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 β 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: β integrin(CD18), pathogenicity, Platelets, Sickle cell disease

Introduction:
Sickle cell disease is a genetic disorder caused by point mutation in theβglobin 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 world1.
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 damage2.

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.3 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 occlusion4.

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 β2-integrin (CD18) that anchors leukocytes to the vessel wall under the force of blood flow, this may significantly contribute to vascular occlusion (VOC)5.But in SCD patients have altered aggregation and increased adhesiveness. β2 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 β2 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 response5.

The β2 (CD18) subgroup found exclusively on leukocytes are the major contributes to leukocytes motility and function6. 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. Movement of leukocytes through the endothelium monolayers is mediated by activating β2 integrin(CD18) receptors. Leukocytes that lack β2-integrin receptors are unable to complete a multistep adhesion process responsible for their recruitment to site of inflammation.

Although the activation of monocytes, neutrophils, and eosinophils 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. 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 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) 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.

| Group         | N   | Mean  | 99% Confidence Interval of the Difference | Std. Error Mean | t       | df  | Sig.(2-tailed) |
|---------------|-----|-------|------------------------------------------|-----------------|---------|-----|----------------|
| 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 - 45.2969 | 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   | Std. Error Mean | t    | df | Sig.(2-tailed) |
| Steady group   | 15 | 374.933| 25.0824         | 14.984| 14 | 0.00          |
| Crisis group   | 15 | 646.333| 84.5681         | 7.643 | 14 | 0.00          |
| Contro group   | 15 | 216.267| 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 | 16.97948 | 4.38408 |
| Crisis group | 85.85260 | 26.576302 | 6.861972 |
| Pair 2 Steady group | 48.8693 | 16.97948 | 4.38408 |
| Control group | 36.85780 | 12.280637 | 3.170847 |
| Pair 3 Crisis group | 85.85260 | 26.576302 | 6.861972 |
| Control group | 36.85780 | 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 | 97.1438 | 25.0824 |
| Crisis group | 646.333 | 327.5310 | 84.5681 |
| Pair 2 Steady group | 374.933 | 97.1438 | 25.0824 |
| Control group | 216.267 | 38.7639 | 10.0088 |
| Pair 3 Crisis group | 646.333 | 327.5310 | 84.5681 |
| Control group | 216.267 | 38.7639 | 10.0088 |

Sig P ≤0.01, non sig P ≥0.01

Table 5. CD18 Paired Samples Test

| Paired Differences | Mean | Std. Deviation | Std. Error Mean | 99% Confidence Interval of the Difference | t    | Df | Sig. (2-tailed) |
|--------------------|------|----------------|-----------------|----------------------------------------|------|----|----------------|
| Pair 1 Steady group Crisis group | -36.983267 | 32.383859 | 8.361476 | -61.874067 - 12.092466 | -4.423 | 14 | 0.001 |
| Pair 2 Steady group Control group | 12.011533 | 21.762469 | 5.619045 | -4.715481 28.738547 | 2.138 | 14 | 0.051 |
| Pair 3 Crisis group Control group | 48.994800 | 32.452561 | 8.379215 | 24.051194 73.938406 | 5.847 | 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 6. Platelets Paired Samples Test**

| Paired Differences | Mean  | Std. Deviation | Std. Error Mean | 99% Confidence Interval of the Difference | T    | Df    | Sig. (2-tailed) |
|--------------------|-------|----------------|-----------------|------------------------------------------|------|-------|----------------|
| 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 |

**Table 7. CD18 Paired Samples Correlations**

| N Correlation Sig | Paired Differences |
|-------------------|--------------------|
| Pair 1 steady group & Crisis group | 15 0.060 0.832 |
| Pair 2 steady group & Control group | 15 0.083 0.770 |
| Pair 3 Crisis group & Control group | 15 0.300 0.277 |

**Table 8. Platelets Paired Samples Correlations**

| N Correlation Sig | Paired Differences |
|-------------------|--------------------|
| Pair 1 steady group & steady group | 15 0.006 0.982 |
| Pair 2 steady group & control group | 15 0.072 0.798 |
| Pair 3 steady group & control group | 15 0.319 0.247 |

**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.  

The choice of SCD patients in this study based on the clinical importance of inflammation state from more than 30 patients in previous study.
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 VOC21.

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 – communication22.

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 reflected 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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