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Sex and Age-Independent Variations in Biomarkers among Sickle Cell Patients in Ibadan, Oyo State

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Introduction: Sickle cell disease is a genetic disorder predominant among people of African origin. Nigeria has the highest burden of the disease in the world. Demographic factors have been shown to influence the clinical manifestation of the disease. Understanding sex and age-related variations in key makers such as C-reactive protein and FDP would significantly improve personalized management.
Aim/Objectives: This study aimed to evaluate the Sex and Age-related Variations in C – Reactive Protein and Fibrinogen Degradation Products Among Sickle Cell Patients in University College Hospital, Ibadan, Oyo state.
Method: A descriptive cross-sectional study was conducted at University College Hospital, Ibadan, Nigeria, from March to July 2019. A total of 91 Sickle cell disease patients and 40 normal participants were used. C - reactive protein and fibrinogen degradation products levels were measured and data were stratified by age and sex. Data analysis was done using the Statistical

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Package for Social Sciences (SPSS) version 21.0. Data was summarized into Tables and Graphs as appropriate.

Results: The study included 91 sickle cell disease (SCD) patients and compared their levels of C-reactive protein (CRP) and fibrinogen degradation product (FDP) to those of controls. CRP levels showed no significant difference between SCD patients and controls (2.31 vs. 2.10, p = 0.400) and were not influenced by age or sex (p = 0.454). In contrast, FDP levels were significantly lower in SCD patients compared to controls (0.66 vs. 1.28, p = 0.001), with no variation based on sex (p = 0.873). FDP levels were also found to be independent of age and sex in SCD patients.

Conclusion: This study demonstrates that CRP and FDP levels in SCD patients are not influenced by demographic factors such as age and sex. While CRP levels were similar between SCD patients and controls, suggesting limited utility as a biomarker, FDP levels were significantly lower in SCD patients. These findings indicate that FDP could serve as a potential biomarker for understanding disease mechanisms or monitoring SCD progression, but further research is needed to establish its clinical relevance.

Keywords: Sickle cell disease; Ibadan; C-reactive protein (CRP); Fibrinogen Degradation Products (FPD); sex; age.

1. INTRODUCTION

Sickle cell disease (SCD) pathophysiology is greatly influenced by chronic inflammation, with biomarkers including fibrinogen degradation products (FDPs) and C-reactive protein (CRP) being important contributors. Studies have shown that a significant proportion of patients had high-risk levels of CRP, an acute-phase protein, which has been linked to enhanced inflammatory responses and problems in SCD [1]. Furthermore, SCD patients have significantly higher levels of inflammatory cytokines and chemokines, including as IL-18 and MCP-2, suggesting a complicated interaction between inflammation and disease severity [2].

Hemolysis and oxidative stress cause SCD patients to become hypercoagulable, which worsens inflammation and increases the risk of vascular problems [3]. In order to manage SCD-related problems, tracking these biomarkers may offer insights into the course of the disease and treatment targets [4]. According to research, people with sickle cell disease (SCD) frequently have higher levels of fibrin degradation products (FDPs) and C-reactive protein (CRP), which are correlated with the severity of the disease and the incidence of vaso-occlusive crises (VOCs) [5,6]. Age and sex are two important factors that affect these coagulation patterns and inflammatory responses. Due to differences in comorbidities and illness care, younger patients may show different inflammatory markers than older patients [6,7].

Males exhibit higher levels of inflammatory indicators, such as CRP and monocyte counts, while females often have higher amounts of fetal hemoglobin and respond better to therapy with hydroxyurea [8]. According to Hamali (2024) and Mauro et al. (2023), these differences underscore the need for customized treatment approaches by highlighting the intricate interactions among biological sex, age, and the inflammatory and coagulation pathways in SCD.

This study's main goal is to assess how age and sex affect the C-reactive protein and fibrinogen degradation products of sickle cell patients at University College Hospital in Ibadan, Oyo state.

With the ultimate goal of improving our understanding of these biomarkers and guaranteeing better clinical management of SCD patients attending the University College Hospital, Ibadan, Oyo State, the study also attempts to offer evidence-based insights into the relationship between age, sex, and levels of C-reactive protein (CRP) and fibrinogen degradation product (FDP) in SCD patients.

2. MATERIALS AND METHODS

2.1 Study Design and Area

This study was carried out in the Department of Haematology, University College Hospital, Ibadan, Ibadan North Local Government area longitude 7.3569°N and latitude 3.8743°E. It is bordered to the East by Ibadan North East Local government and to the West by Ibadan North West Local Government.

2.2 Study Design

This was a descriptive cross-sectional 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

A total number of Ninety-One (91) sickle cell disease patients attending the Haematology day care unit and who were confirmed to be HbS by Haemoglobin electrophoresis were enrolled for the study. A structured questionnaire was used to obtain demographic information from consenting subjects for the study, both verbal and written consent in form of signature was obtained from all participating subject.

2.4.1 Inclusion criteria

- Only individuals who consent to participate in the study were enrolled.
- Consenting participants were within the age bracket 18-60 years accessing care in the study location.
- They were confirmed Sickle Cell Disease patients

2.4.2 Exclusion criteria

- Patients with infection, chronic inflammatory condition other than Sickle Cell Disease, renal disease unrelated to SCD, symptomatic heart disease, rheumatoid arthritis or other autoimmune diseases, hypothyroidism, diabetes mellitus, or steroid therapy were excluded.
- All the studied patients were ensured that they are in a steady state at the time of sample collection, and those who had sickling crisis will be excluded.
- Non-consenting individuals and others who do not meet up with the selection criteria were not enrolled for the study.
- Patients with any clinical evidence of infection or Hs-CRP >10 mg/L were excluded.

2.5 Materials and Equipment

Needle and syringes, alcohol pads, hand gloves, vacutainer needles, vacutainer EDTA tubes, plain vacutainer tubes, cotton wools, tourniquet, Human Fibrinogen Degradation product analyzer (Cobas C311), Haemoglobin electrophoresis tank, ELISA washer, ELISA Reader.

2.6 Clinical Laboratory Investigation

2.6.1 Sample collection and analysis

Fibrin degradation product sample was collected from each consenting respondent into EDTA bottle (3ml) by venepuncture and stored at 4^0C. Also, sample for C Reactive protein sample was collected into Lithium Heparin bottle (3ml) and was subsequently spun; plasma was separated from the blood, aliquot in 2 vials and stored at -80^{o}C at the Blood bank, University College Hospital Ibadan. Samples were brought out and thawed before analysis.

2.6.2 Analysis of C - reactive protein

Analysis of C-reactive protein was done using chemistry analyzer Roche (COBAS 311). The Roche Cobas C311, a part of the Cobas 4000 family, is a mid-size, robust and easy to use Floor Model Chemistry analyzer. This is an open reagent system boasts 91 assays with a maximum throughput of 300 tests per hour. The cobas c 311 analyzer is a stand-alone system that offers consolidated testing from a broad menu of clinical chemistry applications. This analyzer has the capacity for ion-selective electrode (ISE) determination of sodium, potassium, and chloride in serum, plasma, and urine.

2.6.3 Haemoglobin electrophoresis

Haemoglobin electrophoresis was done using the cellulose acetate method.

Haemoglobin electrophoresis at pH 8.4–8.6 using cellulose acetate membrane is simple, reliable and rapid. It is satisfactory for the detection of most common, clinically important haemoglobin variants.

Principle: At alkaline pH, haemoglobin is a negatively charged protein, and when subjected to electrophoresis will migrate toward the anode (+). Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from haemoglobin A. Haemoglobin variants that have an amino acid substitution that is internally sited may not separate, and those that have an amino acid substitution that has no effect on overall charge will not separate by electrophoresis.

2.6.4 Fibrin Degradation Product Assay (ELISA)

Principle: The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human FDP antibody. FDP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human FDP Antibody is added and binds to FDP in the sample. Then Streptavidin-HRP is added and binds to the biotinylated FDP antibody. After incubation unbound Streptavidin-HRP is washed away during the washing step. Substrate solution is then added and color develops in proportion to the amount of human FDP. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Summary of procedure:

1. Reagents, samples and standards were all prepared.
2. Sample and ELISA reagent were added into each well and incubated for 1 hour at 37^{o}C
3. The plate was washed 5 times
4. Substrate solution A and B were added and incubated for 10 minutes at 37^{o}C
5. Stop solution was added and there was color development
6. Optical density value was read within 10 minutes

2.7 Statistical Analysis

Data analysis was done using the Statistical Package for Social Sciences (SPSS) version 21.0. Data was summarized into Tables and Graphs as appropriate.

3. RESULTS

There is no significant difference in mean **C**-reactive protein levels (CRP) between male sickle cell patients and female sickle cell patients (2.40 vs 2.12, p=0.454 >5%). There is no significant difference in mean fibrinogen degradation product (FDP)between male sickle cell patients and individual female sickle cell patients (0.71 vs .57, p=0.873>5%). The implication of the results was that outcome of C-reactive protein levels or fibrinogen degradation products (FDP) were subjected to gender difference.

4. DISCUSSION

The results of this study cast doubt on long-held beliefs on the differences in inflammatory responses across genders in chronic illnesses such sickle cell disease (SCD). In particular, there were no discernible variations in the mean levels of fibrinogen degradation product (FDP) and C-reactive protein (CRP) between male and female SCD patients (p = 0.873 for FDP and p = 0.454 for CRP). This is consistent with earlier studies showing that SCD patients of both sexes had higher levels of inflammatory markers, pointing to a systemic inflammatory state that is common in this population [4,9].

Despite the fact that biological sex is known to affect immune responses, hormonal variables like progesterone and estrogen frequently cause females to demonstrate increased inflammatory activity [10].

Table 1. Comparing difference in mean CRP and FDP between the male and female

Variables	Group	N	Mean	Std. Deviation	95%CI	t-test	p-value
C-reactive protein levels	Male	62	2.40	1.30	2.01-2.4	0.932	.354
	Female	29	2.12	1.32	1.61-2.30		
FDP	Male	62	.71	.78	.41-.71	.873	0.385
	Female	29	.57	.59	1.02-1.43		

These distinctions might not be as noticeable in long-term illnesses like sickle cell disease. Estrogens affect vulnerability to infections and immune-related disorders by modifying immunological function [11]. However, the lack of sex-based variation in CRP and FDP levels suggests that these sex-specific immune modulations may be overridden by the chronic inflammatory milieu of SCD. Understanding sex-based immune responses is made more complex by hormonal changes and epigenetic variables, such as differences in immune gene expression [12,13].

Recent research showing that there are no sex-based differences in sickle cell disease (SCD) care points to a move away from gender-specific treatment regimens and toward a more uniform strategy that focuses on patients' overall inflammatory status. Because treatments may be customized to meet the needs of each patient based on inflammatory indicators and clinical presentations, this could improve care equity [7,8]. Since high levels of fibrin degradation products (FDP) are associated with thrombotic consequences such acute chest syndrome and stroke, it is imperative to regularly evaluate these biomarkers [8,14].

Additionally, the promise for personalized therapy in SCD is highlighted by developments in gene editing and new therapeutic choices, including as combination medicines, which target the particular difficulties presented by the illness [14,15]. All things considered, these advancements highlight how crucial it is to combine biomarker monitoring with cutting-edge therapies in order to enhance patient outcomes in SCD [14,16].

Even while no sex differences have been noted, more research is necessary to fully understand the intricacy of inflammatory responses in SCD. According to research, different gene expression profiles may result from sex-specific transcriptional responses to immunological stimuli like lipopolysaccharide (LPS), which could explain subtle inflammatory variations in other situations like autoimmunity [17,18]. Furthermore, during acute inflammatory conditions, chemokines that affect neutrophil recruitment, such as Cxcl5, may show slight sex-dependent changes [19]. These findings highlight the significance of tailored treatment strategies that take into consideration environmental, hormonal, and genetic variables that affect inflammation in patients with sickle cell disease (SCD) [7].

5. CONCLUSION

According to this study, patients with chronic illnesses like sickle cell disease (SCD) have different inflammatory responses depending on their sex. C-reactive protein (CRP) and fibrinogen degradation product (FDP) levels in male and female SCD patients do not differ significantly, according to the results, which implies that the systemic inflammatory state of SCD may take precedence over normal sex-based immunological variations. In light of each patient's distinct inflammatory profile, this emphasizes the necessity of a change in clinical focus from gender-based therapy regimens to a more individualized approach.

6. RECOMMENDATION

Personalized care techniques should take precedence over sex-based treatment approaches when it comes to treating thrombotic and inflammatory risks in individuals with sickle cell disease. It is crucial to regularly monitor biomarkers like CRP and FDP in order to detect and prevent problems including acute chest syndrome and stroke. The functions of sex-specific transcriptional responses, hormonal effects, and epigenetic alterations are among the immune regulatory mechanisms in SCD that require more investigation. These observations ought to guide the creation of fair, uniform care guidelines to enhance patient outcomes worldwide.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author (s) hereby declare that generative AI technologies such as large language models, etc. have been used during the writing or editing of manuscript. This explanation will include the name, version, model and source of the generative AI technology and as well as all input prompt provided to the generative AI technology.

Details of the AI usage are given below:

1. Open AI’s ChatGPT-4.1 and Perplexity
2. Summarise, Paraphrase and correct grammatical errors

ETHICAL CONSIDERATION

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.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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