International Journal of Multidisciplinary and Current Research ISSN: 2321-3124
Research Article Available at: http://ijmcr.com

Association of L-Selectin gene polymorphism with Clinical Severity of sickle Cell Disease among Sudanese Patients in Khartoum 2016

Alaaeddin M.Elzubeir[1, 3], Alaa Ibrahim[1], Amna Mohammed[1], Noha Osama [1], Yasser Salem[1], Dhafer Salem[1], Hosham Abdalrahman[2], Nazik Husain[4] , Abu Elgasim Awad elkareem[5], Hind M.Ahmed[6]

[1]Department of Haematology and Immunohaematology, FMLs, University of Khartoum-Sudan
[2]Department of Molecular-biology, FMLs, University of Khartoum - Sudan
[3]Department of Haematology and Immunohaematology, FMLs, Sudan International University-Sudan
[4]Department of Pathology, Faculty of medicine, Omdurman Islamic University -Sudan
[5]Department of Histopathology, FMLs, Sudan University of Science and Technology -Sudan
[6]Department of Pediatric Gafar Ibn-Aoaf Pediatrics teaching hospital Khartoum-Sudan

Accepted 15 July 2016, Available online 18 July 2016, **Vol.4 (July/Aug 2016 issue)**

Abstract

Background: *Sickle cell disease (SCD) is an inherited disease characterized by a complex and varied physiopathology and exhibits wide clinical diversity. SCA's variability and pose significant management challenges to caregivers and physicians alike.*
Objectives: *We therefore introduced to correlate the clinical severity of SCD in Sudanese Patients and L.selectin gene polymorphisms.*
Result: *There was significant association between these gene polymorphisms and high expression of L-selectin by leukocytes, or the development of complications in SCD, Chi squared (χ^2) = 0.000 (P < 0.05).*
Conclusion: *These findings indicated that the (P213S) polymorphism of L-selectin gene may contribute to susceptibility to vasoocclusive crisis and more severe situation in the Sudanese patients with homozygous Sickle Cell disease.*

Keywords: *Sickle Cell Disease, Severity, L-Selectin gene, P213S, polymorphism.*

1. Introduction

Sickle Cell Disease (SCD), also known as sickle cell anemia (SCA), is a group of autosomal recessive genetic blood disorders characterized by a single point mutation in the sixth codon of the β-globin gene. Under low oxygen tension, the resultant abnormal hemoglobin S polymerizes and causes rigid and sickle-shaped red blood cells [(1)]. Sickle haemoglobin (HbS) is the most common pathological haemoglobin mutation worldwide.

The pathogenesis of sickle cell disease is based on the increased adhesiveness of sickle cells to the endothelium of blood vessels causing vaso-occlusion which is the major cause of chronic organs damage in SCD [(2)]. Individuals with SCA suffer a wide range of complications: increased susceptibility to infections, chronic hemolytic anemia, recurrent periodic acute vaso-occlusive events and chronic damage affecting almost every organ system [(3)].
Sickle cell disease crises (pain crisis or vaso-occlusive crisis), are still responsible for high morbidity and early mortality. Blood transfusions remain the mainstay of therapy for all severe acute crises [(4)].

Selectins are a family of transmembrane molecules, expressed on the surface of leukocytes and activated endothelial cells, these molecules generates ligand-specific outside-in signals to modulate neutrophil apoptosis, a critical control point in the resolution of inflammation [(5)].

The selectin family consists of three closely related cell-surface molecules with differential expression by leukocytes (L-selectin), platelets (P-selectin), and vascular endothelium (E- and P-selectin).

The selectins mediate neutrophil, monocyte, and lymphocyte rolling along the venular wall. These receptors regulate inflammatory processes.

Study by Aslihan et al., demonstrated that leukocytes play a direct role in the vascular occlusions caused by sickle cell disease. A number of clinical observations have linked leukocytosis with symptomatic sickle cell disease but it has been unclear whether the leukocytosis was simply a surrogate marker of inflammation rather than the leukocytes being directly engaged in the pathogenesis of vasoocclusion. Thus leukocytosis in SS patients is associated with increased mortality [(6)].

Another study by Okpala etal revealed High steady-state l-selectin by leukocytes predisposes to severe manifestations, increased leukocyte adhesion molecules expression above steady-state levels could be important

in the genesis of crisis. The early symptomatic improvement that follows Hydroxyurea therapy is mediated via mechanisms independent of increased HbF, and may involve reduced adhesion molecules expression in leukocytes. Other treatment modalities that reduce leukocyte adhesion molecules expression might also confer clinical benefit [7].

Blood cell adhesion to endothelium would promote sickling, by fulfilling the requirement for slowed flow to accommodate the polymerization delay time [8].Competition between the delay time for HbS polymerization and the RBC transit time in microcirculation is likely a key determinant of disease severity [9].

These findings have led to the current postulation that an enhanced tendency of red blood cells (sickled and nonsickled) to adhere to vascular endothelium and activation of leukocytes and platelets are the primary causative factors of vaso-occlusion and haemolysis [10], [11]. But Ugochukwu et al., found that there was high expression of l-selectin (SELL) on leukocytes in patients with complications of sickle cell disease but there was no association between any of these gene polymorphisms and high expression of l-selectin by leukocytes, or the development of complications in SCD [12].

This study may provide a possible way to connect gene polymorphisms with complications in sickle cell disease, and may reveal different result because the disease itself affected by many genetic and environmental factors and the outcome will help to develop a novel individualized treatment, which uses an individual's genome to provide a more informed and tailored drug prescription according to severity to reduce haemolysis, vaso-occlusive events and associated pathogenic complications, incidence of premature birth, maternal and neonatal morbidity and mortality.

Material and Methods

This is a descriptive cross-sectional and analytical case: control hospital based study, conducted at Gafar Ibn-Aoaf Pediatrics Teaching hospital, during the period from 1/3/2016 to 30/3/2016. The local ethics committee and ministry of health approved the research conducted in accordance with WMA Declaration of Helsinki (2008).

Subjects

The study population comprised; patients with Sickle cell disease, regardless to age, gender and ethnic group. The diagnoses of patients with sickle cell were confirmed using cellulose acetate electrophoresis having Hb SS disease.

Clinical data collected by enclosed questionnaire and recording form and authorized clinician who carry out the clinical examination. After informed consent a sample of venous blood (5mL) collected under specific condition into two EDTA container (whole blood) one for DNA extraction and the second for CBC and electrophoresis**.** Apparently Healthy clinical controls were selected to be similar as sex, age and residence place with the patients.

DNA genotyping

The detection of L-selectin (SELL) genes is based on examination of the size of the polymerase chain reaction (PCR) products.

From each subject, 3 mL of whole blood collected in (EDTA) containers were used to extract genomic DNA using a small amount of whole blood quickly with (innuPREP) whole Blood DNA (Mini Genomic DNA extraction Kit).

The Pro213Ser (P213S) SELL (rs4987310) polymorphism genotyping has been achieved by PCR-RFLP technique, using the following protocol: Each amplification reaction (total volume 25 µl L) contained, The primer sequence for detecting the polymorphism were 5'- TGATTCAGTGTGAGCCTTTG -3' (forward primer) and 5' CTTGACAGGTTGGTTCTG-3' (reverse primer), 1µl of forward primer, 1ul of reverse primer and 2 µl of DNA is added to the other PCR components needed for the reaction (Maxime PCR PreMix, i-Tag).

Initial denaturation in 95c for 5 min. Denaturation in 94c for 30 sec. annealing in 54c for 30 sec, extension in 72c for 30 sec, 30 cycle and then final elongation in 72c for 5 min. The genotypes were determined by digestion of each amplicon (5 µL) with 5U of (Hph1 Thermo fisher Scientific) followed by PAGE (8%). The genotypes have been established after ethidium bromide staining.

Laboratory investigation

Standard laboratory methods were used to determine haemoglobin concentration, hematocrit concentration, red cell count, total white blood cells count, platelets count, red cell indices, and reference neutrophils and lymphocytes values at the time of presentation. (Table 2).

Statistical analyses

Data analysis was performed using SPSS for Windows software version 20. Means, standard deviations (SD), and percentages were determined. Means ± SD were compared using independent t-test or one-way analysis of variance (ANOVA) as appropriate. Ratios were compared using the Pearson Chi squared (χ^2) test. The relationship between disease severity score and continous variables such as age and laboratory findings was assessed using Pearson correlation analysis. Values of P less than (0.05) were considered statistically significant.

Table 1: Clinical Severity of Sickle Cell Disease among study population

Clinical Data		Severity of Disease			Total	P.value
		Mild	Moderate	Severe		
Gender	Female	7	23	46	76	0.456
	Male	7	12	38	57	
Age (mean) years		7.2	8.2	6.8	7.2	0.001
Stroke		2	18	67	87	0.000
Sequestration/vasoocclusion		4	21	81	106	0.000
Hemolytic Crisis		14	35	84	133	*a
Leg ulcer		0	1	3	4	0.768
Muco-skeletal pain		9	35	79	123	0.000
Acute Chest Syndrome		8	23	76	107	0.001
Positive PCR Result		10	31	80	121	0.013

Bolded values indicate statistical significance.
*a No statistics are computed because Hemolytic Crisis is a constant,

Table 2: Relationship between laboratory findings and disease severity

Laboratory parameters	Mild disease N=14 (mean)	Moderate disease N=35 (mean)	Severe disease N=84 (mean)	P
TWBCs ($\times10^9$/L)	12.2	13.8	15.1	0.007
Neutrophils ($\times10^9$/L)	6.8	7	7.5	0.000
Lymphocyte($\times10^9$/L)	5.9	5.7	5.1	0.001
RBCs ($\times10^{12}$/L)	2.6	2.7	2.3	0.045
Haemoglobin (g/dL)	7.0	8.2	7.3	0.000
Haematocrit (%)	22.7	22.9	21.3	0.034
MCV(fL)	89	86.3	94	0.017
MCH(pg)	32.4	30.7	34.3	0.000
MCHC(g/dL)	35.8	35.8	36.5	0.001
Platelet($\times10^9$/L)	572.6	450	525	0.044

N: Number of cases, TWBCs, total white blood cells count; RBCs, red blood cells count; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin; **Bolded values** indicate statistical significance.

Table3: Distribution of L-selectin gene genotypes between patients and Healthy controls

Sample		Frequency	Percent	P.value
Patients	SS	6	4.5%	0.000
	PP	49	36.8%	
	PS	78	58.6%	
Controls	SS	3	3.3%	
	PP	73	81.1%	
	PS	14	15.6%	

Results

Clinical parameters of the subjects were recorded before P213S polymorphism genotyping. The mean age of each group was included in the range 1 to 37 years; SCD duration was about 7.2 years as a mean.

Vasoocclusion and sequestration diseases were detected for 79 % of the sickler patients and those without crisis about 21% of all selected cases. We focused on more severe cases in studding of severity course of the disease, haematological values as showed in **table 2.**

The distribution of genotypes frequencies were compared using chi-square values calculated for each of the analyzed groups were **(0.000)** with p-values < 0.05, as it can be noticed in **table 3.**

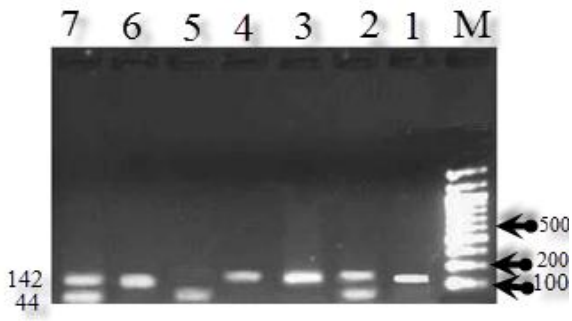

Figure 1: The electrophoresis results for Pro213 Ser polymorphism (lines M: DNA leader 100 bp, line 1: control sample, 2&7 ProSer (PS) genotype; lines 3, 4&6: ProPro (PP) genotype - lines 5: SerSer (SS) genotype)

PCR-RFLP analysis has showed the presence of all three genotypes of P213S polymorphism in the L-selectin gene. The presence of T (213Ser) allele generates a restriction site for *Hph I* enzyme and the amplicon digestion generates two fragments of 142 bp and 44 bp. When C (Pro213) allele is present, the restriction site is not created, the amplicon is not digested and it maintains its size of 186 bp **(Figure 1).**

Discussion

Leukocyte selectin (L-selectin) is an important adhesion molecule that mediates initial contact between white blood cells and vascular endothelium during diapedesis, homotypic aggregation of leukocytes, and heterotypic aggregates that include white, and other types of blood cells.

These leukocyte adhesive interactions contribute to blood vessel occlusion–the major mechanism of organ damage in SCD.

Information on how genetic polymorphisms of adhesion molecules affect clinical outcome in SCD could facilitate development of new therapies for this haemoglobinopathy. The SNPs analysed in this study were chosen because previous reports on non-SCD patients had linked them with two prominent features of SCD-vasculopathy and nephropathy, and other studies in Sickle Cell disease complication.

There was significant association between these gene polymorphisms and high expression of L-selectin by leukocytes, or the development of complications in SCD; Chi squared (χ^2) = 0.000 $P < 0.05$).

These findings indicated that the P213S polymorphism of L-selectin gene may contribute to susceptibility to vasoocclusive crisis and more severe situation in the Sudanese population, and showed possible contribution of this polymorphism to the increased risk for severe complication of sickle cell disease in patients with stroke. Due to the relatively limited number of patients, only the allelic positivity test and the heterozygous and homozygous condition for (P213S) (PP, PS and SS) have reached the statistical significance for increasing the risk. Regarding all possibilities.

Conclusion

Our findings suggest that these gene polymorphisms are predispose to high leukocyte expression of L-selectin, or development of complications in sudanese patients with SCD and severity course of the disease, despite all the possible inconveniences.

Acknowledgement

It's with vast gratitude that we acknowledge the infinite support and help of our supervisors who supplied and guided us with their expertise insights; Gafar Ibn-Aoaf Pediatrics teaching hospital; University of Khartoum, Faculty of Medicine and Faculty of Medical Laboratory Sciences and Sudan University of Science and Technology for their permissions to carry out the work.

References

[1]. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. The Lancet. 2010 Dec 17;376(9757):2018-31.
[2]. Sankaran VG, Nathan DG. Thalassemia: an overview of 50 years of clinical research. Hematology/oncology clinics of North America. 2010 Dec 31;24(6):1005-20.
[3]. Rumaney MB, Bitoungui VJ, Vorster AA, Ramesar R, Kengne AP, Ngogang J, Wonkam A. The co-inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients and could improve their survival. PloS one. 2014 Jun 30;9(6):e100516.
[4]. Novelli EM, Gladwin MT. Crises in Sickle Cell Disease. CHEST Journal. 2016 Apr 1;149(4):1082-93.
[5]. Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HH, Elbashir MI, Crawford M. Effect of omega-3 (n− 3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. The American journal of clinical nutrition. 2013;97(1):37-44.
[6]. Turhan A, Weiss LA, Mohandas N, Coller BS, Frenette PS. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. Proceedings of the National Academy of Sciences. 2002 Mar 5;99(5):3047-51.
[7]. Okpala I, Daniel Y, Haynes R, Odoemene D, Goldman J. Relationship between the clinical manifestations of sickle cell disease and the expression of adhesion molecules on white blood cells. European journal of haematology. 2002 Sep 1;69(3):135-44.
[8]. Kato GJ, Hebbel RP, Steinberg MH, Gladwin MT. Vasculopathy in sickle cell disease: Biology, pathophysiology, genetics, translational medicine, and new research directions. American journal of hematology. 2009 Sep 1;84(9):618-25.
[9]. Du E, Diez-Silva M, Kato GJ, Dao M, Suresh S. Kinetics of sickle cell biorheology and implications for painful vasoocclusive crisis. Proceedings of the National Academy of Sciences. 2015 Feb 3;112(5):1422-7.
[10]. Okpala I, Ibegbulam O, Duru A, Ocheni S, Emodi I, Ikefuna A, Umar G, Asinobi I, Madu A, Okoye A, Nwagha T. Pilot study of omega-3 fatty acid supplements in sickle cell disease. Apmis. 2011 Jul 1;119(7):442-8.
[11]. Frenette PS, Atweh GF. Sickle cell disease: old discoveries, new concepts, and future promise. The Journal of clinical investigation. 2007 Apr 2;117(4):850-8.
[12]. Ugochukwu CC, Okpala I, Pantelidis P, Inusa B, Ibegbulam O, Onyekwere O. l-selectin gene polymorphisms and complications of sickle cell disease. International journal of laboratory hematology. 2008 Aug 1;30(4):312-6.