### Discussion:

The clinical severity of the SCA is remarkably variable. Factors underlying such heterogeneity are not yet fully understood. The study of various modulating factors, and in particular genetic factors, affecting the clinical severity of SCA is interesting research focus especially in communities with a distinct genetic background. The Xmn1 polymorphism is a common genetic variation that was reported in previous studies to increase the HbF level and therefore, ameliorate the severity of the SCA. In the present study, we focused on studying the genotype frequency of the Xmn1  $\gamma^G$  globin polymorphism (-158C>T) in Sudan sickle cell anemic patients as an example of a closed community. Further, we tried to study any possible effect of the presence of the Xmn1 polymorphic site on HbF level.

Our study was conducted on 60 sickle cell anemic patients from Ahmed Gasim Children's Hospital, Khartoum, Sudan during the period from May to Sep 2018. DNA polymorphism of the XmnI site at -158 C→T in the  $\gamma^G$  promoter was found to be associated with higher expression of HbF in sickle cell anemic patients.

Statistical analysis has identified strong evidence for dominance at the locus, suggesting an effect of the  $\gamma^G$ -158 (C→T) polymorphism. The finding of the present study shows that 3 % of patients with SS had one XmnI (+/-) site, and the absence of site XmnI (-/-) were showing the difference in HbF levels. Statistical analyses have identified no evidence for dominance at the locus, suggesting an additive effect of the  $\gamma^G$ -158 (C→T) polymorphism. In agreement with our results, (Rahimi *et al.* (2007)) have performed a study on sickle cell patients of Iran, They found that among patients with HbSS, the HbF level was significantly higher in those who had one (P = 0.04) or two (P = 0.01) Xmn1 polymorphic sites as compared to those with no polymorphic site.

The XmnI polymorphism is known to influence the  $\gamma^G$  gene expression in sickle cell anemia and to increase HbF concentrations in particular when they are under conditions of erythropoietic stress. In Sudanese sickle cell anemic patients, the presence of this polymorphism is associated with high HbF levels. This is the first report of the frequency of the -158

XmnI  $\gamma^G$ -globin polymorphism in patients with sickle cell anemia (SCA) in Sudan.

### Conclusion:

Studying the genotype frequency of the XmnI  $\gamma^G$  globin polymorphism ( $-158C>T$ ) in Sudan, with a distinct genetic background can be considered as a starting point for further research.

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