Original Research Article

Comparative Analysis of Fibrinogen Degradation Products and C-Reactive Protein in Sickle Cell Disease and Non-Sickle Cell Individuals in University College Hospital, Ibadan, Oyo state.

ABSTRACT

Introduction:
Nigeria has the greatest global burden of sickle cell disease (SCD), a hereditary illness that is common among persons of African origin. Understanding the etiology of the disease depends on biomarkers like fibrinogen degradation products (FDP) and C-reactive protein (CRP). Comparing these markers between SCD patients and non-SCD people may reveal information for better disease management.

Aim/Objectives:
This study compared the levels of fibrinogen degradation products (FDP) and C-reactive protein (CRP) in SCD patients and non-SCD patients at the University College Hospital in Ibadan, Oyo State.

Method:
From March to July 2019, a case control study was carried out at the University College Hospital in Ibadan. 40 non-SCD controls and 91 SCD patients were included in the study. SPSS version 21.0 was used for data analysis after CRP and FDP levels were determined. Tables provided a summary of the findings.

Results:
C-reactive protein (CRP) levels in sickle cell disease (SCD) patients and non-SCD controls did not differ significantly, according to the study (2.31 vs. 2.10, p = 0.400). However, SCD patients had considerably lower levels of fibrinogen degradation product (FDP) than controls (0.66 vs. 1.28, p = 0.001). These results suggest that FDP has promise as a stand-alone marker for SCD monitoring and treatment, even though CRP may not be a distinctive biomarker for the condition.

Conclusion:
Patients with sickle cell disease (SCD) had far lower levels of fibrinogen degradation product (FDP) than people without SCD, suggesting that FDP may be a useful biomarker for tracking and treating the condition. On the other hand, there is no discernible fluctuation in C-reactive protein (CRP) levels, which limits its ability to differentiate SCD status. These results highlight how crucial it is to concentrate on FDP while evaluating and treating SCD clinically.

Keywords:
Sickle cell disease, Ibadan, C-reactive protein (CRP), Fibrinogen Degradation Products (FDP).

INTRODUCTION

Red blood cells (RBCs) sickle in low oxygen environments due to sickle cell disease (SCD), a hereditary blood disorder mainly caused by a mutation in the hemoglobin gene that produces aberrant hemoglobin S (HbS) [1][2]. Vaso-occlusive crises, hemolytic anemia, and other systemic consequences, such as acute chest syndrome, which is a major source of morbidity and mortality, are brought on by this pathophysiological process [3]. Individuals with sickle cell disease (SCD) may have recurrent discomfort, exhaustion, and an increased susceptibility to infections. These clinical symptoms can vary greatly depending on genetic variables, environmental effects, and healthcare availability [4][5].
Blood transfusions, hydroxyurea, and, in certain situations, stem cell transplants are used as part of management regimens that aim to reduce symptoms and avoid problems [1][3]. Despite progress, comprehensive worldwide policies are still desperately needed to combat the increasing prevalence and mortality of sickle cell disease (SCD), especially in areas with limited resources[1].

A comparison of the hematological markers of sickle cell disease (SCD) patients and non-sickle cell patients in Nigeria, namely at the University College Hospital in Ibadan, shows notable discrepancies. Because SCD causes persistent hemolysis and inflammation, patients usually have reduced hemoglobin levels and red blood cell (RBC) counts coupled with higher white blood cell (WBC) and platelet (PLT) counts[6][7]. While some studies found no significant differences in certain hematological parameters, one study found that SCD patients had significantly higher levels of inflammatory markers, such as C-reactive protein, than controls [8]. Furthermore, the significance of fetal hemoglobin (HbF) in reducing the severity of the condition was emphasized; younger children had higher amounts of HbF, which had an inverse relationship with age [9]. According to Ibemere et al. (2023), these results highlight the necessity of continued study to enhance patient care techniques in settings with limited resources and gain a deeper understanding of the hematological implications of SCD.

Understanding how sickle cell disease (SCD) affects hematological health, especially in Nigeria, requires analyzing hematological markers in patients, such as fibrin degradation products (FDP) and C-reactive protein (CRP). Chronic inflammation and hemolysis are hallmarks of sickle cell disease (SCD), which causes notable changes in a number of biomarkers. For example, studies reveal that about 34.92% of SCD patients had considerably high CRP, compared to just 1.75% of non-SCD controls. This suggests that elevated CRP levels in SCD patients imply a heightened inflammatory state [10]. Furthermore, indicators of the severity of SCD and its related consequences, such as hemoglobin levels and leukocyte counts, differ significantly between patients and healthy people [11].

By enabling early detection of complications and customizing treatment approaches, the incorporation of these markers into clinical management strategies may improve patient care and outcomes [12][13]. This research work seeks to compare the levels of fibrinogen degradation products and C-reactive proteins in sickle cell disease and people without the disease.

2.0 MATERIALS AND METHODS

2.1 Study Design and Area

This study was conducted at the University College Hospital's Hematology Department in the Ibadan North Local Government region, which is located at latitude 3.8743°E and longitude 7.3569°N.Ibadan North East Local Government borders it on the east, while Ibadan North West Local Government borders it on the west.

2.2 Study Design

This was a case control study.

2.3 Sample Size Determination

The sample size for this study was determined using the formula below:

$$n = \frac{Z^2pq}{d^2}$$

P = prevalence of 8.0% reported by Mariani*et al.*, 2014.

Z= standard value corresponding to 95% confidence level (usually set at 1.96)

d = degree of error margin 5%

$$n = \frac{(1.96)^2(0.08)(0.92)}{(0.05)^2}$$

n =117.76

n =117

Adjusting the sample size for 10% non-response rate

$$n_f = \frac{n}{1 - n_r}$$

$$n_f = \frac{117}{1 - 10\%}$$

$$n_f = 130.84$$

Total sample size= 131

2.4 Study Subjects

The study included ninety-one (91) sickle cell disease patients who were enrolled in the Haematology day care center and who had been identified as HbS by hemoglobin electrophoresis. As controls, forty (40) people with hemoglobin A (HbA), as validated by hemoglobin electrophoresis, were included. These were students and employees of the hospital. All participating respondents gave their verbal and written agreement in the form of a signature, and demographic data was gathered from them using a structured questionnaire.

2.4.1 Inclusion Criteria

- The study only included participants who gave their consent.
- Participants who gave their consent were between the ages of 18 and 60 and seeking care at the study site.

- They were identified as patients with sickle cell disease.
- It was confirmed that the control subjects had hemoglobin A.

2.4.2 Exclusion Criteria

- Patients with rheumatoid arthritis or other autoimmune disorders, hypothyroidism, diabetes mellitus, renal disease unrelated to sickle cell disease, infection, chronic inflammatory conditions other than sickle cell disease, or steroid therapy were not included.
- All of the patients in the study were guaranteed to be in a stable state when the samples were taken, and those who experienced a sickling crisis would not be included.
- Participants in the study were excluded if they did not give their consent or did not fit the selection criteria.
- Patients having Hs-CRP greater than 10 mg/L or any clinical indication of infection were not included.

2.5 Materials and Equipment

Human Fibrinogen Degradation product analyzer (Cobas C311), hemoglobin electrophoresis tank, ELISA washer, ELISA reader, alcohol pads, hand gloves, vacutainer needles, vacutainer EDTA tubes, plain vacutainer tubes, cotton wool, tourniquet, and needle and syringes.

2.6 Ethical Consideration

Prior to the study's start, a request for ethical permission was made to the State Ministry of Health's Joint Ethical Committee and granted by the Oyo State Hospital Management Board. Additionally, all individuals gave their agreement before being included in the study.

2.6 Clinical Laboratory Investigation

2.6.1 Sample collection and analysis

Venipuncture was used to obtain a sample of the fibrin degradation product from each willing respondent, which was then placed in a 3 ml EDTA container and kept at 40C. Additionally, the C Reactive Protein sample was taken into a 3 ml lithium heparin bottle and spun; the plasma was extracted from the blood, aliquoted in two vials, and kept at -80 °C at the University College Hospital Ibadan Blood Bank. Prior to analysis, samples were removed and allowed to thaw.

2.6.2 Analysis of C - reactive protein

The chemical analyzer Roche (COBAS 311) was used to analyze the C-reactive protein. A member of the Cobas 4000 series, the Roche Cobas C311 is a reasonably sized, sturdy, and user-friendly Floor Model Chemistry analyzer. With 91 assays and a maximum throughput of 300 tests per hour, this open reagent system is impressive. Consolidated testing from a wide range of clinical chemistry applications is provided by the standalone Cobas C 311 analyzer. Ion-selective electrode (ISE) analysis of sodium, potassium, and chloride in serum, plasma, and urine is possible with this analyzer.

2.6.3 Hemoglobin Electrophoresis

The cellulose acetate method was used for hemoglobin electrophoresis. Using a cellulose acetate membrane for hemoglobin electrophoresis at pH 8.4–8.6 is quick, easy, and dependable. It is adequate for identifying the majority of prevalent, clinically significant hemoglobin variations. Because hemoglobin is a negatively charged protein at alkaline pH, it will migrate toward the anode (+) when electrophoresed. Haemoglobin A will separate from structural variations that have a change in the charge on the molecule's surface at alkaline pH. Internally sited amino acid substitutions in hemoglobin variations may not separate, and electrophoresis will not separate hemoglobin variants with an amino acid substitution that has no effect on overall charge.

2.6.4 Fibrin Degradation Product Assay (ELISA)

Principle:

ELISA stands for Enzyme-Linked Immunosorbent Assay. The human FDP antibody has already been applied to the plate. When FDP from the sample is added, it attaches itself to the antibodies that have been coated on the wells. After that, the sample's FDP is bound by the biotinylated human FDP antibody. Following its addition, streptavidin-HRP attaches itself to the biotinylated FDP antibody. Unbound Streptavidin-HRP is removed during the washing phase following incubation. Following the addition of the substrate solution, color changes in accordance to the quantity of human FDP. By adding an acidic stop solution, the process is stopped, and the absorbance is measured at 450 nm.

Summary of procedure

1. All of the standards, samples, and chemicals were ready.
2. Each well received the sample and ELISA reagent, which were then incubated for an hour at 37 °C.
3. Five washes were performed on the plate.

4. After adding substrate solutions A and B, the mixture was incubated at 37°C for 10 minutes.
5. After adding stop solution, color development occurred.
6. Within ten minutes, the optical density value was obtained.

2.7 Ethical clearance

Ethical approval request was sought for and obtained from the Joint Ethical committee of the State Ministry of Health Oyo State Hospital Management board before the commencement of the study. Also, consent from all the participants was obtained prior to their inclusion into the study.

2.8 Statistical Analysis

Version 21.0 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. As needed, data was condensed into table.

3.0 RESULTS

Table 1: Descriptive statistics of CRP and FDP of subjects and control

Statistics	Subjects		Control	
	CRP	FDP	CRP	FDP
Mean	2.31	0.66	2.10	1.28
Median	2.10	0.40	1.85	1.32
Mode	2.10	0.01	3.10	0.35
Std. Deviation	1.30	0.62	1.24	0.75
Minimum	0.01	0.01	.01	0.12
Maximum	5.10	3.2	4.30	2.89

The table showed the descriptive statistics of CRP and FDP of subjects and control in which mean ±standard deviation of CRP of subject vs control (2.31 ±1.30vs 2.10±1.24). Also mean ±standard deviation of FDP of subject vs control (0.66 ± 0.62vs 1.28± 0.75). The mode and

median of subjects CRP was same at value 2.10 while the mode and median of control CRP was at value 3.10 and 1.85respectively. The mode and median of subjects FDP was at value 0.1 while the mode and median of control FDP was at value 1.32 and .35 respectively. The range of CRP of subjects was 0.01 to 5.10 while control was 0.01 to 4.30. Also the range of FDP of subjects was 0.01 to 3.23 while control was 0.12 to 2.89.

4.0 Discussion

Examining the levels of C-reactive protein (CRP) and fibrinogen degradation products (FDP) in sickle cell disease (SCD) patients relative to non-SCD controls provides important information about the hemolytic and inflammatory processes linked to SCD. In the pathophysiology of sickle cell disease (SCD), which is typified by persistent hemolysis and vaso-occlusive crises, elevated levels of FDP and CRP are suggestive of systemic inflammation and vascular dysfunction [15][16].

Furthermore, the correlation between fetal hemoglobin (HbF) levels and biomarkers like lactate dehydrogenase (LDH) and total bilirubin (T.bili) implies that although HbF can lessen certain complications, it does not always correlate with all disease biomarkers, suggesting a complex interaction of factors affecting disease severity[17]. Additionally, the discovery of certain plasma proteins linked to pulmonary hypertension and renal illness in SCD patients emphasizes the necessity of a thorough biomarker panel to improve treatment and disease management [16].

The liver produces C-reactive protein (CRP), a sensitive biomarker for inflammation, in response to inflammatory cytokines. It is frequently used in clinical settings to evaluate a range of diseases, such as infections and systemic inflammation [18][19]. Studies on sickle cell disease (SCD) show that although CRP levels can increase significantly during acute vaso-occlusive crises (VOCs), they do not significantly differ from controls during steady-state conditions. For example, mean CRP levels in SCD patients were 2.31 ± 1.30, while those in controls were 2.10 ± 1.24 ($p = 0.400$)[20]. This variation emphasizes that CRP is not a valid baseline diagnostic tool for SCD, but rather a marker for disease exacerbations [21].

Furthermore, even though its pathogenic role is still unclear and sometimes overinterpreted, CRP's rise during acute infections emphasizes its value in tracking the severity and course of disease [19][22].

Fibrinogen degradation products (FDP) are important biomarkers for determining thrombotic risk and fibrinolysis, especially in sickle cell disease (SCD), where endothelial dysfunction and persistent hemolysis can cause a dysregulated fibrinolytic system. FDP levels in SCD patients are substantially

lower (0.66 ± 0.62) than in controls (1.28 ± 0.75, p = 0.001), which may indicate a distinct coagulation profile and a prothrombotic condition marked by microvascular occlusions and thromboembolic events [23]. Careful monitoring is necessary to prevent thrombotic problems, as the severity of the disease and treatment plans may have an impact on the variability in FDP levels among SCD patients [24].

FDP measures may be used in conjunction with high C-reactive protein (CRP) during crises to assess disease activity [25]. However, limitations like the cross-sectional study design and the exclusion of patients in acute vaso-occlusive crises call for more longitudinal research to clarify the function of FDP in SCD management[26][27].

5.0 Conclusion

The substantial decrease in FDP in patients as compared to controls in this study supports its potential as a stand-alone biomarker for sickle cell disease. CRP's usefulness in normal SCD care is limited since, although it can be used to detect inflammation during acute crises, it did not significantly differ between SCD patients and controls in steady state. The results imply that routine FDP evaluations may improve SCD clinical care, especially in terms of tracking the course of the illness and averting thromboembolic episodes. Future studies should investigate the combination of these indicators with additional coagulation and inflammatory indices to create all-encompassing diagnostic and prognostic instruments for the treatment of sickle cell disease.

6.0 Recommendation

According to the study's findings, FDP should be regarded as a standard biomarker for sickle cell disease (SCD) monitoring in order to detect prothrombotic states and direct anticoagulant medication to avoid thromboembolic sequelae. CRP is still useful in identifying inflammation during acute crises and infections, even though it did not demonstrate any discernible changes in steady-state settings. As such, it should be used in combination with other clinical markers. When combined with other clinical measures, biomarkers such as FDP and CRP may offer a more thorough method of evaluating the course of the disease. To learn more about how these biomarkers change over time, especially during acute episodes, longitudinal research is required.

The main goal of public health campaigns should be to inform patients and medical professionals about the value of early diagnosis and routine biomarker monitoring in environments with limited resources. Additionally, standardizing and lowering the cost and increasing the accessibility of biomarker testing in clinical settings—especially in areas with high prevalence—will improve patient outcomes and disease management.

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