

Correlation of NLR with Oxidative Stress Markers in Sickle Cell Anemia with Vasoocclusive Crisis

Satarupa Dash¹, Atanu Kumar Thakur², Sumitra Bhoi³

¹Assistant Professor, Physiology, ²Assistant Professor, Medicine, ³Assistant Professor Biochemistry, VIMSAR, Burla, Odisha

Abstract

Introduction- Sickle cell disease has long been recognized as an inflammatory condition and oxidative stress play an important role in pathophysiology of SCD. Several biomarkers have been associated with SCA clinical prognosis. This study was designed to determine the oxidative stress & inflammatory biomarker that can be used in monitoring the prognosis & management of SCA patients.

Aim & Objective- The present study is aimed to assess the role of NLR along with CRP, MDA and erythrocyte GSH in vaso-occlusive (HbSS) crisis patients compared to steady state (HbSS) patients.

Materials & Method- A total of 100 sickle cell anemia patients aged between 20-40 years of either sex were evaluated and divided into 2 groups. Group A-sickle cell anemia patients with vasoocclusive crisis(N=50) Group B- sickle cell anemia patients with steady state as control group(N=50). We analysed the oxidative stress marker i.e. reduced glutathione (GSH) & MDA along with inflammatory markers NLR,CRP and compared between both A& B groups.

Result- The Unpaired t test between A & B group shows significant increase in NLR,MDA, CRP Level in VOC patients as compared to steady state sickle cell patients($P \leq 0.05$). Compared with values of controls, SCA subjects with VOC had significantly lower erythrocyte GSH ($P \leq 0.05$). In the present study positive correlation of NLR with MDA and CRP in sickle cell anemia patient with vasoocclusive crisis is evaluated.

Conclusion- So the early prediction of vaso-occlusive crisis by the detection of the NLR count along with MDA,CRP,GSH level might be beneficial in the management sickle cell anemia patient. Additional studies are warranted to test this hypothesis.

Keyword- Sickle Cell anemia,vasoocclusive crisis(VOC),NLR(neutrophil to lymphocyte ratio), CRP, MDA, GSH

Introduction

Sickle cell anemia (SCA) is characterized by chronic hemolytic anemia, a point mutation in the beta-globin gene of hemoglobin at the sixth amino acid (Glu6Val). It is a multisystem disease which include chronic hemolysis, repeated infections, growth retardation in addition to acute life threatening complications called crisis.

In India prevalence of Sickle Cell Disease of 1-40% and the State of Odisha falls in the high prevalence zone (21-40%)^[1]. High frequency and clinical severity of the sickle cell anemia, make it a major public health problem, due to the presence of different types of crisis. Most frequent crisis is the vaso-occlusive crisis(VOC) which accounts for the majority of SCD (Sickle cell disease)-related hospital admissions.^[2]

The vaso-occlusive crisis results from the polymerization of deoxy-hemoglobin S, as a consequence there is tissue ischemia leading to acute and chronic pain as well as organ damage that can affect any organs in the body, including bones, joints, brain, eyes, liver, kidneys, and lungs.^[3]

Corresponding Author:

Dr Sumitra Bhoi,

Assistant Professor, Biochemistry, VIMSAR, Burla

E-mail: drsumitrabhoi09@gmail.com

Vaso-occlusive crisis activate and damages the endothelial cells leading to inflammation; as a result inflammatory biomarkers are released.¹⁴¹ Production of C-reactive protein (CRP) is a part of a nonspecific acute phase response to inflammation and tissue necrosis. Most studies find that levels of CRP increase during vaso-occlusion and may be of value in anticipating the development of acute chest syndrome.^{15,61}

Several studies have shown that white blood cells (WBC) particularly neutrophils may be involved in the initiation and propagation of vaso-occlusive events.¹⁷¹ Adhesion of activated neutrophils to endothelium in SCA may lead to endothelial damage because neutrophils do not lyse easily as RBCs do, thereby leading to obstruction of blood flow within the microcirculation.¹⁸¹ A number of studies have identified blood NLR as an important marker of inflammation, which has significant prognostic implications in a number of disease states, particularly those that involve the cardiovascular, renal and gastrointestinal intestinal systems.^{19,101} It is generally believed that higher is the NLR, the worse is the clinical outcome.

SCD has long been recognized as an inflammatory condition and oxidative stress play important role in pathophysiology of SCA.¹¹¹¹ It is now well established that reactive oxygen species (ROS) mediate inflammatory process and may be involved in oxidative reactions such as lipid peroxidation and protein oxidation.¹¹²¹ Although results are sometimes contradictory, patients with SCA are shown to have high oxidative stress. Normal RBCs are usually, subjected to oxidative stress as a result of continuous ROS production that accompanies Hb autoxidation, a condition that increases two times more in SCA, leading to a continuous inflammatory response, oxidative stress and multiple organ damage. To counter the destructive effects of these oxidants, there are endogenous antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, which help to detoxify ROS. In the cell, glutathione (GSH) is considered to be the most sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. GSH depletion in cell can trigger suicide of the cell by a process known as apoptosis.¹¹³¹

As the frequency of vaso-occlusive episodes was a marker of poorer survival in patients with sickle cell anemia the study of the biomarkers may helps in prevention of vaso-occlusive crisis. The present study is

aimed to assess the role of NLR along with CRP, MDA and erythrocyte GSH in vaso-occlusive (HbSS) crisis patients compared to steady state (HbSS) patients.

Materials & Method

The prospective single centered study was conducted in the Department of Physiology, Biochemistry, in collaboration with department of General Medicine VIMSAR, Burla over a period of 6 month from 1st August 2017 to 5th January 2018. The study was approved by the institutional ethical committee and informed consent was obtained from the study group.

A total of 100 sickle cell anemia patients aged between 20-40 years of either sex were evaluated clinically and were screened by sickling test and diagnosis is confirmed by haemoglobin electrophoresis. The cases divided into 2 groups

Inclusion Criteria-

Group A-sickle cell anemia patient with vasoocclusive crisis (hospitalised) as case

Group B- sickle cell anemia patient with steady state as control group

[Steady state condition was defined as no manifestation of crisis for at least 4 weeks after the last episode, 3 or more months after the last blood transfusion and no febrile episode for at least 2 weeks]

Exclusion Criteria- SCA Patients with systemic diseases like diabetes mellitus, hypertension, neoplasm, thyroid disorder and other haemoglobinopathy.

Sampling Method

CBC, CRP, MDA & GSH(oxidative marker) were studied and compared in all groups.(A&B)

5 ml of venous blood were drawn from all participants after taking aseptic precaution. Complete blood count(CBC), were done using automated hematology analyzer (ACCULAB CBC-360). NLR was calculated by dividing the value of absolute neutrophil count(ANC) by absolute lymphocyte count(ALC). Malondialdehyde (MDA) were evaluated by Satoh et al method. Estimation of C-Reactive Protein was done by using standard nephelometry procedure. The assay of GSH with DTNB was performed by a standard Beutler method (1963).

Statistical analysis was done using a SPSS version 20, IBM, IL, USA. Unpaired t test was used to compare test results between SCA patients with VOC and steady state. Statistical significance was set at p value ≤ 0.05 . Correlation analysis was carried out to test the relationship between NLR and MDA, NLR & CRP and NLR& GSH in VOC patients.

Results

Table 1- Comparison of Haematological Parameters in Study Groups

No. of subjects	A=50	B=50	P value
Age (years)	21.68±3.1	28.7±2.3	≥ 0.05
Hematocrit (%)	13.6±1.3	18.9±2.75	≤ 0.05 *
Hemoglobin (g/dl)	6.2±0.24	8.82±1.05	≤ 0.05 *
Total leukocyte Count (x10 ⁹ /l)	3.12±0.83	2.05±0.23	≤ 0.05 *
Red blood cells Count (x10 ¹² /l)	1.49±0.1	2.43±0.6	≤ 0.05 *
Platelet count (x10 ⁹ /l)	430±93	360±97	≥ 0.05
Mean cell volume(fl)	82.2±6.9	84.9±4.2	≥ 0.05
Mean cell hemoglobin Conc. (g/dl)	36.4±2.1	30.7±3.3	≤ 0.05 *
Absolute lymphocyte Count (x10 ⁹ /L) (ALC)	2.55±0.33	3.13±0.17	≤ 0.05 *
Absolute neutrophil Count (x10 ⁹ /L) (ANC)	6.1±0.41	5.31±0.34	≤ 0.05 *

* - Significant 'p' value

Table-2 : Comparisons of inflammatory & oxidative stress parameters between A & B group

	A=Cases (SCD) (n=50) (Mean ± SD)	B= Control (SCD) (n=50) (Mean ± SD)	p value
NLR	2.44±0.097	1.78±0.049	0.006 *
MDA($\mu\text{mol/l}$)	3.69±0.03	2.9±0.02	0.02 *
CRP (mg/dl)	14.49±0.24	7.68±0.89	0.001 *
GSH($\mu\text{mol/l}$)	7.06±1.02	15.6±1.3	0.04 *

* - Significant 'p' value

Table 3: Correlation of NLR with oxidative stress marker(MDA,GSH) & inflammatory marker CRP.

SCA with VOC(n=50)	MDA ($\mu\text{mol/l}$)	GSH($\mu\text{mol/l}$)	CRP(mg/dl)
Mean ± SD	3.69±0.03	7.06±1.02	14.49±0.24
r	0.66	-0.83	0.77
p	≤ 0.001	≤ 0.001	≤ 0.001

Out of 100 HbSS patients 50 were with VOC and 50 were in steady state. The mean age of both steady state and voc patient with in normal range.

There was a significant decrease in the level of Hb in sickle cell anemia with VOC (6.2 ± 0.24) while a moderate decline in sickle cell anemia(ss) in steady state (8.82 ± 1.05). The total leucocyte count was found to be higher in VOC (11.7 ± 4.05) comparison to steady state .

The mean level of ANC, NLR were significantly higher in VOC ($6.1 \pm 0.41, 2.39 \pm 0.11$). But the mean level of ALC was higher in control group (3.13 ± 0.17) as compared to VOC group (2.55 ± 0.33).

The level of MDA was higher in A & B group with mean value ($3.69 \pm 0.03, 2.9 \pm 0.08$). Similarly the level of CRP was also higher in A group with mean value (14.49 ± 0.24) as compared to control group (7.68 ± 0.89). But the mean value of GSH in the SCA group with VOC was less than that of control.

Table 3 show a strong positive correlation between NLR & MDA (r^2 linear 0.661) & also between NLR & CRP (r^2 linear 0.778) in sickling patient with VOC. There was a strong negative correlation between NLR & GSH (r^2 linear- 0.83) in sickling patient with VOC.

Discussion

The sickle cell disease patients due to chronic hemolysis are constantly exposed to the increased generation of ROS and vessel walls are the primary exposed tissue. Studies have reported that oxidative stress and inflammation may contribute directly to pathophysiological events in SCA. ^[14]

In this study, the blood counts, were significantly higher while the PCV was significantly lower in VOC subjects compared with controls which is same as study in Lagos, Nigeria. ^[15]

The data presented in this study showed that NLR values in SCA patients with VOC were significantly higher ($P < 0.006$) compared with steady state subjects. There was also a positive correlation between NLR and CRP in SCA patients with VOC which is similar to study by C. Aneke John et al ^[16].

Neutrophil represents the active nonspecific inflammatory mediator of cellular immunity (innate)

while lymphocytes mediate the adaptive or protective aspect of inflammation ^[17]. Elevated NLR and PLR values may be used to distinguish patients who do not have physiological capacity to withstand the inflammatory injury and low survival outcomes ^[18]. Studies that have evaluated NLR and PLR ratios in SCA patients are very few. In this study NLR was high in VOC patients compared to steady state .

MDA and 4 hydroxynoneal(4-NHE) which can be measured as oxidative stress biomarker in urine or blood ,to indicate the degree of oxidative stress and have been demonstrated to be increased in SCD. ^[19] In our study MDA was found to be increased in SCD patients with VOC as compared to steady state P value 0.02. Some previous studies among sickle cell found similar results ^[20].

In this study, we found that CRP level was higher in Vaso-occlusive crisis patients than in Steady state patients and this result is in agreement with studies ^[21,22]. Also there is a significant positive correlation between CRP level and NLR count in vaso-occlusive crisis patients. Krishnan et al found a strong positive correlation between CRP and vaso-occlusion ^[6].

Chronic hemolysis and painful episodes (vaso-occlusive crisis) in SCD stimulates vascular tissue to counteract the pro-oxidative and pro-inflammatory environment created by free heme or haemoglobin. In the present study data showed that there were increases in oxidative stress (MDA) and inflammatory markers (CRP & NLR) in SCD patients with painful episode (VOC). Studies have shown increased serum levels of acute phase protein and oxidative stress parameters in SCD patients in a steady state ^[23], and sickle cell crisis ^[24] which is similar to our study.

In this study, subjects with SCA had markedly lower erythrocyte concentrations of GSH compared with controls (≤ 0.05) this was similar to the study reported by Morris C R (2008) ^[24]. Previous research shows that sickle red blood cells are more susceptible to oxidative lipid damage ^[25].

In this study, we found a significant positive correlation between NLR and MDA ,also between NLR & CRP & negative correlation between NLR and GSH. This indicate inflammation and oxidative stress both are the culprit of vaso occlusive crisis in sickle cell anemia.

Conclusion

The patients with sickle cell anemia easily develop the complications specially the vaso-occlusive crisis which is the most frequent, and if not diagnosed early they may develop organ damage and die in early age.

The NLR is an index that can be calculated very simply and it was significantly higher in SCA subjects with VOC. It was significantly correlated with MDA & GSH which are oxidative stress markers and CRP an inflammatory marker. NLR is a cheap & useful marker that can be easily calculated from CBC.

So, the early prediction of vaso-occlusive crisis by the detection of the NLR count along with MDA, erythrocyte GSH & CRP level might be beneficial in the management sickle cell anemia patient.

Financial support and sponsorship- Nil

Conflicts of Interest- There are no conflicts of interest

Ethical Clearance- Approved by IEC Vimsar Burla.

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