الاستـهلال

قال تعالى:

﴿ يَٰٓأَيُّهَا ٱلنَّاسُ قَدْ جَآءَتْكُم مَّوْعِظَةٌ مِّن رَّبِّكُمْ وَشِفَآءٌ لِّمَا فِى ٱلصُّدُورِ وَهُدًى وَرَحْمَةٌ لِّلْمُؤْمِنِينَ ﴿٥٧﴾ ﴾

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Dedication

This thesis is dedicated to the **soul** of my beloved **Mother** (May Allah have mercy on her) and to my **Father** (may Allah restores his health). To my **brothers** who have supported me along the way, especially **Abdelrahman**.

Also, it is dedicated to my wife **Shima** who has been a great source of motivation and inspiration.

Finally, this thesis is dedicated to all friends in **batch 14 FMLS –SUST**, who endured this long process with me, always offering support and love and to **all** those who believe in the richness of learning.

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Abstract

This was analytical, hospital-based, case-control study, conducted at Gaafar Ibn-Auf Paediatric Tertiary Hospital in Khartoum from June 2015 to June 2017.

The present study aimed to study the association of integrin alpha 2 (*ITGA2*) C807T and L-selectin (*SELL*) P213S Alleles polymorphism with clinical severity of sickle cell disease (SCD) among Sudanese patients.

Venous blood samples were collected from homozygous SCD patients (n=133) and from apparently healthy, age and sex matched, Sudanese individuals (n=112) as controls to compare them with cases. Sociodemographic and clinical data were collected by a questionnaire and from the reporting forms. A modified scoring system was used to assess severity. It includes number of transfusions, hospitalizations and lifetime cumulative incidence of specific complications of SCD as described by the SCD cooperative study group.

Blood was genotyped by polymerase chain reaction-restriction fragment length polymorphism. Complete blood counts were measured by automated hematology analyzer (Sysmex KX 21-N), and retics were counted by the manual method.

Patients' age ranged from 1 year up to 37 with median ages of 7 years, (n=76)57% were females and (n=57)43% were males and came from different areas in Sudan. The genotype and alleles frequencies of *ITGA2* C807T and L-Selectin *SELL* P213S were found to be significantly different between patients and controls (P=0.002 and 0.000, respectively). In *ITGA2* relative risk analysis of alleles, frequency showed that patients with the T allele were 5.4

times more likely to suffer from the hemolytic crisis, vaso-occlusive and ischemic stroke rather than patients with the C allele.

There was a significant association between these gene polymorphisms and high expression of L-selectin by leukocytes, or the development of complications in SCD (P= 0.000). Hematologic parameters (Hb, RBCs, WBCs and PCV) were found to be statistically significantly higher in controls than in patients (P values = 0.000, 0.000, 0.045, 0.034, respectively), while retics significantly lower in controls (P= 0.034) and also when compared with severity groups for SCD, all of them gave statistically significant results with P=0.000 for each.

These results indicated that the (P213S) polymorphism of *SELL* gene and (C807T) polymorphism of *ITGA*2 are associated with more complications and crisis, with *ITGA*2 T allele that appears to deliberately increase susceptibility to ischemic stroke and vasoocclusive and hemolytic crisis in Sudanese patients with SCD. Hematologic markers and CBC are useful markers for assessment of erythropoietic bone marrow activity in SCD patients with different severities. These alleles can be a target for new therapeutic approaches of SCD.

المستخلص

هذه الدراسة التحليلية أُجريت كدراسة حالة وحالة ضابطة للحالات بالمستشفى ومقارنتها بالضوابط ، في عيادات أمراض الدم الخارجية والعيادات المحولة بمستشفى جعفر بن عوف المرجعي للأطفال بالخرطوم في الفترة من يونيو 2015 إلى يونيو 2017.

هدفت الدراسة الحالية إلى التعرف على العلاقة بين تعدد أشكال البدائل الجينية للإنتغرين ألفا 2 و السيليكتين-L والشدة السريرية لمرض الأنيميا المنجلية في المرضى السودانيين، بالاضافة لمعرفة المتغيرات الدموية والربط بينها.

جمعت عينات الدم الوريدي من المرضى السودانيين الذين يعانون من مرض الأنيميا المنجلية (عددهم =133) ، كما تم جمع عينات من الأفراد السودانيين الأصحاء (عددهم = 112) كعينات ضابطة مع مراعاة مطابقة العمر والجنس للمرضى عند مقارنتهم بالحالات.

تم ترسيم الجينات من خلال جهاز التوليف متعدد البلمرة المتسلسلة ، وتم قياس عد الدم الكامل بواسطة محلل الدم الذاتي(سيسمكس KX 21-N) كما تم عد الخلايا الشبكية بواسطة الطريقة اليدوية.

المرضى المحولين إلى عيادة أمراض الدم وجد ان أعمارهم تتراوح من سنة حتى 37سنة حيث ان الوسيط للاعمار 7 سنوات من كلا الجنسين (57% اناث عددهم 76 و 43% ذكور عددهم 57) وجاءوا من مناطق مختلفة في السودان. كما وجد أن التراكيب والبدائل الوراثية من *ITGA2* C807T و-L سيلكتين *SELL* P213S تختلف اختلافا كبيرا بين المرضى والاصحاء بقيم ذات دلالة معنوية تساوي(P=0.002) و (P=0.000) على التوالي. في *ITGA2* أظهر تحليل المخاطر النسبية لعدد البدائل الجينية أن المرضى الذين يعانون من البديلة الجينية T كانوا أكثر عرضة بعدد 5.4 مرات لان يصابوا بنوبات التحلل الدموي، انسداد الأوعية الدموية والسكتة الإقفارية بدلا من المرضى الذين لديهم البديلة الجينية C. بالاضافة لذلك كان هناك ارتباط كبير بين هذه الأشكال الجينية والظهور العالي من -L سيلكتين على سطح كريات الدم البيضاء ، أو زيادة المضاعفات في الانيميا المنجلية (P0.000) .

كل القيم المقاسة للدم كالهيموغلوبين، عدد كريات الدم الحمراء والبيضاء و حجم الخلايا المضغوطة كلها أعلى بكثير في العينات الضابطة من التي في المرضى وايضا عند مقارنتها بين مجموعات شدة الحالة المرضية(P=0.000 للهيموغلوبين، 0.045 =P لعدد كريات الدم الحمراء ، وP=0.034 لحجم الخلايا المضغوطة) وايضا عندما تم مقارنتها بين المجموعات المختلفة للشدة السريرية للأنيميا المنجلية كلها اعطت نتائج ذات دلالة احصائية بقيمة معنوية وصلت P=0.000.

أشارت هذه النتائج إلى أن تعدد الأشكال (P213S) للجين-L سيلكتين و تعدد أشكال *ITGA2* (C807T) يرتبط بمزيد من المضاعفات والنوبات، مع البديل الجيني *ITGA2* Tالذي يبدو أنه يتعمد زيادة قابلية الإصابة بالسكتة الإقفارية والنوبات الوعائية والتحللية في المرضى السودانيين المصابين بالانيميا المنجلية.

كذلك فان الموسمات التحلليلة للدم ذات فائدة للتحقق من فعالية نخاع العظم في انتاج خلايا الدم الحمراء وذلك عند مقارنتها بين الاصحاء والمرضى المصابين بالانيميا المنجلية ولديهم درجات متفاوتة من الشدة السريرية.

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Abbreviations

Abbreviation	Term
AA	Arachidonic Acid
ACS	Acute Chest Syndrome
ADP	Adenosine Di-Phosphate
ARC	Absolute Reticulocyte Count
AVN	Avascular Necrosis
BM	Bone Marrow
CBC	Complete Blood Count
CD	Cluster of Differentiation
CRC	Corrected Reticulocyte Count
CSSCD	Cooperative Study of Sickle Cell Disease
DNA	Deoxy-Ribonucleic Acid
HA	Hemolytic Anemia
Hb A	Hemoglobin phenotype A
Hb C	Hemoglobin phenotype C
Hb D	Hemoglobin phenotype D
Hb E	Hemoglobin phenotype E
Hb S	Hemoglobin phenotype S
HbF	Fetal hemoglobin
Hct	Hematocrit
HU	Hydroxyurea
ICAM	Intra Cellular Adhesion Molecule
*ITGA*2	Integrin Alpha 2
KD	Kilo Dalton

MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NO	Nitric Oxide
PAGE	Poly acrylamide Gel Electrophoresis
PBP	Peripheral Blood Picture
PCV	Packed Cell Volume
Pt.	Patient
RBCs	Red Blood Cells
RNA	Ribo-Nucleic Acid
RPI	Reticulocyte Production Index
RI	Reticulocyte Index
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SELL	L- selectin
VOC	Vasoocclusive Crisis
VAM	Vascular Adhesion Molecule
WBCs	White Blood Cells