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## Evaluation of Beta-2 Integrin and Platelets Roles in Sickle Cell Disease Pathogenicity in Basrah Governorate Patients

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### Abstract:

Sickle cell disease (SCD) comprises an inherited blood disorder that is life long and affects many people globally. In spite of the development in treatment, SCA is a considerable cause of mortality and morbidity. The present study tries to assess the role of leukocytes represented by  $\beta$  integrin(CD18) and platelets and their productivity in the pathogenicity of disease during the steady state and crisis in comparison with the healthy as-control group, SCD patients (15) enrolled during crisis and steady state (follow up) showed a significant increase in leukocytes and platelets cells productivity during crisis when compared to the steady state and in the steady state when compared to the healthy control group. In this study, SCD pathophysiology in steady state and crisis affected by the platelets account and leukocyte activation triggered by inflammatory factors and reflex on the adherence and attachment between cells and blood vessel led to vascular occlusion (VOC).

**Keywords:**  $\beta$  integrin(CD18), pathogenicity, Platelets, Sickle cell disease

### Introduction:

Sickle cell disease is a genetic disorder caused by point mutation in the  $\beta$ globin gene on chromosome 11 resulting in the replacement of adenine by thymine in the sixth codon of the gene which leads to the replacement of glutamine by valine amine acid. Sickle cell disease is the most common hemoglobineopathy; >70% of sickle cell disease in the world<sup>1</sup>.

This mutation causes the polymerization of hemoglobin molecules to rigid fiber in the red blood cells (RBCs), transforming to (sickle shape). The sickling RBC leads to the occlusion of the microvasculature with acute and chronic end organ damage<sup>2</sup>.

The vascular endothelium plays an important role in vaso-occlusion and ischemic-organ damage, this is done by several mechanisms and steps, which start from endothelial activation leading to the recruitment of adherent leukocytes, triggering interaction among sickled RBC with adherent leukocytes and activating inflamed and damaged endothelium.<sup>3</sup> Lysis sickled RBC release adenosine 5-diphosphate (ADP) which potentially results in the activation and aggregation of platelets. This together with sickled RBC and

activated leukocytes contribute to micro-vascular occlusion<sup>4</sup>.

In the acute inflammatory response such as crisis event in the SCD patients the leukocytes recruit to inflamed endothelium in response to chemotactic stimulation, which in turn activates  $\beta$ 2-integrin (CD18) that anchors leukocytes to the vessel wall under the force of blood flow, this may significantly contribute to vascular occlusion (VOC)<sup>5</sup>. Platelets in SCD patients have altered aggregation and increased adhesiveness.  $\beta$ 2 integrin or CD18 (ITGB2) gene located on chromosome 21922 are non-covalently associated, heterodimer cell surface receptors. They are composed of one subunit (CD11, CD11b, or CD11c) and a common  $\beta$ 2 chain which is required for surface expression of the CD11 chains. These proteins mediate leukocytes adhesion to the endothelium and other leukocytes to play significant roles in cellular adhesion and cell signaling as well as important role in immune response<sup>5</sup>.

The  $\beta$ 2 (CD18) subgroup found exclusively on leukocytes are the major contributors to leukocytes motility and function<sup>6</sup>. Because vascular occlusion plays a critical role in pathogenicity of

SCD so the current study aims to estimate the CD18 marker value and platelets account in SCD patients during crisis and follow up the steady state as well as compare the results with healthy individuals as a control to evaluate the role of leukocytes and platelets in the VOC. Strong sticking of circulating leukocytes to inflamed vascular endothelium is an important step of multistep adhesion cascade that results in the aggregation and eventual migration of leukocytes to the wall vessel<sup>7</sup>. Movement of leukocytes through the endothelium monolayers is mediated by activating  $\beta$ 2 integrin(CD18) receptors Leukocytes that lack  $\beta$ 2- integrin receptors are unable to complete a multistep adhesion process responsible for their recruitment to site of inflammation<sup>8-10</sup>.

Although the activation of monocytes, neutrophils, and eosinophil's has been stabilized in human with SCD, the previously studies focused on heteroaggregates which contain platelets which are those formed between platelets and monocyte or platelets and sickled RBCs and also because of complicated analysis of platelets function in SCD due to the fact that sever chronic inflammation may lead to platelets depletion, desensitization and margination<sup>11</sup>. So the current study is interested in platelets account and leukocytes value estimation and related the results with the pathogenicity of SCD

### Materials and Methods:

Patients with sickle cell disease registered at the Hereditary Blood Disease Centre (HBDC) at Basrah Maternity and Children Hospital total of 15 blood samples were enrolled as 15 patients in crisis and steady state (follow up), their ages ranged from 16 to 55 years and from 15 healthy individuals matched in age and sex with patients group as control group A designed questionnaire was used which includes the date of birth, sex site and frequency of vasoocclusion crisis, hydroxy urea

intake. All patients had history of admission to Hereditary Blood Diseases Ward for the management of VOC. These patients were assessed initially clinically and by selected laboratory data during VOC, and then they were in the steady state (follow up). All the patients in the study were not treated by hydroxy urea., platelets count using Hematology Analyzer Mindray-BC 5300. Leukocyte Evaluation BD Accrui C6 flow cytometry (BD, Accuri C6, Accuricytomters, Inc. Ann Arbor 21, MI 48103, USA) and (BD, Accuri C6 software version 1.0.264.21) are used for cell acquisition and events analysis. The machine is calibrated using 6 peaks and 8 peaks calibration beads (BD, Accuri C6, Accuri cytometers, Inc. Ann Arbor, MI48103 USA).

Flow Cytometry Reagents Anti-Human CD18 FITC and Flow Cytometry Reagents Anti-Human CD3 APC cell acquisition and events analysis according to the procedure fixed in kits manuals, All the items have been used by BD Accuri C6 flow cytometry (BD, Accuri C6, Accuricytomters, Inc. Ann Arbor 21, MI 48103, USA) and (BD, Accuri C6 software version 1.0.264.21).

### Statistical analysis

Statistical analysis was done using SPSS program V23 at P value < 0.01. Data were expressed by means and Standard Deviation (SD). One-Sample t -test to compared between one group and the paired samples t-test was used for quantitative comparison between two means of different group. Paired Samples Correlations were evaluated.

### Results:

Our results showed a significant value to all parameters enrolled in this study (CD18 and platelets count) in different groups (steady, crisis and control group) as shown in Tables 1, 2.

**Table 1. CD18 One-group Statistics**

Group	N	Mean	99% Confidence Interval		Std. Error Mean	t	df	Sig.(2-tailed)
			of the Difference					
			Lower	Upper				
Steady group	15	48.8693	35.8186	61.9201	4.3840	11.147	14	0.00
Crisis group	15	85.8526	65.4255	106.2796	6.8619	12.511	14	0.00
Control group	15	36.8578	27.4186	24.2969	3.1708	11.624	14	0.00

SSig P ≤0.01, non sig P ≥0.01

**Table 2. Platelets one group Statistics**

Test Value = 0								
Group	N	Mean	99% Confidence Interval of the Difference		Std. Error Mean	t	df	Sig.(2-tailed)
			Lower	Upper				
Steady group	15	374.933	300.274	449.600	25.0824	14.984	14	0.00
Crisis group	15	646.333	646.333	898.079	84.5681	7.643	14	0.00
Contro group	15	216.267	216.266	246,061	10.0088	21.608	14	0.00

Sig P ≤0.01, non sig P ≥0.01

When comparing between two groups in a pair test, the statistical analysis showed a significant difference between steady and crisis groups in CD18 marker with 36.98 mean and 0.001

significant P value which refers to an increase in CD18 level in crisis group compared with steady state group (Tables 3, 5).

**Table 3. CD18 Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Steady group	48.8693	15	16.97948	4.38408
	Crisis group	85.85260	15	26.576302	6.861972
Pair 2	Steady group	48.8693	15	16.97948	4.38408
	Control group	36.85780	15	12.280637	3.170847
Pair 3	Crisis group	85.85260	15	26.576302	6.861972
	Control group	36.85780	15	12.280637	3.170847

The same result showed that in a pair test for platelets count statistical analysis there was a significant difference between groups specifically between steady and crisis with 271.40 mean and

significant P value which refers to an increase in platelets count level in crisis group compared with steady state group (Tables. 4, 6).

**Table 4. platelets Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Steady group	374.933	15	97.1438	25.0824
	Crisis group	646.333	15	327.5310	84.5681
Pair 2	Steady group	374.933	15	97.1438	25.0824
	Control group	216.267	15	38.7639	10.0088
Pair 3	Crisis group	646.333	15	327.5310	84.5681
	Control group	216.267	15	38.7639	10.0088

Sig P ≤0.01, non sig P ≥0.01

**Table 5. CD18 Paired Samples Test**

		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Steady group	-36.983267	32.383859	8.361476	-61.874067	-12.092466	-4.423	14	0.001
	Crisis group								
Pair 2	Steady group	12.011533	21.762469	5.619045	-4.715481	28.738547	2.138	14	0.051
	Control group								
Pair 3	Crisis group	48.994800	32.452561	8.379215	24.051194	73.938406	5.847	14	0.000
	Control group								

Sig P ≤0.01, non sig P ≥0.01

**Table 6. Platelets Paired Samples Test**

		Paired Differences					T	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	steady group crisis group	-271.4000	341.0465	88.0578	-533.5343	-9.2657	-3.082	14	0.008
Pair 2	steady group control group	158.6667	101.9563	26.3250	80.3012	237.0321	6.027	14	0.000
Pair 3	crisis group – control group	430.0667	317.3055	81.9279	186.1801	673.9532	5.249	14	0.000

Sig P ≤0.01, non sig P ≥0.01

Statistical analysis as paired sample correlations between groups showed no significant value between them which observed there was no correlation linking between an increase in one group and decrease in the other one at one study. (Tables. 7, 8)

**Table 7. CD18 Paired Samples Correlations**

		N	Correlation	Sig
<b>Pair 1</b>	steady group & Crisis group	15	0.060	0.832
<b>Pair 2</b>	steady group & Control group	15	0.083	0.770
<b>Pair 3</b>	Crisis group & Control group	15	0.300	0.277

Sig P ≤0.01, non sig P ≥0.01

**Table 8. Platelets Paired Samples Correlations**

		N	Correlation	Sig
<b>Pair 1</b>	steady group & steady group	15	0.006	0.982
<b>Pair 2</b>	steady group & control group	15	0.072	0.798
<b>Pair 3</b>	steady group & control group	15	0.319	0.247

Sig P ≤0.01, non sig P ≥0.01

### Discussion:

This study supports other hypothesis about the role of leukocytes and platelets in VOC and how they can enhance the inflammatory vascular endothelium by activating the adherence to the reticulum endothelium. Hem and Dead – associated molecules pattern (DAMP) released from skilled RBCs activated the immune system, activated leucocytes, monocytes, and neutrophils to release inflammatory cytokines which promote the inflammatory state, activation of endothelium cells, and activation of platelets to enhance their adhesion to neutrophils and other leucocytes<sup>12</sup>.

The choice of SCD patients in this study based on the clinical importance of inflammation state from more than 30 patients in previous study

<sup>13</sup>. We have chosen 15 patients of them who had the highest level of IL-6 important inflammatory cytokine to evaluate the platelets count and CD18 marker of them during steady and crisis state and compare the result with other healthy one as control to show the importance of this parameters in the pathophysiology of the disease. The activation of platelets and leukocytes led to enhance the production of pro inflammatory cytokines<sup>14</sup> increased levels of IL6 and other inflammatory cytokines have been reported in serum from patients with SCD<sup>15</sup>.

The present study showed a significant increase value of platelets count in crisis compared to steady state patients and those with healthy group indicating activation of platelets as significant factor to the pathogenesis and outcome to SCD patients. They showed that SCD patients during VOC had higher level of PDGF compared to healthy control and steady state. Also<sup>16</sup> in vitro assays showed that endothelium stimulated monocytes productions of inflammatory cytokines (IL-6, IL1B, IL8, etc.), neutrophil production of platelet-activating factor (PAF), and enhanced monocyte and neutrophil chemotaxis<sup>17</sup>. Activated, adherent platelets by cytokines supported all steps required for accumulation and emigration of neutrophils at site of vesicular injury which suggest that deposit may be used as alternative for endothelial cells in their ability to recruit circulating leukocyte through selection and integrin-dependent adhesion<sup>18</sup>.

Levels correlated significantly with the numbers of total leukocytes in SCD<sup>19</sup> patients and also can be responsible for the leukocytosis observed as proinflammatory cytokine like IL-6 which are increased in SCD can be associated with elevated level of GM-CSF. A lot of studies have shown that whole blood tissue factor with procoagulant activity associated with mononuclear leukocytes is elevated in sickle disease<sup>20</sup>.

Tissue factor expression like β2- integrin is significantly elevated on circulating endothelial cells isolated from SCD patients compared with

normal subject. Expression is greater when patients have acute VOC, elevated tissue factor expression on activated monocytes and endothelium in SCD are activated providing ideal condition of VOC<sup>21</sup>.

Platelets from patients with VOC are activated and can adhere to monocytes through thrombospondin cross-linking of glycoprotein on the surface of both kinds of cell Neutrophils and platelets aggregate at site of VO, interaction between neutrophil and platelets is required in the production of chemo attractants thus adhesive interaction may be a pre requisite for promoting neutrophil/platelets cross – communication<sup>22</sup>.

### Conclusion:

As discussed above and by our results a significant value has been shown to all the parameters enrolled in this study (CD18 and platelets count) in different groups (steady, crisis and control). It can be concluded that SCD pathophysiology in steady state and crisis is affected by the platelets account and leukocyte activation triggered by inflammatory factors and reflexed on the adherence and attachment between cells and blood vessel leading to VOC.

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### Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- Author sign on ethical consideration's approval
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

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## تقييم بيتا 2 انتجرين والصفائح الدموية في مرضى فقر الدم المنجلي بمحافظة البصرة ودراسة دورهما في الأمراض

### مها خلف المشري

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#### الخلاصة:

يتكون مرض فقر الدم المنجلي (SCD) من اضطراب دم وراثي يستمر مدى الحياة ويؤثر على العديد من الأشخاص على مستوى العالم. على الرغم من التطور في العلاج، فإن توقف القلب المفاجئ هو سبب كبير للوفاة والمرض. نحاول في هذه الدراسة تقييم دور كريات الدم البيضاء التي تمثلها  $\beta$  إنتجرين (CD18) والصفائح الدموية وإنتاجيتها في أمراضية المرض أثناء الحالة المستقرة والأزمات ومقارنتها بالأفراد الأصحاء كمجموعة تحكم مرضى داء الكريات المنجلية (15 مريضاً) المسجلون أثناء الأزمات وفي الحالة المستقرة (متابعة) زيادة ملحوظة احصائياً في إنتاجية الخلايا الدم البيضاء والصفائح الدموية أثناء الأزمات مقارنة بمرحلة الثبات وفي مرحلة الثبات عند مقارنتها بالمجموعة الضابطة. في هذه الدراسة الفيزيولوجيا المرضية لفقر الدم المنجلي في حالة مستقرة وأزمة متأثرة بحساب الصفائح الدموية وتنشيط الكريات البيض التي تسببها العوامل الالتهابية وردود الفعل على الالتصاق والتعلق بين الخلايا والأوعية الدموية مما أدى إلى انسداد الأوعية الدموية (VOC).

الكلمات المفتاحية:  $\beta$  إنتجرين (CD18)، الأمراض، الصفائح الدموية، مرض فقر الدم المنجلي.