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OPTIMIZING HEMATOLOGICAL PROFILES AND REDUCING VASO-OCCLUSIVE CRISES: HYDROXYUREA THERAPY'S IMPACT ON COMPLETE BLOOD COUNT PARAMETERS IN SICKLE CELL DISEASE PATIENTS.

Sura Amarendar[1], Shalini Chandra*[2], Srinivasu Karedla[3], Ashok kumar jyothi[4], Deepa Gupta[5], Kalyani Amit Jagadale[6].

[1]Ph.D., Research scholar, Department of Pharmacology, Rohilkhand Medical College & Hospital, Bareilly International University, Bareilly, Uttar Pradesh, India, Orcid ID: 000-0001-6144-7445
Email ID: amarendarsura@gmail.com.
[2*]Professor & Head, Department of Pharmacology, Rohilkhand Medical College & Hospital, Bareilly International University, Bareilly, Uttar Pradesh, India, Orcid ID: 000-0002-1074-472X
Email ID: dr.shalini.chandra@gmail.com
[3]Department of Pharmacology, Rohilkhand Medical College & Hospital, Bareilly International University, Bareilly, Uttar Pradesh, India, Orcid ID: 000-0002-5394-8255
Email ID: karedlasrinivasu@gmail.com
[4]Assistant Professor, Department of Anatomy, Government Medical College, Khammam,Telangana. India, Email ID: jyothiashok.anatomist@gmail.com, Orcid ID:0000-0003-4453-9262.
[5]Assistant Professor, Department of Biochemistry, NAMO Medical Education And Research Institute, Silvassa, DNH (U.T), India. Email id: drdeepag003@gmail.com
[6]Tutor, Department of Biochemistry, NAMO Medical Education And Research Institute, Silvassa, DNH (U.T), India. Orcid ID: 0000-0003-4205-3234, Email id: kalyani21021995@gmail.com

***Corresponding author:** Shalini Chandra.
*[*]Professor & Head, Department of Pharmacology, Rohilkhand Medical College & Hospital, Bareilly International University, Bareilly, Uttar Pradesh, India, Orcid ID: 000-0002-1074-472X
Email ID: dr.shalini.chandra@gmail.com.

Abstract

Background: Hydroxyurea (HU) is a vital medication for sickle cell disease (SCD) since it has been shown to enhance hematological parameters and lessen the frequency of excruciating crises. In this study, the effects of HU on different complete blood counts (CBC) in individuals with sickle cell disease are examined. To evaluate the variations in CBC values between responders and non-responders to treatment among SCD patients receiving HU therapy.

Methods: A cross-sectional research with 255 SCD patients from Dadra & Nagar Haveli region was carried out. Based on their reaction to HU, participants were split into five groups 51 in each group: Group 1 responders (painful crises reduced by 50% or more), Group 1 non-responders (painful crises reduced by less than 50%), Group 2 responders (complete independence from transfusions), and Group 2 non-responders (no complete independence from transfusions). Patients with SCD who were not getting HU treatment made up the control group. ANOVA and t-tests were

used to evaluate and compare several CBC parameters, such as ferritin levels, hemoglobin, hematocrit, HbF percentages, platelet count, mean platelet volume (MPV), platelet percentage, and platelet distribution width (PDW).

Results: Study participants with sickle cell anemia using hydroxyurea had significantly different complete blood count and a higher risk of painful vaso-occlusive crises. Group 1 responders had lower ferritin levels than controls, but Group 2 responders and non-responders had greater levels. These differences were statistically significant. Group 2 respondents had the greatest levels of hemoglobin, hematocrit, and HbF percentages among the two groups of respondents. The mean platelet volume (MPV) and platelet counts of Group 1 and Group 2 responders were likewise greater than those of controls. In all groups, platelet distribution width (PDW) and platelet percentages were also much higher than in the control group. Moreover, after receiving hydroxyurea therapy, the incidence of vaso-occlusive painful crises was notably decreased in Group 1 responders by 50% or more, demonstrating the therapeutic advantage of hydroxyurea in treating these crises.

Conclusion: In SCD patients, hydroxyurea treatment has a major impact on several CBC markers. In comparison to non-responders and the control group, HU treatment responders have superior hematological profiles in terms of less painful crises and transfusion independence. These results underline the significance of CBC value monitoring to maximize treatment outcomes and support the effectiveness of HU in controlling SCD.

Keywords: Hydroxyurea, Sickle Cell Disease, Complete Blood Count, Hematological Parameters, Vaso-occlusive Painful Crises,

Introduction

The sickle cell, or "S," hemoglobin is a defective hemoglobin that is present in various concentrations in the erythrocytes of patients with sickle cell disease (SCD) (1). The cause of sickle cell anemia (SCA) is the replacement of adenine with thymine in the glutamic DNA codon (GAG→GTG), which leads to the replacement of glutamic acid with β6 valine. Many persons with SCA reach a steady state level of fitness and are generally in satisfactory health. African, Arabian, and Indian populations are afflicted with Sickle Cell Disease (SCD), an inherited disease (2). The most prevalent of the five major haplotypes and the one linked to elevated baseline HbF levels in Indian individuals suffering from sickle cell anemia is the Arab-Indian haplotype (3,4). All Indian ethnic groups share SCD, with tribal populations reportedly having a higher frequency than other ethnic groups (5). Odisha has been found to have the greatest prevalence of the sickle cell gene in India, followed by Assam, MP, UP, TN, and Gujarat. In India, the average frequency of SCD is 4.3%, however in Odisha, it is 9.1%. Children under the age of fifteen had a 16.55% prevalence of SCD, with a wide range of clinical manifestations (5).

The vaso-occlusive crisis (VOC), which is most prevalent and characteristic of SCD patients, is one of the recurrent crises that disrupt this state of relative well-being (6). Being subjected to familiar with the patient's stable state substantially facilitates the importance of early detection and subsequent clinical and hematological assessment of the disease. When an infection, acute complicating factors, acute clinical symptoms, or a crisis has not occurred for at least three months, a patient with SCA is said to be in a steady state (7). The term "crisis" describes periods of acute sickness related to the sickling phenomenon when there is an abrupt deterioration or a rapid worsening of symptoms and indicators in SCA patients who had previously been in stable health (8). When a crisis occurs, the illness renders the patient helpless, upsetting both parents and medical professionals.

The hematological factors in steady state and crisis have often been combined in earlier research. If there are any differences in the hematological factors between the steady state period and VOC, this

investigation will find them. Moreover, a large number of the established values were decided upon more than ten years ago. Since then, there have been numerous advancements in technology, social, economic, and medicinal fields. It has been demonstrated that treating sickle cell anemia patients with hydroxyurea (HU) reduces excruciating crises and associated consequences (9). However, it has been difficult to anticipate how each patient would react to HU because there is no obvious correlation between HU response and certain blood values (10). HU has been observed to elevate the average corpuscular volume (MCV) of red blood cells and the levels of fetal hemoglobin (HbF) nonetheless (10). Moreover, elevated glutathione levels have been linked to HU therapy, indicating a possible antioxidant impact (11). Additionally, it has been demonstrated that HU considerably lowers the frequency of blood transfusions and hospitalizations while raising hematocrit and lowering platelet and white blood cell counts (12).

The most frequent reason for problems in sickle cell disease is the painful vaso-occlusive crisis (13,14). Patients with sickle cell disease attend the emergency department between 79% and 91% of the time, and they stay in the hospital between 59% and 68% of the time, with an average stay of 8 to 11 days (15). Individuals with elevated hemoglobin levels and reticulocyte counts are more likely to experience painful episodes (14).

Adults who have experienced painful episodes have shown changes in their complete blood count (CBC) levels. Because sickling red blood cells cause hemolysis, most patients have a reduction in total hemoglobin levels throughout the painful episode. Certain individuals may have elevated reticulocyte counts as a result of their bodies' reaction to the sickling process. When infection is prevalent, leukocyte counts rise, with a notable increase in band neutrophils. It is assumed that the rise in leukocyte counts in the absence of infection is a result of an inflammatory response to bone marrow. Throughout the excruciating event, platelet levels may or may not fluctuate (16,17).

Ballas and Smith (1992) reported in their studies that the painful episode is a process whose features develop in a systematic two-phase development. An excruciating crisis usually has two stages. The initial phase is characterized by maximal discomfort, decreased hemoglobin levels, and increased reticulocyte counts. The painful episode's second phase is marked by a progressive reduction in pain and a gradual recovery of hemoglobin and reticulocyte counts to their pre-crisis levels (18). Additionally, a prior study found that painful episodes, independent of infection, are associated with a modest but substantial rise in leukocyte counts (19).

The previously described CBC value alterations were seen in adult longitudinal investigations (18–20), which contrasted rheological changes during painful episodes with steady states. The resolution of the hemolytic and inflammatory processes that precede an acute painful episode is frequently gauged by CBC levels (18). Nevertheless, no research has looked at CBC value variations and how they relate to acute painful episodes and their level of pain. As a component of a bigger research project that looked at hospitalized children's pain perception and pain control during excruciating vaso-occlusive episodes (21,22).

Numerous studies have demonstrated HU's therapeutic effects in SCA. Despite the fact that SCA is rather common in this area, no significant amount of literature was found assessing the advantages of HU in this area. Hence this conducted this investigation to assess the hematological and clinical response of HU in individuals with SCA.

Materials and methods

Study population:

This prospective research was conducted at the Shri Vinoba Bhave Civil Hospital in Silvassa, Dadra & Nagar Haveli (DNH), in collaboration with the NAMO Medical Education & Research Institute and the Sickle Cell Anemia Project. 300 people with sickle cell disease who visit the sickle cell outpatient department at SVBCH Silvassa and are permanent residents of DNH.

Screening of sickle cell anemia

First, sickle cell anemia was screened for using the sickling slide test. Agarose gel Hb electrophoresis in an alkaline medium (pH 8.6) was performed on those who tested positive. Using established methods, the Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) was utilized to validate the sickle cell disease [codon 6 (GAG>GTG) mutation] (23,24). High-Performance Liquid Chromatography was used to confirm sickle cell disease (HPLC).

Grouping:

Control group: Sickle cell anemia cases Hydroxyurea therapy is not taken, matched for age, were selected, who is having more than three Vaso-occlusive crises per year and they were followed every 3 months for 1 year.

Group I: Sickle cell anemia patients treated with hydroxyurea:

The primary indicator was the decrease in the incidence of painful crises (25).

i) Responders (painful crises): Patients with sickle cell anemia who received HU treatment had a 50% or greater reduction in painful crises;

ii) Non-responders (painful crises): Sickle cell anemia patients who did not have a 50% or greater decrease in painful crises with HU treatment.

Group II: Sickle cell anemia patients treated with Hydroxyurea.

The reduction in blood transfusion is serving as the second indication. Responders were included are patients who achieved total independence from transfusions.

i) Responders (blood transfusion requirement): sickle cell anemia patients treated with HU treatment who were entirely independent of transfusions;

i) Non-responders (blood transfusion requirement): Patients with sickle cell anemia who did not achieve full independence from transfusions after HU treatment.

Study design

This prospective open-label observational study was carried out to assess the effusiveness of hydroxyurea as measured by clinical and laboratory response in patients with 255 SCD patients from Dadra & Nagar Haveli region was carried out. Based on their reaction to HU, participants were split into five groups 51 in each group, to evaluate the impact of hydroxyurea.

Inclusion Criteria

Patients diagnosed with sickle cell anemia (SCA) were classified as experiencing vaso-occlusive crises (VOC) based on established clinical criteria, including bone and joint pain or pain in multiple locations, the need for analgesic medication, and self-perception of the episode as typical of a crisis requiring hospitalization.

Exclusion criteria

Patients with the following criteria were excluded from the study: Any patient suffering from conditions like kidney disease that might impact their hematological levels. Pregnancy, Children under the age of 3 years and adults over 60 years of age; Pregnancy, Concomitant diseases, such as neutropenia and thrombocytopenia may have increased the hydroxyurea's toxicity; The concurrent use of another "anti-sickling" substance; Patients who refuse to come in for routine check-ups or who could not ready for follow-ups frequently due to the distance they would have to travel from home to the hospital; Patients who refused to consent to be part of the study.

Ethics

The study was approved by the Institutional Ethical Committee of NAMO Medical Education & Research Institute, Dadra & Nagar Haveli, prior to its commencement. Additionally, each participant provided their informed written consent.(DMHS/IEC/2016/214/2521).

Sample Size:
All populations of DNH were screened by field survey under the Sickle Cell Anemia Control Programme. The sample size is calculated in consultation with the guide and institute statistician. For the assessment of the safety and efficacy of HU, the sample size is calculated based on the following formulae as described earlier (26).

Sample collection
5ml of venous blood was collected by clean venepuncture from each patient via the antecubital vein using a plastic syringe with minimum stasis, into commercially prepared concentrations of sequestered Ethylene Di-amine Tetracetic Acid (EDTA) bottles. Each sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation. An aliquot was used to determine complete blood counts (CBC) within 2 hours of collection.

Methodology
The complete blood counts (CBC) were analyzed using the automated Analyzer. The CBC includes hemoglobin, hematocrit, total white blood cell count and differential, platelet count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and red cell distribution width.

Data analysis
Data was analyzed by SPSS version 3.0. An initial frequency count of all variables was done. The means ranges and standard deviations (SD) of the hematological values were calculated. One-way ANOVA was used to find the variation among the groups. Student t-test was employed to estimate the difference between groups. P-value < 0.05 was considered Statistically significant.

Results:

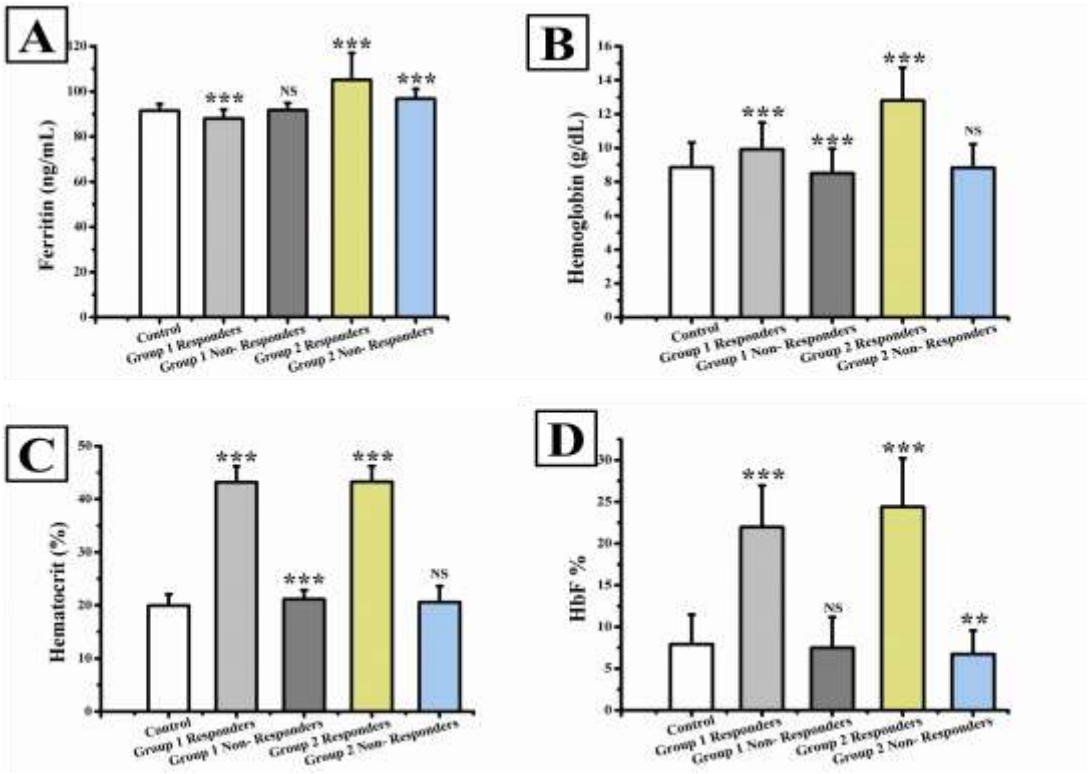

Figure 1: Haematological parameters in different groups of sickle cell anemia patients A] Ferritin levels, B] Hemoglobin, C] Hematocrit D] HbF%. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Ferritin levels:

A significant variation in ferritin levels was found across the groups of sickle cell anemia patients according to the ANOVA test findings ($F(4, 256) = 4.96$, $p = 0.00001$). Ferritin levels in group 1 responders (sickle cell anemia patients who saw a 50% or more decrease in excruciating crises following hydroxyurea (HU) treatment) were significantly lower than in the control group (sickle cell anemia patients who did not receive HU therapy) [($t(50) = 4.96$, $p = 0.00001$)] and the other groups. Ferritin levels were raised in group 1 non-responders (individuals who did not have a 50% or larger decrease in excruciating crises following HU therapy); however, this elevation was not statistically significant [($t(50) = 0.45$, $p = 1/32$)]. The ferritin levels in group 2 non-responders ($t(50) = 7.39$, $p = 0.00001$) and group 2 responders (patients who did not achieve full independence from transfusions after HU treatment) were significantly higher than those in the control group [($t(50) = 7.98$, $p = 0.00001$) and ($t(50) = 7.39$, $p = 0.00001$), respectively]. Additionally, group 2 responders had greater ferritin levels than group 2 non-responders [($t(50) = 4.85$, $p = 0.00001$)] (Figure 1A).

Hemoglobin:

A substantial variation in hemoglobin levels was found across the different groups of sickle cell anemia patients according to the findings of the ANOVA test ($F(4, 256) = 147.14$, $p < 0.00001$). When compared to the control group, which consisted of sickle cell anemia patients not receiving HU therapy, hemoglobin levels were significantly higher in Group 1 responders ($t(50) = 5.949$, $p < 0.00001$) and Group 2 responders ($t(50) = 21.26$, $p < 0.00001$), as well as other groups. Group 1 responders experienced a 50% or greater reduction in painful crises following HU treatment, and Group 2 responders became completely independent of transfusions following HU treatment. Among the groups, Group 2 responders had significantly higher hemoglobin levels than the other groups [$t(50) = 21.26$, $p < 0.00001$]. On the other hand, patients in Group 2 non-responders (those who did not become fully independent from transfusions following HU treatment) had hemoglobin levels that were comparable to those in the control group [$t(50) = 0.06$, $p = 0.473$] (Figure 1B).

Hematocrit:

Based on the ANOVA test findings, there was a significant difference ($F(4, 256) = 1148.510$, $p < 0.00001$) in the hematocrit levels between the various groups of sickle cell anemia patients. The hematocrit values of patients in Group 1 (those who experienced a 50% or greater reduction in painful crises following hydroxyurea treatment) and Group 2 (those who became completely independent of transfusions following HU treatment) were significantly higher than those of the control group (those who did not receive HU therapy) [$t(50) = 45.08$, $p < 0.00001$ and $t(50) = 45.74$, $p < 0.00001$, respectively], as well as in comparison to the other groups. Furthermore, patients in Group 1 non-responders (those who did not have a 50% decrease in excruciating crises after receiving HU therapy) showed noticeably higher hematocrit levels than those in the control group [$t(50) = 3.106$, $p = 0.001233$]. On the other hand, there was no discernible change in Group 2 non-responders (individuals who did not obtain complete independence from transfusions after receiving HU therapy) [$t(50) = 0.93419$, $p = 0.176184$] (Figure 1C).

The HbF percentages of the various groups of sickle cell anemia patients varied significantly, according to the findings of the ANOVA test ($F(4, 256) = 218.97$, $p < 0.00001$). In comparison to the control group, the HbF percentage was significantly higher in Group 1 responders and Group 2 responders [$t(50) = 16.37$, $p < 0.00001$ and $t(50) = 17.57$, $p < 0.00001$, respectively]. However, in Group 2 non-responders, it was significantly lower than in the control group [$t(50) = 2.44$, $p < 0.008193$)], and in Group 1 non-responders, it was similar to the control group [$t(50) = 0.58$, $p < 0.281422$)] (Figure 1D).

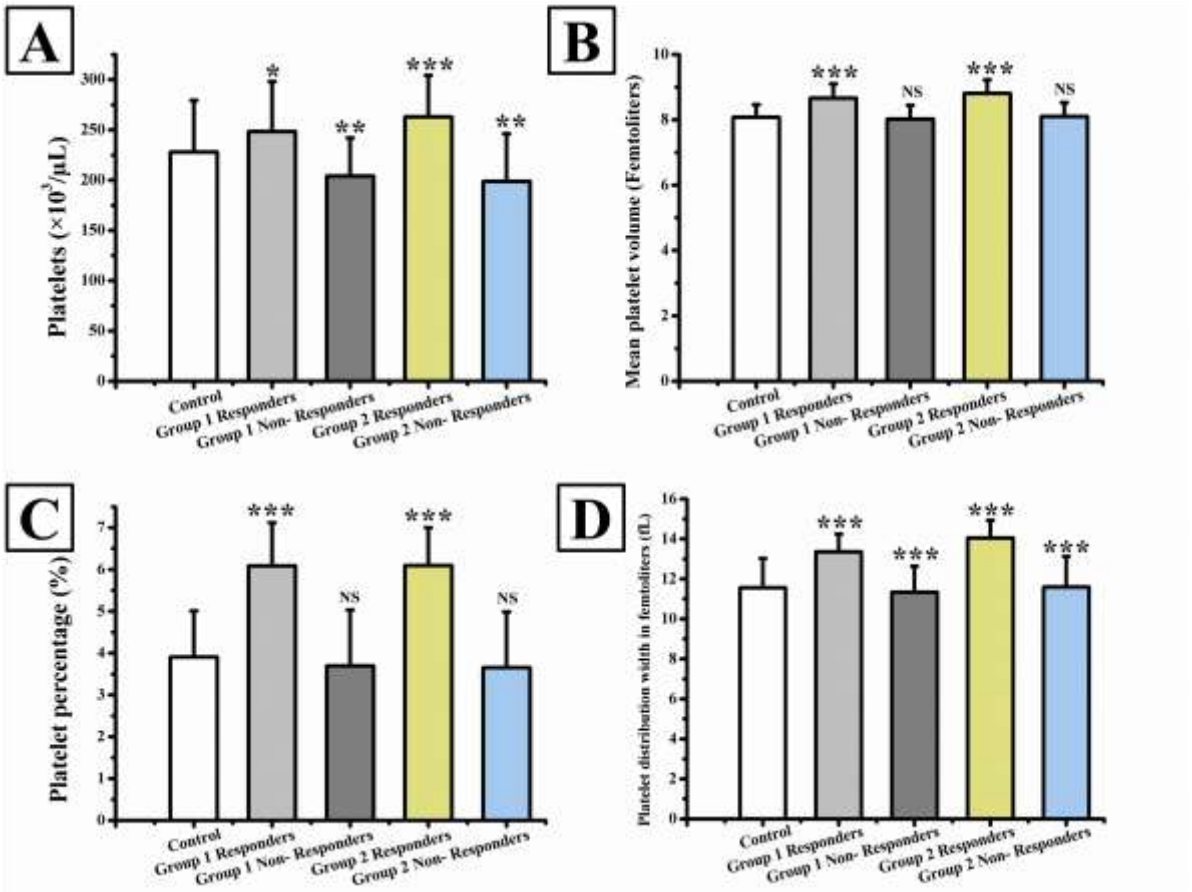

Figure 2: Haematological parameters in different groups of sickle cell anemia patients, indices of platelet A] Platelet count, B] Mean platelet volume (MPV), C] Platelet percentage D] Platelet Distribution Width (PDW). Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Platelet count:

The findings of the ANOVA test revealed a significant variation in platelet count between different cohorts of sickle cell anemia patients ($F(4, 256) = 18.34$, $p < 0.00001$). In comparison to the control group, the platelet count increased significantly in both Group 1 and Group 2 responders [$t(50) = 2.04$, $p < 0.021$ and $t(50) = 3.98$, $p < 0.000064$, respectively], as well as in both Group 1 and Group 2 non-responders [$t(50) = 2.67$, $p < 0.00441$ and $t(50) = 2.60$, $p < 0.005273$, respectively] (Figure 2A).

Mean platelet volume (MPV):

The findings of the ANOVA test showed that there was a significant difference in mean platelet volume (MPV) between the different groups of patients with sickle cell anemia ($F(4, 256) = 42.15$, $p < 0.00001$). When compared to the control group, MPV showed a significant increase in Group 1 responders and Group 2 responders [$t(50) = 7.06$, $p < 0.00001$ and $t(50) = 9.34$, $p < 0.00001$, respectively]. In Groups 1 and 2, the difference was not considered statistically significant among non-responders [$t(50) = 0.73$, $p < 0.232937$ and $t(50) = 0.24$, $p < 0.402303$, respectively] (Figure 2B).

Platelet percentage:

According to the findings of the ANOVA test, there was a significant difference in the platelet percentage between the different groups of patients with sickle cell anemia ($F(4, 256) = 64.73$, $p < 0.00001$). In contrast to the control group, the platelet percentage was considerably greater in Group 1 responders and Group 2 responders [$t(50) = 10.33$, $p < 0.00001$ and $t(50) = 11.11$, $p < 0.00001$,

respectively]. Nevertheless, among non-responders of both Groups 1 and 2, the variations in platelet % were not statistically significant [t(50) = 0.85622, p = 0.19 and t(50) = 0.83606, p = 0.20, respectively] (Figure 2C).

Platelet Distribution Width (PDW):

A significant difference in Platelet Distribution Width (PDW) was found between the groups of sickle cell anemia patients according to the findings of the ANOVA test (F(4, 256) = 51.58, p < 0.00001). PDW was significantly higher in non-responders of both Group 1 and Group 2 [t(50) = 23.69, p < 0.00441 and t(50) = 22.85, p < 0.00001, respectively], as well as in responders from Groups 1 and 2 compared to the control group [t(50) = 7.47, p < 0.021 and t(50) = 45.55, p < 0.000064, respectively] (Figure 2D).

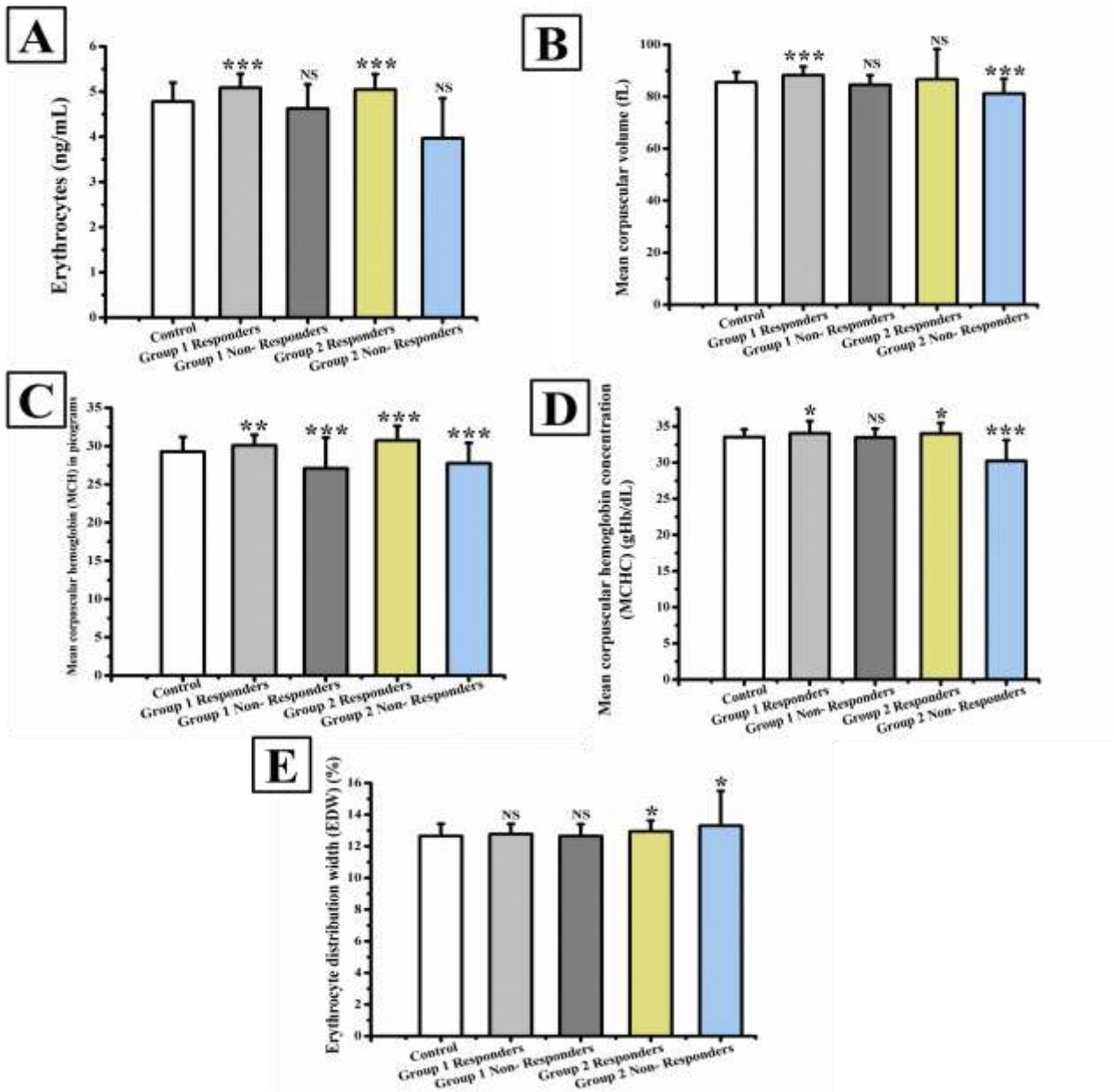

Figure 3: Haematological parameters in different groups of sickle cell anemia patients, indices of Erythrocyte A] Erythrocyte count, B] Mean corpuscular volumes (MCV). C] Mean corpuscular hemoglobin (MCH), D] Mean corpuscular hemoglobin concentration (MCHC), E] Erythrocyte distribution width (EDW). Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Erythrocyte count:
Significant differences in erythrocyte count were found across the various groups of sickle cell anemia patients according to the findings of the ANOVA test ($F(4, 256) = 1.49$, $p = 0.204269$). However, in contrast to the control group, the erythrocyte count in Group 1 responders and Group 2 respondents was substantially greater [$t(50) = 4.22$, $p = 0.000026$ and $t(50) = 3.71$, $p = 0.000164$, respectively]. On the other hand, neither Group 1 nor Group 2's non-responders' erythrocyte count showed any discernible variations [$t(50) = 1.64$, $p = 0.051206$ and $t(50) = 0.67546$, $p = 0.250442$, respectively] (Figure 3A).

Mean corpuscular volumes (MCV):
Subjects with sickle cell anemia showed significantly different mean corpuscular volumes (MCV) among groups ($F(4, 256) = 10.09$, $p = 0.00001$), according to the findings of the ANOVA test. Group 1 responders and Group 2 non-responders had MCVs that were considerably higher than those of the control group [$t(50) = 3.71$, $p = 0.000169$ and $t(50) = 4.97$, $p = 0.00001$, respectively]. With regard to Group 2 responders and Group 1 non-responders, however, no discernible difference was seen [$t(50) = 0.65$, $p = 0.257975$ and $t(50) = 1.3373$, $p = 0.09208$, respectively] (Figure 3B).

Mean corpuscular hemoglobin (MCH):
Mean corpuscular hemoglobin (MCH) varied significantly across groups of sickle cell anemia patients, according to the findings of the ANOVA test ($F(4, 256) = 19.96$, $p < 0.00001$). Respondents in Groups 1 and 2 had significantly higher MCH than the control group [$t(50) = 2.50$, $p < 0.006893$ and $t(50) = 4.12$, $p < 0.000037$, respectively]. On the other hand, $t(50) = 3.46$, $p < 0.000388$, and $t(50) = 3.40$, $p < 0.00001$, respectively, for non-responders in Groups 1 and 2 in comparison to the control group showed notable reductions in MCH (Figure 3C).

Mean corpuscular hemoglobin concentration (MCHC):
The mean corpuscular hemoglobin concentration (MCHC) of sickle cell anemia patients varied significantly between groups, according to the findings of the ANOVA test ($F(4, 256) = 10.09$, $p < 0.00001$). When compared to the control group, the corpuscular hemoglobin concentration (MCHC) was significantly higher in Group 1 responders and Group 2 responders [$t(50) = 1.87$, $p < 0.03213$ and $t(50) = 7.52$, $p < 0.00001$, respectively], but it was significantly lower in Group 2 non-responders [$t(50) = 2.03$, $p < 0.022032$)], and it was similar in Group 1 non-responders [$t(50) = 0.21$, $p < 0.415748$)] (Figure 3D).

Erythrocyte distribution width (EDW):
Based on the findings of the ANOVA test, there was a significant difference between the groups of sickle cell anemia patients in terms of erythrocyte distribution width (EDW) ($F(4, 256) = 2.55$, $p < 0.039467$). In Group 2's responders and non-responders, erythrocyte distribution width (EDW) was significantly higher than in the control group ($t(50) = 2.00$, $p < 0.02376$ and $t(50) = 1.91$, $p < 0.02882$, respectively); in Group 1's responders and non-responders, it was not statistically significant ($t(50) = 0.85$, $p < 0.198168$) ($t(50) = 0$, $p < 0.5$) respectively) (Figure 3E).

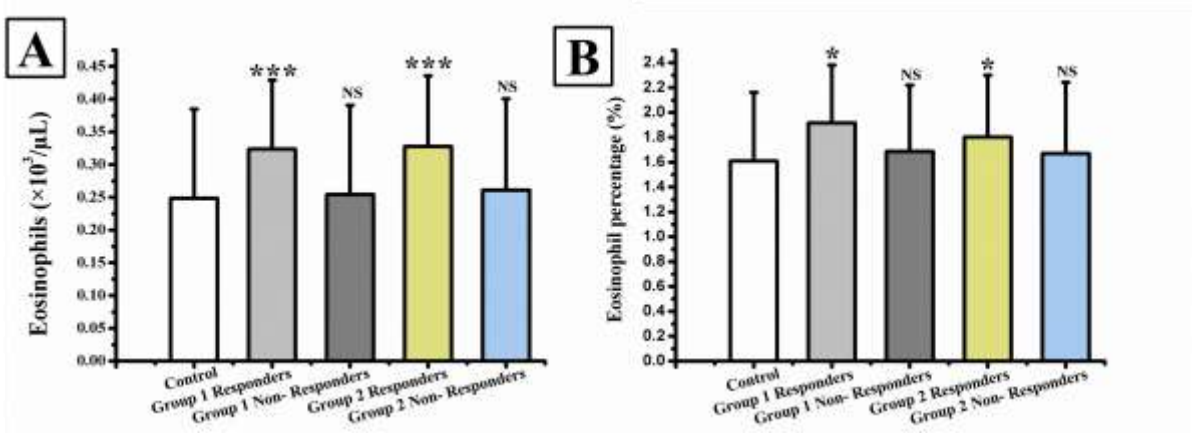

Figure 4: Haematological parameters in different groups of sickle cell anemia patients, indices of Eosinophil A] Eosinophil count, B] Eosinophil percentage. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Eosinophil count:

Significant differences in Eosinophil counts were found between the groups of sickle cell anemia patients according to the findings of the ANOVA test ($F(4, 256) = 5.11$, $p = 0.000558$). But when compared to the control group, the Eosinophil count was significantly higher in Group 1 responders and Group 2 responders [$t(50) = 3.12$, $p = 0.001174$ and $t(50) = 3.17$, $p = 0.000988$, respectively], but it did not differ significantly in Group 1 and Group 2 non-responders [$t(50) = 0.21$, $p = 0.413961$ and $t(50) = 0.2361$, $p = 0.406911$, respectively] (Figure 4A).

Eosinophil percentage:

A significant variation in the proportion of Eosinophils was found across several groups of sickle cell anemia patients, according to the findings of the ANOVA test ($F(4, 256) = 2.74$, $p = 0.02918$). In contrast, the Eosinophil percentage was not statistically significant in the non-responders of either Group 1 or Group 2, but it was significantly elevated in the responders of Groups 1 and 2 when compared to the control group [$t(50) = 3.02$, $p = 0.001597$), $t(50) = 1.85$, $p = 0.033218$, respectively] (Figure 4B).

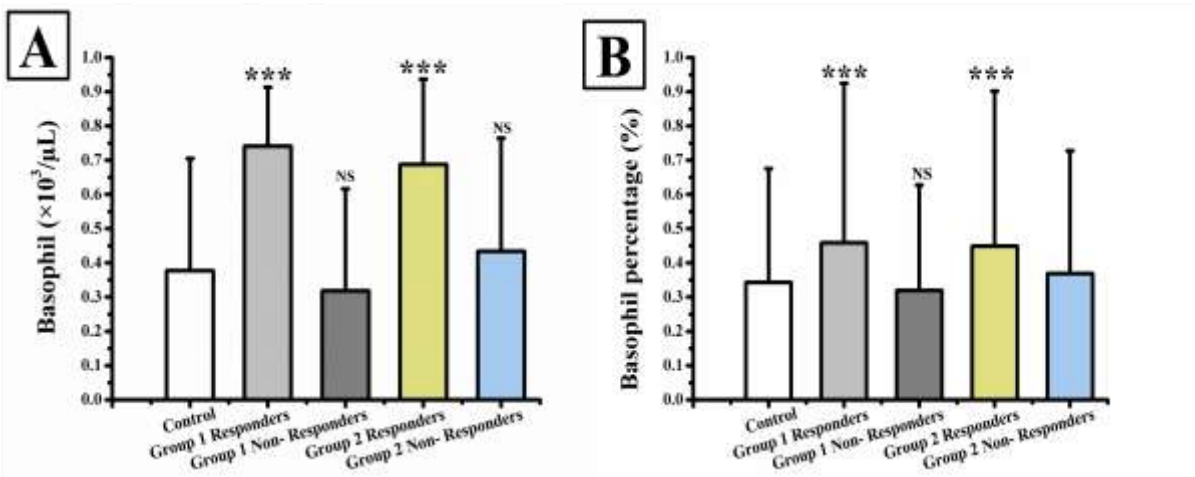

Figure 5: Haematological parameters in different groups of sickle cell anemia patients, indices of Basophil A] Basophil count, B] Basophil percentage. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Basophil count:

Significant differences in basophil count were found between the groups of sickle cell anemia patients according to the findings of the ANOVA test (F(4, 256) = 24.37, p = 0.00001). But in contrast to the control group, the basophil count was significantly higher in Group 1 responders and Group 2 responders [t(50) = 7.02, p = 0.00001) and t(50) = 5.49, p = 0.00001, respectively], but it was not significantly higher in Group 1 and Group 2 non-responders [t(50) = 0.9454, p = 0.173368 and t(50) = 0.53, p = 0. 296412, respectively] (Figure 5A).

Basophil percentage:

Significant differences in basophil percentage were found between the several groups of sickle cell anemia patients according to the findings of the ANOVA test (F(4, 256) = 5.35, p = 0. 000373). While it was not significant in Group 1 and Group 2 non-responders [t(50) = 0.65, p = 0. 25809 and t(50) = 0.82, p = 0. 205118, respectively], the basophil percentage was significantly higher in Group 1 responders and Group 2 responders compared to the control group [t(50) = 3.10, p = 0.001253) and t(50) = 2.84, p = 0. 002693, respectively] (Figure 5A).

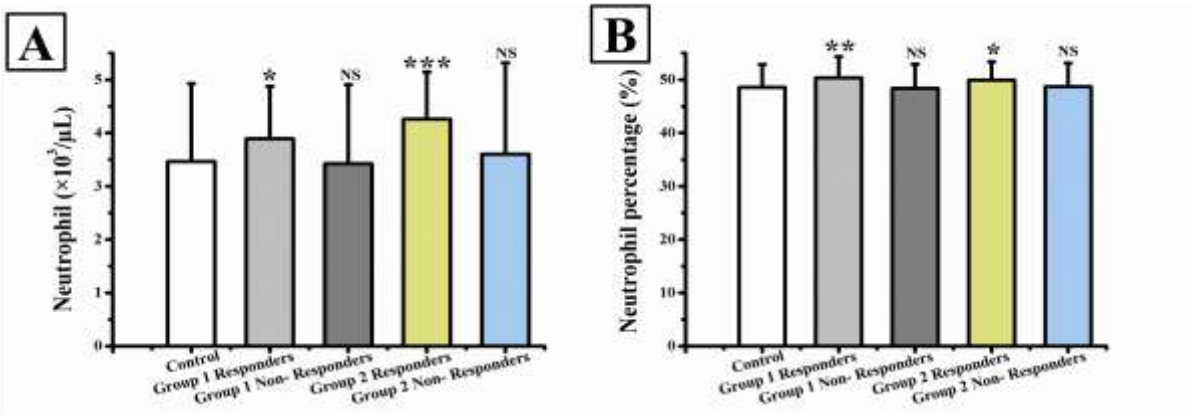

Figure 6: Haematological parameters in different groups of sickle cell anemia patients, indices of Neutrophil A] Neutrophil count, B] Neutrophil percentage. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Neutrophil count:

Significant differences in neutrophil counts were found between the groups of sickle cell anemia patients according to the findings of the ANOVA test (F(4, 256) = 3.59, p = 0.007206). Nonetheless, in comparison to the control group, the neutrophil count was significantly higher in Group 1 responders and Group 2 responders [t(50) = 1.74, p = 0.041752) and t(50) = 3.41, p = 0.000453, respectively], but not significantly higher in Group 1 and Group 2 non-responders [t(50) = 0.12781, p = 0.449278 and t(50) = 0.6186, p = 0.268766, respectively] (Figure 6A).

Neutrophil percentage:

The findings of the ANOVA test showed a significant difference in the proportion of neutrophils between the various patient groups with sickle cell anemia (F(4, 256) = 2.19, p = 0.015072). But in contrast to the control group, the Neutrophil percentage was significantly higher in Group 1 responders and Group 2 responders [t(50) = 2.19, p = 0.015072) and t(50) = 1.81, p = 0.03603, respectively], but it was not significantly higher in Group 1 and Group 2 non-responders [t(50) = 0.20, p = 0.42 and t(50) = 0.06, p = 0.475771, respectively] (Figure 6A).

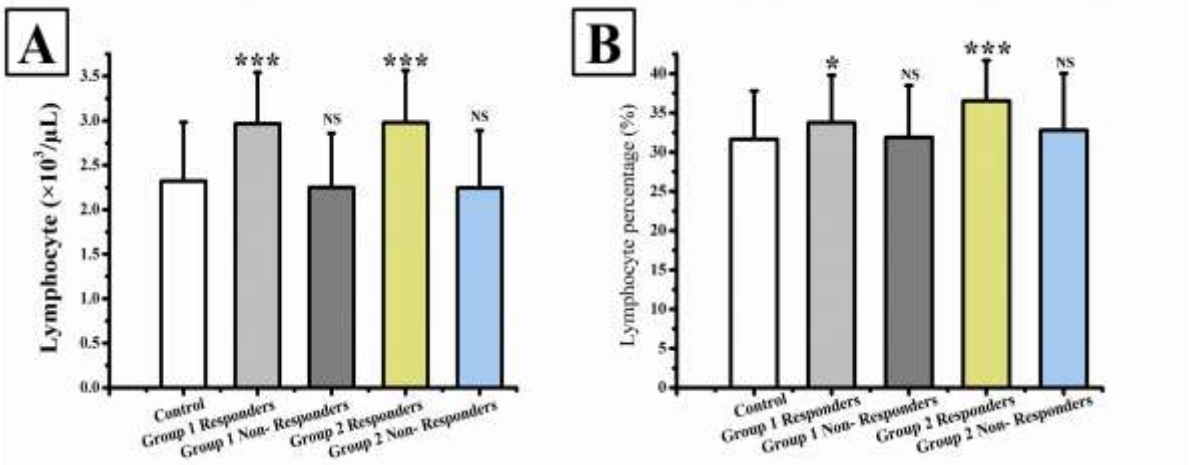

Figure 7: Haematological parameters in different groups of sickle cell anemia patients, indices of Lymphocyte A] Lymphocyte count, B] Lymphocyte percentage. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Lymphocyte count:

According to the ANOVA test findings, there was a significant difference in the lymphocyte count across the various patient groups with sickle cell anemia (F(4, 256) = 20.04, p = 0.0000). Even so, the lymphocyte count was not statistically significant in the non-responders of either Group 1 or Group 2, but it was significantly elevated in responders of Group 1 and Group 2 compared to the control group [t(50) = 5.24, p = 0.00001) and t(50) = 5.32, p = 0.00001, respectively] (Figure 7A).

Lymphocyte percentage:

ANOVA test findings showed a significant difference in lymphocyte percentage across the several patient groups with sickle cell anemia (F(4, 256) = 5.18, p = 0.000494). Lymphocyte percentage, however, was not significant in non-responders of either Group 1 or Group 2, but it was significantly higher in responders of Group 1 and Group 2 compared to the control group [t(50) = 1.76, p = 0.04031) and t(50) = 4.32, p = 0.000018, respectively] (Figure 7A).

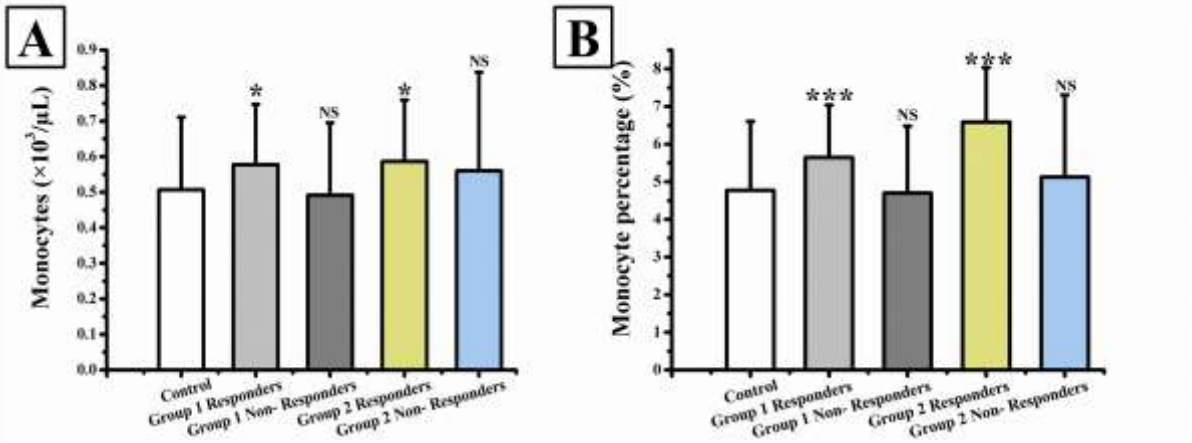

Figure 8: Haematological parameters in different groups of sickle cell anemia patients, indices of Monocyte A] Monocyte count, B] Monocyte percentage. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Monocyte count:

According to the findings of the ANOVA test, there was no discernible difference in the monocyte count across the various patient groups with sickle cell anemia (F(4, 256) = 2.15, p = 0.074938).

Nonetheless, in comparison to the control group, the monocyte count was significantly higher in Group 1 responders and Group 2 responders [t(50) = 1.90, p = 0.029538) and t(50) = 2.11, p = 0.018601, respectively], but not significantly higher in Group 1 and Group 2 non-responders [t(50) = 0.3691, p = 0.356418 and t(50) = 1.18, p = 0.119586, respectively] (Figure 8A).

Monocyte percentage:

According to the ANOVA test findings, there was a significant difference in the proportion of monocytes between the various groups of sickle cell anemia patients (F(4, 256) = 11.37, p = 0.00001). In contrast, the monocyte percentage was not significantly different in non-responders of either Group 1 or Group 2, but it was significantly higher in responders of Group 1 and Group 2 compared to the control group [t(50) = 2.70, p = 0. 003967) and t(50) = 5.81, p = 0.00001, respectively] (Figure 8B).

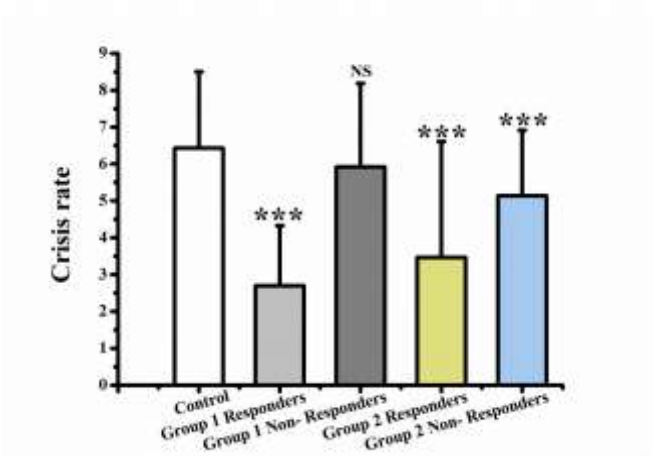

Figure 9: Vaso-occlusive pain crisis in different groups of sickle cell anemia patients. Data represent mean ± S.D. The superscripted stars (*) indicate statistical significance. ***p<0.001, **p<0.01, *p<0.05, NS- Non-significant.

Vaso-occlusive pain crisis:

There was a significant difference in the vaso-occlusive painful crisis rate between the groups of sickle cell anemia patients, according to the findings of the ANOVA test (F(4, 256) = 27.006, p = 0.00001). Yet, when compared to the control group, the rate of vaso-occlusive painful crises was significantly lower in Group 1 responders and Group 2 responders [t(50) = 10.11, p = 0.00001) and t(50) = 5.80, p = 0.00001, respectively]. However, the rate of crises was also lower in non-responders of both Groups 1 and 2, but it was not statistically significant in non-responders of Group 1 [t(50) = 1.18, p = 0.118975 and t(50) = 3.37, p = 0.000515, respectively]. (Figure 9).

Discussion:

In an effort to bring back a smile to SCD patients, HU has explored novel possibilities for the treatment of the disease (27). Based on clinical trials and medicinal research, HU is the only medication that effectively lowers the frequency of painful episodes. The levels of HbF and Hb are elevated by it. HU reduces the frequency of (acute chest syndrome) and unpleasant episodes by 50%. blood transfusions and Acute chest syndrome (ACS) episodes by about 50% (28). For children over the age of five, HU is safe and well-tolerated; there is no evidence of clinical or test harm. Myelosuppression is the only main toxicity, and it may be reversed when the medication is stopped (28).

The results of our investigation into ferritin levels in various sickle cell anemia patient populations are consistent with other studies. Chand, 2014 discovered that patients' inability to demonstrate a sufficient rise in HbF levels with hydroxyurea therapy was largely due to non-compliance, not genuine non-response. (29). This implies that treatment adherence may have an impact on the

variations in ferritin levels between study participants who responded and those who did not. Our results of greater ferritin levels in sickle cell anemia patients are similar to the observations made by Lin, 2011 (30) and Blrgegård (2009) (31) with regards to raised ferritin levels in patients with certain diseases. The effectiveness of ferric citrate in treating iron deficiency anemia was proven by Fishbane (2017) (32), which may be relevant to our investigation considering the possible influence of iron levels on ferritin.

Bispo (2013) discovered that individuals with sickle cell anemia who received hydroxyurea therapy had much higher hematocrit levels, which decreased the frequency of vaso-occlusive events (33). This is in line with the notable increase in hematocrit levels that Al-Khalidi's study (2022) found in responders to hydroxyurea therapy (34). Valafar (2000) provided more evidence in favor of hydroxyurea's ability to raise hematocrit levels and the percentage of fetal hemoglobin (10). The reaction to hydroxyurea can be influenced by the amount of fetal hemoglobin, as highlighted by Croizat (1999) which raises the possibility that this contributes to the observed variations in hematocrit values between sickle cell anemia patient groups (35).

Since its inception, HU treatment has improved development, preserved splenic function, and decreased the incidence of ACS, painful crises, and dactylitis (28). Leucocyte, PMN, reticulocyte, and dense sickle cell mean levels significantly decreased, whereas hemoglobin, PCV, MCV, HbF, F cells, and F reticulocytes significantly increased It has been discovered that HU can be used for sickle cell stroke instead of blood transfusions. HU is economical and raises life quality (36).

Respondents in group 2 showed a statistically significant decrease in the frequency of blood transfusions; HU had a clear beneficial effect on transfusion frequency; in contrast, the transfusion rate increased in the control group, which may indicate that older SCA patients require more blood transfusions; a related study found that the mean number of transfusions decreased in those subjects receiving HU therapy; a study from Belgium also revealed a similar outcome (37,38). In comparison to the control group, the number of VOCs per year was considerably lower in Group 1 responders and Group 2 responders; however, the reduction in crises was also shown in non-responders of both Group 1 and Group 2, but it was not statistically significant in non-responders of Group 1. Similar results were observed with a 50% decrease in VOC following HU treatment. In our study, there was a rise from two to six months as the median interval between two consecutive VOCs. Compared to the treatment group, the control group had an average yearly increase in VOC incidence. While there is little doubt that HU contributes to a lower annual incidence of VOC events, those who did not get HU may have had more VOC episodes (36,38).

Effect of HU on the frequency of excruciating crises: $P < 0.03.7$ indicated a decrease in the annual number of VOC in participants who received HU (36,39), which is similar to our study findings. In both group 1 and group 2 responders, red cell indices such as erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and erythrocyte distribution width (EDW) showed significant improvements following HU therapy. Some effect was even seen in the HU group that did not respond. According to published research, MCV rose by 14% following two years of HU treatment (40). Similarly, after two years, much additional research also indicates improvements in red cell indices (41). Statistical research revealed that following two years of HU treatment, the MCHC value rose (40–44). While some observational studies have demonstrated that HU is advantageous in raising MCV, MCH, and MCHC, our analysis did not find any discernible improvements in MCV, MCH, or MCHC (40–44).

Research by Ferster et al. (1996) and Kinney et al. (1999) showed that the highest increase in Hb was seen after 12 months of HU therapy, rising from 7.8 grams/dl to 9 grams/dl over the course of two years after HU administration (40,41). Research by Ferster et al. (1996) and Kinney et al. (1999) showed that the highest increase in Hb was seen after 12 months of HU therapy, rising from 7.8 grams/dl to 9 grams/dl over the course of two years after HU administration (40,41,43). These results are in line with our own, but we were unable to detect much of an impact of HU in any of the respondents.

The capacity of HU to raise fetal Hb concentration is the mechanism of action by which it enhances the general wellness of SCA patients. When the number of HU treatment responders increased, the hematological parameters improved noticeably. Even though they are in the cohort of nonresponders, all of the HU-treated patients had some positive clinical recovery in terms of less VOC and fewer transfusions than the control group. These individuals may have profited theoretically from persistent red cell hydration and vasodilatation brought on by the production of nitric oxide. Other research supports the nitric oxide pathway, however further proof with a larger sample size is needed to validate this theory.

Conclusion:

Hydroxyurea therapy significantly influences several complete blood count (CBC) parameters in patients with sickle cell disease (SCD), demonstrating marked improvements in hematological profiles among responders. Notably, Group 1 and Group 2 responders exhibited higher hemoglobin, hematocrit, HbF percentages, platelet counts, mean platelet volume (MPV), and other hematological parameters compared to non-responders and the control group. Additionally, responders to HU therapy showed significantly reduced ferritin levels and a substantial decrease in the incidence of vaso-occlusive painful crises. These findings underscore the therapeutic efficacy of HU in managing SCD, emphasizing the importance of monitoring CBC values to optimize treatment outcomes and improve patient quality of life.

Conflict of interest: None

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