Asian Hematology Research Journal

Volume 7, Issue 4, Page 180-192, 2024; Article no.AHRJ.125186

Assessment of Coagulation Profiles and Investigation of Leukaemia Incidence in Sickle Cell Patients at Lagos University Teaching Hospital

Olusolape Temilola Kayode [a], Arinze Favour Anyiam [a,b*], Daniel Ohilebo Ugbomoiko [a], John Olusola Kayode [c], Oloruntoba Ayodele Ekun [d], Musa Abidemi Muhibi [b], Onyinye Cecilia Arinze-Anyiam [a], Theophilus Ogie Erameh [a] and Rosemary Nwamaka Adebayo [a]

[a] Department of Medical Laboratory Science, Igbinedion University, Okada, Edo State, Nigeria.
[b] Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Uzairue, Edo State, Nigeria.
[c] National Agency for Food and Drug Administration and Control (NAFDAC), Lagos, Nigeria.
[d] Department of Medical Laboratory Science, College of Medicine, University of Lagos, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125186

Original Research Article

Received: 14/08/2024
Accepted: 17/10/2024
Published: 24/10/2024

**Corresponding author: E-mail: anyiam.arinze@iuokada.edu.ng, arinze21.favour@edouniversity.edu.ng;*

Cite as: *Kayode, Olusolape Temilola, Arinze Favour Anyiam, Daniel Ohilebo Ugbomoiko, John Olusola Kayode, Oloruntoba Ayodele Ekun, Musa Abidemi Muhibi, Onyinye Cecilia Arinze-Anyiam, Theophilus Ogie Erameh, and Rosemary Nwamaka Adebayo. 2024. "Assessment of Coagulation Profiles and Investigation of Leukaemia Incidence in Sickle Cell Patients at Lagos University Teaching Hospital". Asian Hematology Research Journal 7 (4):180-92. https://www.journalahrj.com/index.php/AHRJ/article/view/182.*

ABSTRACT

Aims: The purpose of this research was to assess and compare the coagulation and haematological profiles of sickle cell disease (SCD) patients to those without the condition, as well as to ascertain the incidence of leukaemia in SC patients. It specifically examined if there were any observable variations between SCD patients with and without leg ulcers, as well as variances in coagulation parameters including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and D-Dimer levels.

Study Design: In order to ascertain the incidence of leukaemia and evaluate the coagulation and haematological profiles of sickle cell patients (steady state) with leg ulcers, as well as non-leg ulcer and non-sickle cell persons as the control group, this study used a case control design.

Place and Duration of Study: The study was conducted at the Sickle Cell Foundation Nigeria, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria, from May to September 2024.

Methodology:

A questionnaire was developed and administered to both patients with sickle cell disease (SCD) at their steady state and individuals in the control group (non-SCD). Ninety-two (92) participants were recruited for this study, of which 80 met the inclusion criteria. Fifty (50) subjects were of the SCD case group, while 30 were of the non-SCD control group. The 80 consenting participants comprised 44 males and 36 females. The average age for SCD cases was 19.58 ± 9.8 years, while controls averaged 27.4 ± 12.3 years. Investigations were carried out on all the samples for Full Blood Count, coagulation profile, haemoglobin electrophoresis, ABO blood group, and peripheral blood film examination. Ethical approval was obtained from the College of Medicine, University of Lagos, and the Sickle Cell Foundation, Nigeria. Data were analyzed using t-tests to compare haematological and coagulation parameters between groups. All analyses were performed using R and Python to ensure accurate data analysis, which is crucial in clinical research.

Results: While significant differences were found in haematological parameters between SCD patients and controls—specifically lower red blood cell counts, haematocrit, and haemoglobin levels, alongside elevated white blood cell and platelet counts ($P < 0.001$)—no new cases of leukemia were detected. Coagulation profiles, including PT and APTT, were prolonged in SCD patients ($P < 0.001$), though fibrinogen and D-Dimer levels showed no statistically significant differences between SCD and control groups ($P = 0.563$ and $P = 0.223$, respectively).

Conclusion: The absence of leukaemia in our sample highlights the potential rarity of leukaemia in this SCD population. The coagulation profile anomalies highlight the therapeutic significance of monitoring thrombotic risks in the management of sickle cell disease (SCD), even if no significant connection with leukaemia was established. Our results shed light on the haemorrhagic problems associated with sickle cell disease (SCD), even when leukaemia is not present. They also imply that vigilant monitoring of coagulation parameters is still very essential to shield SCD patients from vascular complications.

Keywords: Sickle cell disease; leg ulcer; haemoglobin; leukaemia; coagulation profile; D-dimer.

1. INTRODUCTION

1.1 Background Study

Sickle cell disease (SCD) is a group of genetic blood disorders caused by mutations in the gene responsible for producing the β-haemoglobin subunit. The presence of sickle haemoglobin (HbS) deforms red blood cells (RBCs) into a sickle shape, leading to chronic haemolytic anaemia and organ damage. SCD includes subtypes like sickle cell anaemia (SCA), HbSC, and HbSβ-thalassemia, all resulting from mutations in the HBB gene [1]. Sickle cell anaemia (SCA), the most common form of SCD, causes chronic anaemia, periodic pain, and organ damage. Its severity varies based on genetic factors like elevated foetal haemoglobin (HbF) and co-inheritance of α-thalassemia [2]. Globally, Nigeria has a high prevalence, with 4-6 million affected individuals [3]. A review identified 51 cases of leukemia in SCD, often linked to chronic inflammation and genomic instability due to chronic hemolysis and secondary hemochromatosis [4].

1.2 Sickle Cell Disease with Chronic Leg Ulcer

Sickle Cell Anemia (SCA) increases venous thromboembolism risk due to higher

prothrombotic factors and reduced anticoagulants [5]. Chronic leg ulcers (CLU), common in SCA patients, are recurrent, painful, and slow to heal. CLU prevalence varies: up to 75% in Jamaica, 8-10% in North America, and 10-19% in Ghana [6]. Nearly 97% of healed CLUs recur within a year, often leading to disfigurement, social isolation, and economic hardship.

1.3 Pathophysiology of Sickle Cell Disease

The fundamental pathophysiology involves HbS polymerization during deoxygenation, causing RBC deformity, leading to vaso-occlusion, haemolysis, and inflammation. This results in complications like pain, lung injury, stroke, and hypertension [7]. SCD also presents a hypercoagulable state with altered platelet function and coagulation pathway activation [8].

1.4 Leukaemia and Sickle Cell Disease

Leukemia, a blood cancer involving abnormal proliferation of white blood cells, is linked to SCD. SCD patients may face an increased leukemia risk due to factors like heightened bone marrow turnover and genetic susceptibility [9]. Chronic inflammation and immune dysregulation may contribute to this risk, alongside persistent coagulation pathway activation [10].

1.5 The Role of Coagulation in the Pathogenesis of SCD

In SCD, activated coagulation pathways are present even without vascular occlusions. This is indicated by elevated tissue factor, thrombin generation, and procoagulant microparticles. Endothelial dysfunction due to haemolysis is linked to elevated soluble vascular cell adhesion molecule-1 (sVCAM-1) levels, reflecting haemolysis-induced endothelial activation.

1.6 Statement of Problem

Sickle cell disease (SCD) causes haemostatic abnormalities, leading to thrombotic and bleeding complications. Investigating coagulation profiles and related haematologic abnormalities, including leukemia incidence, is crucial for improving early detection, optimizing diagnostic methods, and developing targeted treatment strategies to manage these haemostatic challenges in SCD patients.

1.7 Justification of Study

Sickle cell disease (SCD) leads to haemostatic abnormalities, causing thrombotic and bleeding complications. Investigating the coagulation profiles and haematologic abnormalities in SCD patients is essential to enhance early detection, refine diagnostics, and improve treatment strategies. Understanding these haemostatic disturbances, including the potential impact of leukemia, is critical for optimizing clinical care in SCD management. Moreover, there is limited research on the specific incidence of leukemia in this population, despite risk factors like chronic bone marrow hyperactivity and inflammation.

1.8 Objectives

This study assessed selected haematological and coagulation profiles of sickle cell patients with and without leg ulcers, at Lagos University Teaching Hospital.

1.9 Literature Review

1.9.1 Sickle cell disease and hypercoagulability

Sickle cell disease (SCD) is a genetic blood disorder caused by a mutation in the β-globin gene, leading to the production of sickle haemoglobin (HbS), which deforms red blood cells [11]. SCD leads to acute and cumulative organ damage, manifesting as stroke, acute chest syndrome, sickle lung disease, pulmonary hypertension, nephropathy, end-stage renal disease, and other complications. [12]. SCD affects over 100,000 Americans and millions globally, particularly in Africa [13].

1.9.2 Coagulation abnormalities in SCD

The hypercoagulable state in SCD arises from haemolysis-induced platelet activation and endothelial dysfunction [1], with elevated procoagulant markers and tissue factor activity contributing to thrombosis [14]. Activated platelets promote vascular adhesion, leading to complications like pulmonary hypertension [15].

2. MATERIALS AND METHODS

2.1 Inclusion Criteria

Male and female subjects aged 10 to 65 years with an established diagnosis of sickle cell

disease at steady state were included in this study. In this study, steady-state SCD was defined as the absence of acute sickling events, infections, or blood transfusions within at least three months prior to sample collection, based on established clinical guidelines for SCD management [16]. To ensure comparability between groups and reduce potential confounding factors, the control group was matched to the SCD group based on age and sex. Control participants who were screened to confirm they had no underlying haematological disorders, chronic illnesses, or any conditions that might affect coagulation profiles were selected to ensure that age distributions and gender ratios were similar to those of the SCD group, thereby minimizing the influence of these variables on the study's outcomes.

2.2 Exclusion Criteria

However, individuals taking anticoagulant medications, those who have received recent blood transfusions, individuals with known liver disease affecting clotting factor production, and pregnant women with sickle cell disease were excluded from participation in this study because pregnancy induces substantial changes in the body's haematological and coagulation systems, including increased blood volume, altered red and white blood cell counts, and a hypercoagulable state. In women with SCD, these changes could exacerbate existing complications such as anaemia, thrombosis, and vaso-occlusive crises, making it difficult to isolate the effects of SCD on the coagulation profile from those caused by pregnancy. Since this study aims to compare steady-state SCD patients with healthy controls, including pregnant women could introduce significant variability and confounding factors. Excluding them ensures a more homogeneous sample, allowing the results to specifically reflect the effects of SCD rather than pregnancy-related changes.

2.3 Sample Collection and Processes

A total of 3.8 ml of blood sample was collected per subject via standard venipuncture, with 2 ml placed in ethylene-diamine tetra-acetic acid (EDTA) tube and 1.8 ml in a sodium citrate tube to prevent clotting. Samples were transported under cold chain conditions, maintained at a temperature range of 2°C to 8°C, to ensure sample integrity. The samples were then processed, and stored at appropriate temperatures for analysis.

2.4 Sample Size

The sample size for this study was determined using the formula outlined by Daniel and Cross [17] based on a 3% prevalence rate of sickle cell disease (SCD) in Nigeria. The formula used for sample size calculation was:

$$n = Z^2P(1-P) / d^2$$

Where:

- n = sample size
- Z = Z-score corresponding to the 95% confidence level (1.96)
- P = estimated population proportion with SCD (3%)
- d = margin of error (5%)

Using this formula, the minimum required sample size for this study was calculated to be 50 participants, which was met and exceeded in this study with a total of 80 participants (50 SCD patients and 30 controls).

2.5 Sample Analysis

The laboratory investigations included a full blood count measuring haemoglobin, haematocrit, white cell, and platelet counts. Coagulation studies assessed APTT, PT, fibrinogen, and D-dimer levels and were assessed using standardized, validated protocols. The assays were conducted in duplicate to ensure the precision and reproducibility of the results. Haemoglobin electrophoresis confirmed haemoglobin genotype, and a peripheral blood film examination was conducted to check for cellular morphological anomalies.

2.5.1 Full Blood Count (FBC)

FBC was analysed using haematology autoanalyzer (Horiba Yumizen h500), haemoglobin was derived from the analysis.

2.5.2 Haemoglobin electrophoresis

1.2 ml of blood was mixed with a haemolysing agent (1:6 ratio). The Haemolysate was loaded onto a cellulose acetate membrane in an electrophoresis apparatus set at pH 8.4. After applying electric current for 15 minutes, the membrane was stained, and bands were compared to control bands to identify haemoglobin variants.

2.5.3 ABO-rhesus blood grouping

One drop of Anti-A, Anti-B, and anti-D sera was placed on a slide, one drop of blood was placed near each serum. It was gently mixed for 2 minutes and observed for agglutination, alongside the controls.

2.5.4 Peripheral blood film examination

A blood drop was spread on a glass slide at a 30–45-degree angle to create a thin smear with a feathered edge, then air-dried. After staining with Leishman stain and rinsing, the slide was examined microscopically. Lower magnification assessed general cell distribution, followed by detailed analysis at 100x. Red blood cells were evaluated for size and shape abnormalities, white blood cells for numerical and morphological issues, and platelets for distribution. Significant findings suggestive of anaemia or infection were recorded.

2.5.5 Prothrombin time

Blood sample was collected in a sodium citrate tube and was centrifuged for 15 minutes at 2500g. 200ul of PT reagent was placed in a test tube, incubated in water bath at 37°C for 2 minutes. 100ul of the plasma was added and the timer was started immediately. Time taken for clot formation was measured and recorded. The PT is reported in seconds.

2.5.6 Activated Partial Thromboplastin Time (APTT)

Blood sample was collected in a sodium citrate tube and centrifuged at 2500g for 15 minutes. 200µl of APTT reagent was incubated at 37°C for 2 minutes, followed by the addition of 100µl of plasma and another 2 minutes incubation. After adding 100 µl of pre-warmed calcium chloride, the timer was started, and the clot formation time was measured and recorded in seconds.

2.5.7 Fibrinogen assay

This assay employs a sandwich ELISA technique:

2.5.7.1 Principle of fibrinogen

This assay employed a sandwich ELISA technique. The plate was pre-coated with an anti-fibrinogen antibody, to which the fibrinogen antigen in the sample binds. A biotinylated secondary antibody was added to form a sandwich complex with the bound antigen. The addition of a chromogenic substrate produced a color change, with the intensity directly proportional to the concentration of fibrinogen in the sample.

2.5.8 D-Dimer assay

This assay employs a sandwich ELISA technique:

2.5.8.1 The principle of d-dimer

Similar to the fibrinogen assay, the D-dimer assay used a sandwich ELISA technique. In this assay, plates were pre-coated with anti-D-dimer antibodies. D-dimer antigens in the sample bind to the plate-bound antibodies, and a biotinylated detection antibody was added to form a sandwich complex. The subsequent addition of a chromogenic substrate produced a color reaction, proportional to the concentration of D-dimer in the sample.

2.6 Statistical Analysis

The statistical analysis was conducted using R and Python.

2.6.1 R

This is a specialized language for statistical computing and graphics, often used in medical research for its comprehensive collection of statistical functions and user-friendly packages like dplyr, ggplot2, and tidyverse. R is well-suited for complex statistical modeling, exploratory data analysis, and visualization, making it popular among statisticians and biostatisticians.

2.6.2 Python

This is a versatile language often used in machine learning, data analysis, and statistical computations. With libraries such as pandas, scipy, statsmodels, and matplotlib, Python is highly flexible and allows for integrating statistical analysis with automation, machine learning, and predictive modeling.

Table 1 shows the distribution of Haemoglobin genotypes by sex and sickle cell disease (SCD) diagnosis**.** The table presents the distribution of haemoglobin genotypes (SC, SS, AA, AC, AS) by sex and disease status (SCD and non-SCD).

Table 1. The distribution of Haemoglobin genotypes by sex and sickle cell disease (SCD) diagnosis

Sex	Case (SCD)		Control (non-SCD)			Total	%
	SC	SS	AA	AC	AS		
Female	1	18	11	1	5	36	45%
Male	4	27	10	1	2	44	55%
Total	5	45	21	2	7	80	100%

Table 2 and Fig. 1 Summary Statistics of coagulation and haematological profiles between the SCD diagnosis groups (CASE and CONTROL).

The reduced HCT in SCD patients is consistent with the lower RBC count and chronic anemia. It indicates the reduced capacity of the blood to carry oxygen, which is a hallmark of SCD.The lower HGB levels in SCD patients are due to chronic hemolysis and reduced RBC count. This may contributes to the symptoms of fatigue and poor oxygenation commonly seen in these patients.

Table 3: T-test results comparing haematological profiles between sickle cell patients with and without leg ulcers

Statistical analysis of variables were compared in SCD with leg ulcer and non-leg ulcer with the corresponding *P* value.

Table 2. Summary Statistics of coagulation and haematological profiles between the SCD diagnosis groups (CASE and CONTROL)

Variable	CASE *(n=50)* Mean ± SD	CONTROL *(n=30)* Mean ± SD	t-statistic	p-value	95% Confidence Interval
RBC ($\times10^{12}$/L)	2.702 ± 0.931	4.567 ± 0.668	-10.388	0.001*	2.5 to 3.0
HCT (%)	21.606 ± 4.762	37.333 ± 4.167	-15.478	0.001*	20.1 to 23.1
HGB (g/dl)	7.356 ± 1.54	12.413 ± 1.413	-14.977	0.001*	7.0 to 7.7
WBC ($\times10^{9}$/L)	11.193 ± 3.294	5.42 ± 1.219	11.182	0.001*	10.5 to 11.9
Platelets ($\times10^{9}$/L)	442.26 ± 218.712	253 ± 77.858	5.560	0.001*	402.6 to 482.0
PT (seconds)	18.56 ± 2.666	15.333 ± 2.67	5.236	0.001*	17.5 to 19.6
APTT (seconds)	46.58 ± 10.272	46.433 ± 5.788	0.082	0.935	44.1 to 49.0
Fibrinogen g/l	3.370 ± 0.692	3.188 ± 0.579	1.264	0.210	3.1 to 3.6
D-Dimer ng/ml	672.947 ± 138.325	637.512 ± 115.849	1.230	0.223	620.0 to 726.0

** p is significant at 0.05*

Key: RBC- Red blood cells, HCT- Haematocrit, HGB-Haemoglobin, WBC-White blood cell, PT-Prothrombin Time, APTT- Activated partial thromboplastin time.

Table 3. T-test results for comparing haematological profiles between sickle cell patients with and without leg ulcers

Variables	t-statistics	*P*-value
RBC	-2.065	0.045*
HCT	-1.129	0.265
HGB	-0.676	0.503
WBC	2.330	0.024*
Platelets	3.303	0.002*
PT	1.281	0.206
APTT	-0.533	0.596
Fibrinogen	0.788	0.435
D-Dimer	0.842	0.406

**p* is significant at 0.05

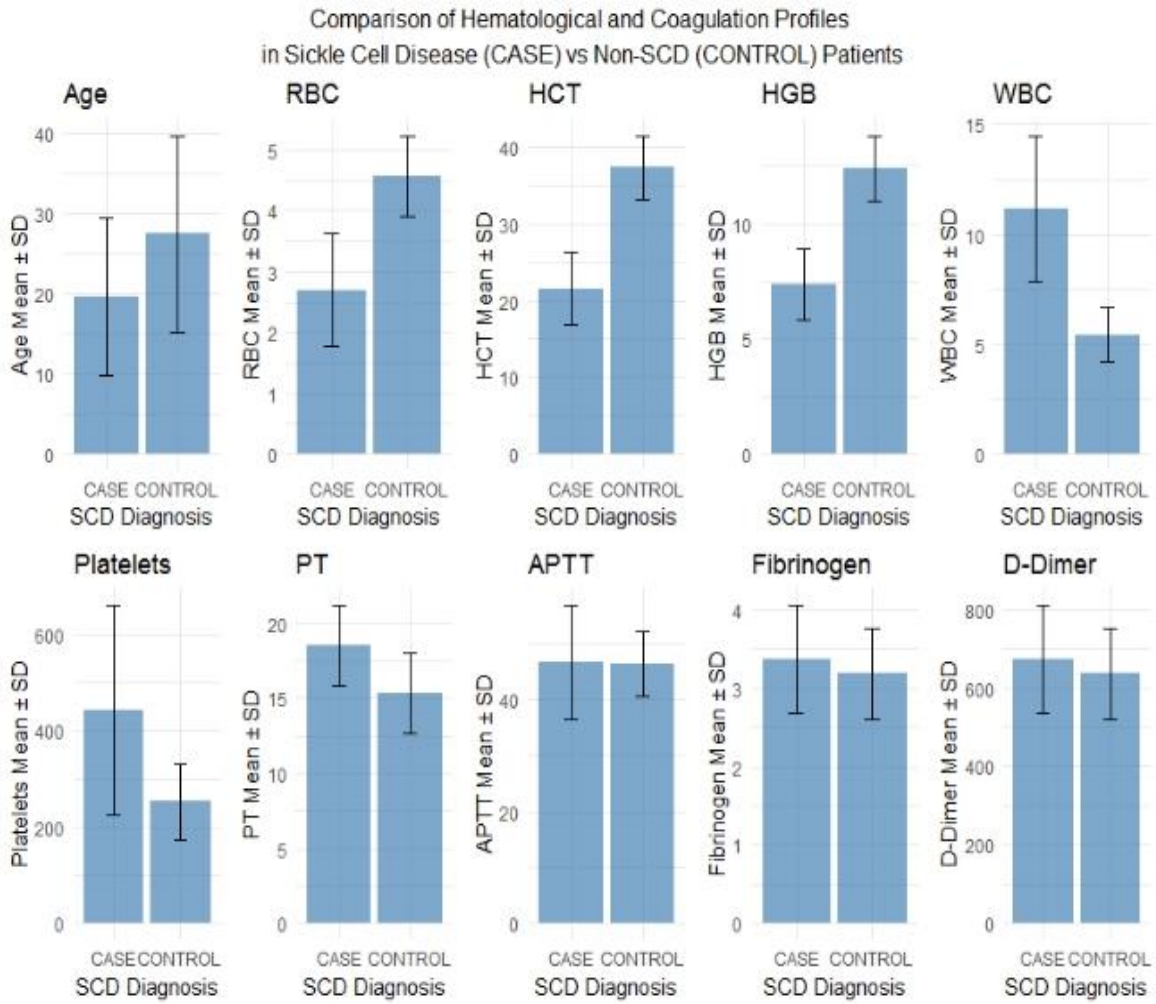

Fig. 1. Comparison of haematological and Coagulation Profiles in Sickle Cell Disease (Case) and Non-Sickle Cell (Control)

Fig. 2. presents a boxplot to compare Prothrombin Time, APTT, Fibrinogen, and D-Dimer levels between SCD patients with and without leg ulcers.

3. RESULTS AND DISCUSSION

3.1 The Distribution of Haemoglobin Genotypes by Sex and Sickle cell Disease (SCD) Diagnosis (Table 1)

The predominance of HbSS in the SCD group aligns with known epidemiology, as HbSS is the most common variant associated with sickle cell anaemia. HbSC, a milder variant, still leads to complications such as haemolysis and pain crises. The control group's high frequency of HbAA reflects the general population. Our study corroborates [1], reporting Sickle cell anaemia as the most common form of SCD. The higher prevalence in males (62%) compared to females (38%) is clinically significant, with studies indicating increased incidence of complications like priapism [16].

3.2 (Table 2 and Fig. 1) Summary Statistics of Coagulation and Haematological Profiles between the SCD Diagnosis Groups (Case and Control)

The summary statistics **(Table 2)** of haematological and coagulation profiles in Sickle Cell Disease (SCD) and controls show significantly lower mean RBC (*P*=.001), HCT (*P*=.001), and haemoglobin (*P*=0.001) in SCD individuals. Sickle cells are fragile, with a lifespan of 10–20 days, leading to chronic haemolysis. This aligns with [18], who found lower Hb levels in HbSS compared to HbSC and HbAA participants.

3.2.1 White cell count

WBC count is significantly elevated in SCD patients compared to controls (*P*=0.001), likely due to chronic inflammation from vaso-occlusion and tissue ischemia. This stimulates increased white blood cell production. Our findings align with [19], who reported mean WBC counts of 10.7±6.3, and [20], who found 12.7±7.6 in Saudi adults with SCD. Increased WBC levels are associated with heightened immune activity and inflammatory responses, which can contribute to complications such as acute chest syndrome, stroke, and increased mortality in SCD patients. Moreover, persistently high WBC counts may indicate a higher risk of organ damage due to ongoing inflammation. Clinically, this suggests that monitoring and managing WBC levels may be critical in preventing or mitigating long-term complications in SCD patients.

3.2.2 Platelet count

The platelet count in SCD patients is significantly higher than in the control group (P=.001), likely due to compensatory mechanisms for chronic anemia and hemolysis or the hypercoagulable state in SCD. This finding aligns with [21], who reported higher platelet counts in SCD participants.

3.2.3 Prothrombin time

Additionally, our study showed a statistically significantly increased PT in SCD patients (P=.001) and a slightly but not statistically significantly higher APTT (*P*=.93). Prolonged PT and APTT may result from reduced plasma levels of factor V [22] and total factor VII [23], consistent with [24] and [18]. Prolonged PT in SCD patients suggests a delay in the blood clotting process, which may be due to abnormal liver function or deficiencies in certain clotting factors. Although this may seem counterintuitive given the hypercoagulable state often seen in SCD, the prolonged PT could indicate a compensatory mechanism or reflect underlying liver dysfunction due to iron overload or chronic hemolysis. Clinically, prolonged PT can increase the risk of bleeding complications, particularly during surgical procedures or in cases of trauma.

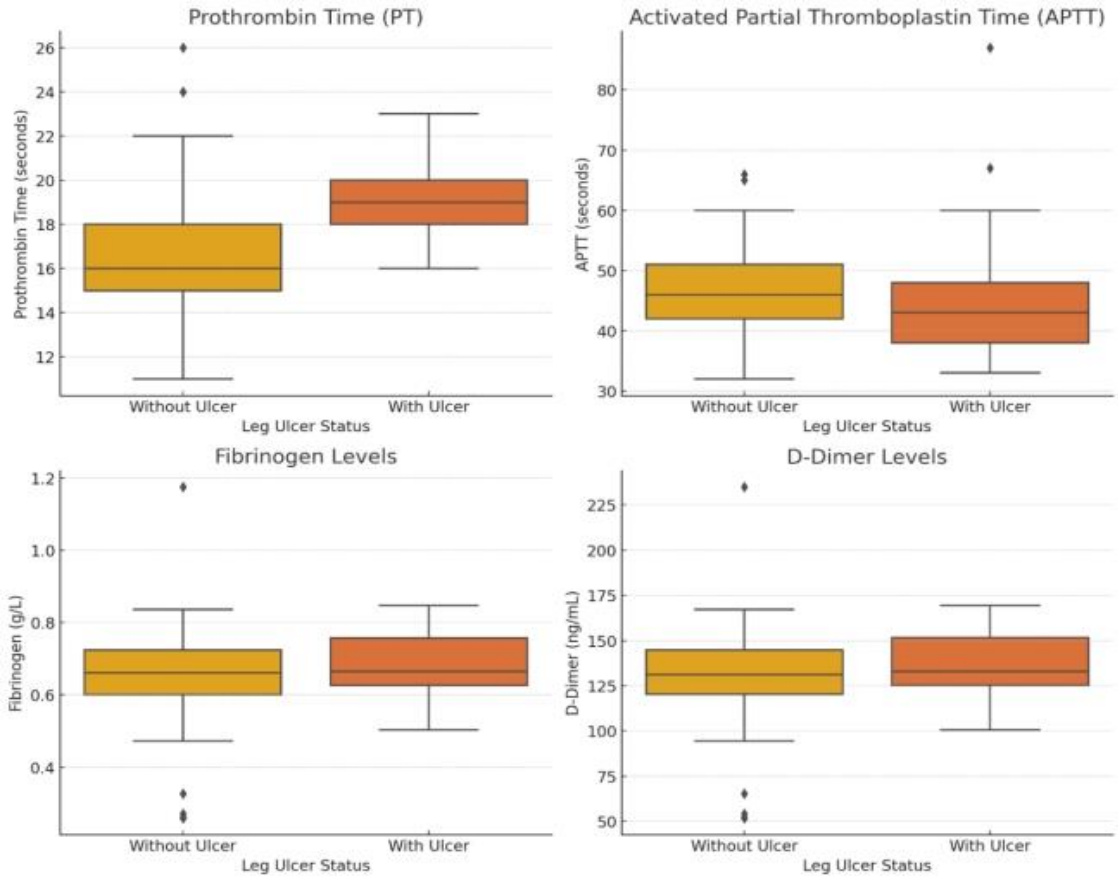

Fig. 2. Coagulation profile in SCD Patients with and without Leg Ulcers

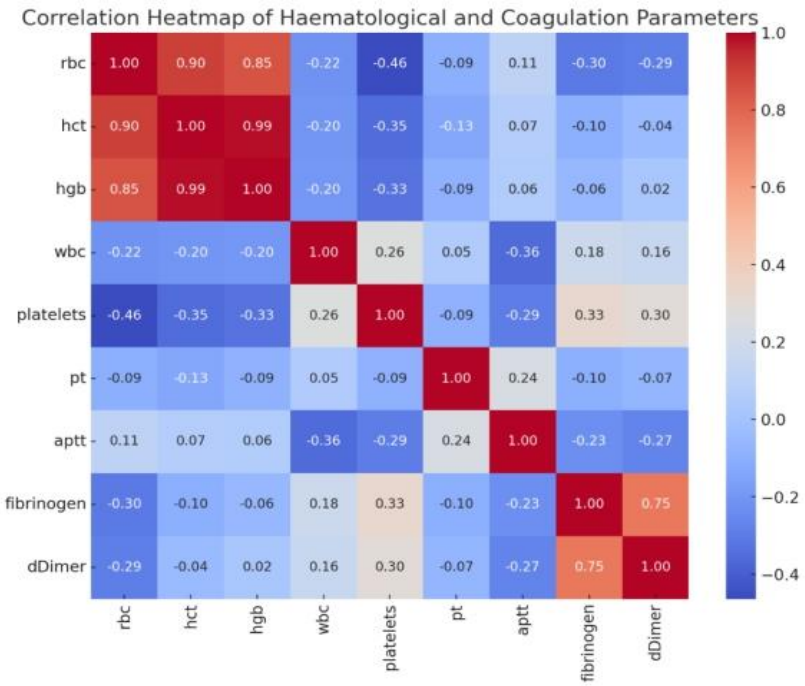

Fig. 3. Shows the correlation heatmap of all the variables. Indicating the correlation among them

3.2.4 Fibrinogen

Fibrinogen levels were slightly higher in the SCD group (mean 3.370 ± 0.692 g/L); however, the difference was not statistically significant (p = 0.210). This finding corroborates with the research done by [23], with mean fibrinogen concentration of 314.3 ± 109.83 and 284.90 ± 83.46 mg/dL for SCA with and without chronic leg ulcer, respectively.

3.2.5 D-dimer

D-Dimer levels were elevated in the SCD group (mean 672.947 ± 138.325 ng/mL) compared to the control group (mean 637.512 ± 115.849 ng/mL), but the difference was not statistically significant (p = 0.223, threshold: p < 0.05). Elevated D-dimer levels have been reported in SCA (HbSS) patients compared to HbAA controls [25]. Similarly, [26] in his study, reported a higher D-dimer in SCA with chronic leg ulcer compared to HbAA controls.

3.3 Analysis of Peripheral Blood Film (PBF) Examination

80 haematological slides were examined for abnormalities in red cell morphology, white cells, and platelets. In the case group, red cell abnormalities included sickle cells, Hb C crystals, target cells, red cell fragmentation, and nucleated red cells. White cell and platelet abnormalities observed included moderate leukocytosis, mild toxic granulation, thrombocytosis, macrothrombocytes, and platelet aggregation. In contrast, the control group exhibited only mild and clinically insignificant abnormalities.

3.4 (Table 2 and Fig. 1) Summary Statistics of coagulation and haematological profiles between the SCD diagnosis groups (CASE and CONTROL)

The summary statistics (Table 2) of haematological and coagulation profiles in Sickle Cell Disease (SCD) and controls show significantly lower mean RBC (*P*=.001), HCT (*P*=.001), and haemoglobin (*P*=0.001) in SCD individuals. Sickle cells are fragile, with a lifespan of 10–20 days, leading to chronic haemolysis. This aligns with [18], who found lower Hb levels in HbSS compared to HbSC and HbAA participants.

3.5 T-test Results for Comparing Haematological Profiles between Sickle Cell Patients with and Without leg Ulcers

T-tests revealed significant differences in red blood cell (RBC) counts (t = -2.065, p = 0.045),

white blood cell (WBC) counts (t = 2.330, p = 0.024), and platelet counts (t = 3.303, p = 0.002) between SCD patients with and without leg ulcers, suggesting that immune and inflammatory responses may play a role in ulcer development. However, other parameters, such as hemoglobin (HGB) and haematocrit (HCT), did not show significant differences (p > 0.05). These non-significant results could be due to a Type II error caused by the relatively small sample size, which may not have provided sufficient power to detect differences in these variables [27].

3.6 Fig. 2 Coagulation profile in SCD Patients with and without Leg Ulcers

Our study evaluated coagulation profiles (prothrombin time, APTT, D-Dimer, and fibrinogen levels) in 25 SCD participants with leg ulcers compared to 25 without. SCD patients with leg ulcers showed a higher median PT (t-statistic: 1.281; P=0.21), but this difference was not statistically significant. APTT medians were similar, with greater variability in patients without ulcers (t-statistic: -0.533; P=0.60). Fibrinogen levels and D-Dimer were slightly higher in patients with leg ulcers, but differences were not significant (t-statistic: 0.788; P=0.44; t-statistic: 0.842; P=0.41). These findings align with [28-42], indicating no significant differences in coagulation profiles between groups. The lack of significance in these comparisons may also reflect the small sample size, raising the possibility of a Type II error. Given that SCD is associated with a hypercoagulable state, larger studies are needed to assess whether more subtle differences in coagulation profiles exist between patients with and without leg ulcers.

3.7 Fig. 3 Correlation Heatmap of Haematological and Coagulation Parameters

The heatmap from our study indicates significant relationships among haematological and coagulation parameters in sickle cell patients. A strong positive correlation exists between red blood cell count (RBC) and haematocrit (HCT) (r = 0.93), indicating that HCT reflects the proportion of RBCs. Haemoglobin (HGB) also shows a strong correlation with RBC count (r = 0.87). HGB and HCT demonstrate a near-perfect correlation (r = 0.97), emphasizing their link to oxygen-carrying capacity. Conversely, a moderate negative correlation exists between platelet counts and prothrombin time (PT) (r = -0.34), while D-Dimer levels decrease with increasing RBC counts (r = -0.28). D-Dimer also exhibits similar negative correlations with HCT and HGB (r = -0.24 in both cases), suggesting reduced clotting activity in patients with better oxygen-carrying capacity. APTT and fibrinogen levels show weak correlations, indicating their independence from basic haematological parameters.

4. CONCLUSION

This study highlights the distinct haematological and coagulation profiles in SCD patients compared to non-SCD controls. Significant differences in RBC, WBC, and platelet counts were observed, while no association with increased leukemia incidence was found. Additionally, the absence of significant differences in coagulation parameters between SCD patients with and without leg ulcers underscores the complexity of managing SCD-related complications. These findings contribute to a better understanding of SCD's haematological profile and emphasize the need for ongoing research and tailored management strategies for affected individuals. Given the rarity of leukemia in SCD patients, future research should involve larger sample sizes and multi-centre collaborations to better assess the incidence of leukemia in SCD populations. This would help to confirm whether the absence of leukemia in our study was due to the small sample size or reflects a genuine trend. To better understand the long-term hematological and coagulation changes in SCD patients, future studies should adopt a longitudinal design. This would help in identifying potential risk factors for malignancies like leukemia that may develop over time, and also assess how coagulation profiles change with age or disease progression. Although our study highlighted significant coagulation abnormalities in SCD patients, further research is needed to explore the underlying mechanisms driving these changes. Investigating molecular and genetic factors that contribute to the hypercoagulable state in SCD could provide valuable insights for developing targeted therapies.

Another major limitation is the lack of control for potential confounding variables, such as the use of hydroxyurea, a common treatment for SCD that is known to affect both blood counts and coagulation profiles. Hydroxyurea increases foetal haemoglobin levels and can reduce white blood cell counts, potentially influencing the study’s results. Additionally, other medications, comorbidities, and lifestyle factors were not controlled for, which could have affected the

coagulation profiles and haematological parameters observed in the SCD group. Future studies should stratify participants based on medication use, including hydroxyurea, to better isolate its effects.

This study was conducted at a single centre, which may limit the generalizability of the findings. Differences in SCD management and patient demographics at other healthcare centres or regions may lead to variations in haematological and coagulation profiles. Multicentre studies involving diverse populations are needed to confirm the generalizability of these results.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT AND ETHICS APPROVAL

Ethical approval was given by the Ethics Committee of the College of Medicine, University of Lagos, Lagos state, Nigeria, and the Sickle Cell Foundation, Nigeria. All participants gave their informed consents before inclusion into the study.

ACKNOWLEDGEMENT

We express our appreciation to the Department of Medical Laboratory Science Igbinedion University, Okada, Edo Stata, Nigeria. We extend our gratitudes to the CEO of Sickle Cell Foundation, Nigeria for granting patient access, and to the management of Federal Fedical Centre, Ebute-Metta, Lagos, for career support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kato GJ, Piel BF, Reid CD, Gaston HM, Frempong KO, Krishnamurti L, et al. Sickle cell disease. Nature News; 2018. [Accaesson 2024 Mar 27]. Available:https://www.nature.com/articles/nrdp201810
2. Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. Am J Hematol. 2012;87:795–803.
3. Aygun B, Odame I. A global perspective on the sickle-cell disease. Pediatr Blood Cancer. 2012;59(2):386–90. Available:https://doi.org/10.1002/pbc.24175
4. Giovanna C, Solène P, Maël H, Hélène L, Marie-Virginie L, Xavier T, et al. Hot. 1. Sickle cell disease and acute leukemia: one case report and an extensive review. Annals of Hematology; 2023. DOI: 10.1007/s00277-023-05294-3
5. Whelihan MF, Lim MY, Mooberry MJ, et al. Thrombin generation and cell-dependent hypercoagulability in sickle cell disease. J Thromb Haemost. 2016;14(10):1941–52. DOI: 10.1111/jth.13416
6. Antwi-Boasiako C, Andemariam B, Colombatti R, et al. A study of the geographic distribution and associated risk factors of leg ulcers within an international cohort of sickle cell disease patients: the CASiRe group analysis. Ann Hematol. 2020;99(9):2073–9. DOI: 10.1007/s00277-020-04057-8
7. van Beers EJ, et al. Imaging flow cytometry for automated detection of hypoxia-induced erythrocyte shape change in sickle cell disease. Am J Hematol. 2014;89(6):598–603.
8. Akinbami A, Dosunmu A, Adediran A, Oshinaike O, Adebola P, Arogundade O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA control in Lagos, Nigeria. BMC Res Notes. 2012; 5:398. Available:https://doi.org/10.1186/1756-0500-5-396
9. Barton JC, et al. Elevated white blood cell count at diagnosis of sickle cell disease predicts early death. Transfusion. 2002;42 (3):370–6. Available:https://doi.org/10.1046/j.1537-2995.2002.00053.x
10. Noubouossie D, Key NS, Ataga KI. Coagulation abnormalities of sickle cell disease: Relationship with clinical outcomes and the effect of disease-modifying therapies. Blood Rev. 2016;30 (4):245–56.
11. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376: 2018–31.

12. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease: Life expectancy and risk factors for early death. N Engl J Med. 1994;330:1639–44.
13. Bunn HF. Pathogenesis and treatment of sickle cell disease. N Engl J Med. 1997; 337(11):762–9.
14. Ataga KI, Moore CG, Hillery CA, et al. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. Haematologica. 2008;93(1): 20–6.
15. Villagra J, Shiva S, Hunter LA, Machado RF, Gladwin MT, Kato GJ. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. Blood. 2007;110 (6):2166–72.
16. Murad MH, Liem RI, Lang ES, Akl EA, Meerpohl JJ, DeBaun MR, et al. 2019 Sickle Cell Disease Guidelines by the American Society of Hematology: Methodology, Challenges, and Innovations. Blood Adv. 2019;3(23):3945-3950.
DOI: 10.1182/bloodadvances.2019000931.
17. Daniel WW, Cross CL. Biostatistics: A Foundation for Analysis in the Health Sciences. 10th ed. Hoboken, NJ: John Wiley & Sons; 2013.
18. Hudnall M, Reed-Maldonado AB, Lue TF. Advances in the understanding of priapism. Translational Andrology and Urology [Internet]. 2017 Apr 1;6(2):199–206.
Available:https://pubmed.ncbi.nlm.nih.gov/28540227/
19. Antwi-Baffour S, Kyeremeh R, Annison L. Severity of anaemia has corresponding effects on coagulation parameters of sickle cell disease patients. Diseases. 2019;7 (59).
Available:https://doi.org/10.3390/diseases7040059
20. Ahmed AE, Ali YZ, Al-Suliman AM, Albagshi JM, Al Salamah M, Elsayid M, Alanazi WR, Ahmed RA, McClish DK, Al-Jahdali H. The prevalence of abnormal leukocyte count, and its predisposing factors, in patients with sickle cell disease in Saudi Arabia. J Blood Med. 2017;8:185–91.
Available:https://doi.org/10.2147/JBM.S148463
21. Alsultan A, Alabdulaali MK, Griffin PJ, et al. Sickle cell disease in Saudi Arabia: The phenotype in adults with the Arab-Indian haplotype is not benign. Br J Haematol. 2014;164(4):597–604.
22. Famodu AA. Coagulation changes in homozygous sickle cell disease in Nigeria. J Clin Pathol. 1987;40:1487.
23. Ajuwon MD, Olayemi E, Benneh AA. Plasma levels of some coagulation parameters in steady state HBSC disease patients. Pan Afr Med J. 2014;19:289.
24. Kurantsin-Mills J, Ofosu FA, Safa TK, Siegel RS, Lessin LS. Plasma factor VII and thrombin-antithrombin III levels indicate increased tissue factor activity in sickle cell patients. Br J Haematol. 1992; 81:539–44.
25. Buseri FI, Jeremiah ZA, Shokunbi WA. Plasma levels of some blood coagulation parameters in Nigerian homozygous sickle cell patients (HbSS) in steady state. Hematology. 2006;11:375–9.
26. Anazoeze M, Madu K, Anigbogu I, Ugwu AO, Okwulehie VA, Ololo U, Ugwu C, Chikezie K. Phenotypic characterisation and associations of leg ulcers in adult sickle cell patients. Wound Repair Regen. 2021;30(1):126–31.
Available:https://doi.org/10.1111/wrr.12970.
27. Laffan M, Manning R, Bain BJ, Bates I, Laffan MA, Lewis MS. Investigation of haemostasis. In: Dacie and Lewis Practical Haematology. 11th ed. Elsevier (*Churchill Livingstone*); 2011. p. 393–445.
28. Duru C, Pughikumo C, Adeyemi O. Acute myeloblastic leukaemia in a patient with Downs syndrome and sickle cell anaemia: A case report. Pediatr Ther. 2013;3:167.
Available:https://doi.org/10.4172/2161-0665.1000167.
29. Stricker RB, Linker CA, Crowley TJ, Embury SH. Hematologic malignancy in sickle cell disease: Report of four cases and review of the literature. Am J Hematol. 1986;21:223–30.
30. Ahmad F, Arbaeen. A clinico-hematological study of sickle cell disease among adult patients in Makkah, Saudi Arabia. Al-Azhar Int Med J. 2023.
DOI: 10.58675/2682-339x.1684.
31. Anwar A, Ali YZ, Al-Suliman AM, Albagshi JM, Al-Salamah M, Elsayid M, et al. The prevalence of abnormal leukocyte count, and its predisposing factors, in patients with sickle cell disease in

Saudi Arabia. J Blood Med. 2017;8:147-156.
DOI: 10.2147/JBM.S148463.

32. Alzahrani F, Fallatah AM, Al-Haddad FM, Khayyat ST, AlMehmadi WM, AlQahtani BG, et al. Risk Factors and Complications Among Pediatric Patients With Sickle Cell Anemia: A Single Tertiary Center Retrospective Study. Cureus. 2021;13(1): e12440.
DOI: 10.7759/CUREUS.12440.
33. Paulukonis S, Harris WT, Coates TD, Neumayr L, Treadwell M, Vichinsky E, et al. Population-based surveillance in sickle cell disease: methods, findings, and implications from the California Registry and Surveillance System in Hemoglobinopathies Project (RuSH). Pediatr Blood Cancer. 2014;61(2):227-231.
DOI: 10.1002/pbc.25208.
34. Omoto A, Mumbo D, Hussein SN, Apela M, Audi G. Analysis of provider-initiated sickle cell disease screening in Mathare slums, Nairobi, Kenya: a case of German Doctors, Baraka Health Centre. J Adv Med Med Res. 2023;35(4):253-?
DOI: 10.9734/jammr/2023/v35i245325.
35. Sostin O, Pai R, Dandwani M, Espana-Schmidt C. Sickle Cell Trait As a Risk Factor for Leukemia: A Nationwide Study. Blood; 2023.
DOI: 10.1182/blood-2023-178275.
36. Chennapragada SS, Thevuthasan S, Savani S, Goyal S, Singh V, Ramphul K, et al. Characteristics and trends of hospitalizations for sickle-cell related complications. Blood; 2023.
DOI: 10.1182/blood-2023-187511.
37. Radhika OH, Krishna N, Bapanpally NB, Fatima SS, Padmaja GV. Quantification of sickle cells in the peripheral smear as a marker of disease severity in sickle cell disease in pediatric patients. J Med Sci Health. 2023;9(1):21-22.
DOI: 10.46347/jmsh.v9i1.21.22.
38. Sackey D, Dei-Adomakoh Y, Olayemi E. Enhanced Hypercoagulability in Sickle Cell Anaemia Patients with Chronic Leg Ulcers. Adv Hematol. 2020;2020:5157031.
DOI: 10.1155/2020/5157031. PMID: 33299420; PMCID: PMC7704197.
39. Camille F, Sparkenbaugh EM, Pawlinski R. Hypercoagulable state in sickle cell disease. Clin Hemorheol Microcirc. 2018; 68(2-3):147-157.
DOI: 10.3233/CH-189013.
40. Gupta G, Dey Sarkar P, Manyal R, Singh Rajput M. Sickle cell disease: Assessment of hemostasis parameters in steady-state patients at Tertiary Care Hospital, Indore, India. Biomed Biotechnol Res J. 2022;6 (2):155-165.
DOI: 10.4103/bbrj.bbrj_162_21.
41. Catella J, Guillot N, Nader E, Skinner S, Poutrel S, Hot A, Connes P, Fromy B. Controversies in the pathophysiology of leg ulcers in sickle cell disease. Br J Haematol; 2024.
DOI: 10.1111/bjh.19584.
42. Santos EdC, Santana PV, Jesus LL, Melo GIV, Yahouédéhou SCM, Guarda CC, et al. Leg Ulcers in Sickle Cell Disease: A Multifactorial Analysis Highlights the Hemolytic Profile. Hematol Rep. 2023;15 (1):13.
DOI: 10.3390/hematolrep15010013.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125186