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Bamgboye M. Afolabi, Ahmed Abubakar, Ramatu Aliyu Zubair, Gloria Yimi Bahago, Monica Stephen Shuaibu, Abdullahi Isah Yusuf, Usman Haruna Nakorji & Tolulope Fagbemi

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ABSTRACT

Introduction: Therapeutic management of sickle cell disease (SCD) has proven to be a major task to both patients and clinicians in Africa. Investigation of blood and serum parameters are essential tools for assessing efficacy of medical interventions and eventual outcome of the disease. There is paucity of studies on modern African medicinal treatment and resulting post-intervention hematological parameters of SCD in Nigeria. This study aimed at determining serum electrolytes and urea and some hematological parameters among SCD patients who were treated with a study agent compared to SCD patients who were not treated with the study agent but given normal hospital care. Objective: The objective of this study was to assess the differences in reticulocytes, thrombocytes, and serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and in control patients.

Keywords: creatinine, electrolytes, nigeria, reticulocytes, sickle cell disease, thrombocytes, urea.

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Serum Electrolytes, Creatinine and Urea Concentrations, Reticulocyte and Thrombocyte Counts in Pediatric and Adult Sickle Cell Disease Patients Placed on Immunozin™ Therapy in Northern Nigeria

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ABSTRACT

Introduction: Therapeutic management of sickle cell disease (SCD) has proven to be a major task to both patients and clinicians in Africa. Investigation of blood and serum parameters are essential tools for assessing efficacy of medical interventions and eventual outcome of the disease. There is paucity of studies on modern African medicinal treatment and resulting post-intervention hematological parameters of SCD in Nigeria. This study aimed at determining serum electrolytes and urea and some hematological parameters among SCD patients who were treated with a study agent compared to SCD patients who were not treated with the study agent but given normal hospital care. Objective: The objective of this study was to assess the differences in reticulocytes, thrombocytes, and serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and in control patients.

Materials and Method: This was a double-blind, two-arm, randomized control pilot study involving a total of 62 subjects, including 33 cases with SCD who were given the study agent and 29 controls with SCD who were not given the study agent. After preliminary evaluation, the study drug was administered at enrollment into the study on Day 1 and each study participant was re-evaluated at each monthly administration of the test drug for 6 consecutive visits conducted monthly. Study drug was administered one month after enrolment on each subsequent month for 5 months. Venous blood sample was collected and all other variables were investigated at each visit. A full blood count (hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cells (WBC), reticulocytes (RTC), platelets (PLT) counts were done within 2 hours of collection, and were recorded. Serum electrolytes and urea, liver enzymes were also investigated. NCSS statistical software was used for analysis.

Results: At the end of study, mean (±sd) reticulocyte count of pediatric cases (1.54 [1.00]) was significantly lower (t-test=4.19, P-value = 0.0002) than the enrolment value (2.44 [0.77]) and greater drop in reticulocyte count occurred among pediatric cases than among control subjects. A significant decrease (t-test=2.07, P-value=0.02) in the mean (±sd) thrombocyte count of adult controls at enrolment (515.0 [77.9]) compared to the value at the end of the study (432.3 [29.4]) was observed. The mean (±sd) creatinine blood level of pediatric cases at enrollment was significantly lower (t-test= -3.12, P-value=0.002) than that at end of study (49.5 [11.2]). Serum potassium levels were elevated in all cases and controls at the end of the study. Simple linear regression analysis showed that the estimated change in total thrombocyte count per unit change in reticulocyte count varied

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between pediatric and adult case and control subjects.

Conclusion: The significant reduction in mean reticulocyte count of pediatric SCD patients on test drug and the difference in the slope of the equation of straight line relating thrombocyte count and reticulocyte count may reflect the therapeutic effect of the test drug among pediatric patients. Clinicians should monitor serum potassium level when managing sickle cell disease patients for cardiac response to hyperkalemia. Further studies are needed to confirm these findings.

Keywords: creatinine, electrolytes, nigeria, reticulocytes, sickle cell disease, thrombocytes, urea.

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I. INTRODUCTION

Sickle Cell Disease (SCD) results from a point mutation where glutamic acid is replaced by valine at position 6 on the β globin. [1,2] The abnormal β^s chains combine with normal α chains to form the sickle hemoglobin (HbS), a less soluble complex compared to the fetal or adult hemoglobin. SCD is a condition consequent to the inheritance of abnormal allelomorphic genes controlling the formation of the beta (β) chains of hemoglobin (Hb). [3] About 5% of the world's population carry at least one of the two alleles responsible for sickle hemoglobinopathies. [4] The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Sahara -n Africa, India, and the Middle East. [5] Migration of substantial populations from these high - prevalence areas to low - prevalence countries in Europe has dramatically increased in recent decades and in some European countries, sickle cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis. [6] In 2015, it resulted in about 114,800 deaths. [7] Organizations such as the World Health Organization (WHO) and United Nations (UN) have recognized SCD as a global health issue. In 2006, the World Health Assembly passed a resolution recognizing SCD as a public health priority and called on countries to tackle the disease. This resolution was also adopted by the United Nations in 2009. [8] Nigeria has the highest burden of the disease in the world with over 150,000 children born every year with SCD. [9]

Clinical studies evaluating modern approaches of managing of sickle cell disease in the African populace are scarce. Although developed countries have access to novel and innovative therapies such as monoclonal antibodies and small molecule hemoglobin S polymerization inhibitors, crizanlizumab and voxelotor respectively, therapies available in developing African countries are stunted at the use of hydroxyurea (HU), an agent with "anti-sickling" effect by inducing fetal hemoglobin (HbF, $\alpha_2\gamma_2$). [10] The gap resulting from the lack of innovative therapies is filled by local complementary and herbal remedies available in respective African countries. Despite the consequent encroachment, data on the use of local complementary and herbal remedies in patients with SCD is also scarce, leading to the objective of this study to evaluate the effect of an indigenous therapeutic agent on metabolic and hematological parameters in pediatric and adult patients with SCD in Nigeria.

II. MATERIALS AND METHODS

This has already been described in a previous publication [11]. Briefly, the initial study, of which this was extracted, was conducted between January and May of 2018, as a double-blind, two-arm, randomized control pilot study. The

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study agent in this trial is a commercially available herbal nutritional capsule supplement with a mixture of Allium sativum, Balanites aegyptiaca, Guiera senegalensis and Azadirachta indica.

Sample size calculation

A universal formula for selecting the sample size for a clinical trial or research problem based on a level of significance and a chosen margin of error was proposed by Cochran [12] and Levy and Lemeshow [13]. Cochran's formula for sample size determination used for determining the sample size has been reported earlier [11].

Study area

The study was conducted at Kaduna City, (10.52° North latitude, 7.44° East longitude and 614 meters elevation above sea level) in Northern Nigeria with a projected population of 1,582,102, based on the 2006 national census figures [14]

Study population

These were patients diagnosed with Sickle Cell Disease attending the pediatric and adult hematology clinics at Barau Dikko Teaching Hospital, Kaduna City in Nigeria.

Recruitment, inclusion and exclusion criteria

Recruitment of participants was carried out at Barau Dikko Teaching Hospital, Kaduna. Participants aged 5 to 45 years were included if they had been diagnosed with SCD, provided informed consent or assent for minors and stated willingness to comply with all study procedures and availability for the duration of the study. Participants were included if they exhibited any clinical signs and symptoms of sickle cell disorder including at list an episode of crisis monthly and were able to take oral medication and compliant with the medication regimen. Individuals with concomitant use of any other medication or medical devices not part of the study were excluded. Other exclusion criteria included those with known allergic reactions to any of the components of study drug, pregnant or lactating women or women who were planning to get pregnant within five months after commencement of the study, patients who had cardiac, hepatic or kidney disease, patients who had one or more episodes of febrile illness within 1 month preceding the study (to exclude patients potentially with malaria, tuberculosis, measles) or those who were alcohol or tobacco users 4 months prior to the start of the study.

Study design and protocol

Following a screening period, patients were enrolled into the study protocol after completing baseline blood and urine sample collection and baseline clinical examination. Participants were then initiated on the intervention, receiving a monthly dose of 500 mg (pediatric patients 5-18 years) or 1000 mg (adult patients>18 years) every 12 hours for a minimum of 120 days, in addition to standard of care practices. After preliminary evaluation, the study drug was administered at enrollment into the study on Day 1 (first visit) and each study participant was re-evaluated at each monthly administration of the test drug for 6 consecutive visits conducted monthly (approximately 30 days apart).

Screening and baseline data at recruitment into study

At the initial visit, inclusion/exclusion criteria and informed consent form for study subjects were verified and urine pregnancy test was conducted for females in the reproductive age group. Other relevant information was recorded, blood was aseptically collected for various analyses and clean-catch mid - stream urine was collected for urinalysis. At the next visit (Visit 2) case subjects were given appropriate dosage of the test drug as specified above.

Hypotheses

There is no difference in mean counts of reticulocytes and thrombocytes of pediatric and adult SCD patients on the study agent and those not on the study agents. There is no difference in mean serum concentrations of serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and those not on the study agents.

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Ethical approval

Each study subject (or caregiver/guardian) signed a consent form to participate in the study and was assured that his/her data will be discreet, coded, and unnamed. The study was approved by the Human Research Ethics Committee (HREC) with a reference number 17-0025 and protocol number 17-0027-1.

Data management and statistical analysis

The coded data was transferred from Excel spreadsheet into NCSS (LLC, Kaysville, Utah, USA) software which was used for further analysis. For the purpose of this study, age (years) was categorized into<10, 10-19.9 and ≥20. Multivariate regression analysis was performed to determine the association between thrombocytes (independent variables) and reticulocyte counts at 1st and 6th visits (dependent variable). Student's t-test was used to evaluate significant differences in means between two continuous variables. Data were presented as numbers and percentages for categorical variables, as mean with standard deviations for continuous variables and as Tables and Figures for all variables. A P-value <0.05 was regarded as statistically substantial.

III. RESULTS

3.1 Demographic characteristics of the study participants. Table 1

Of the 62 SCD subjects included in the study, 33 (53.2%) received the intervention (23 [69.7%] pediatric and 10 [30.3%] adult patients), while 29 (46.8%) were in the control group (22 [75.9%] pediatrics and 7 [24.1%]) adult patients) respectively. There was no significant difference in the means of age and body mass index among the pediatric or adult cases and control (Table 1).

3.2 Reticulocyte count: Table 2, Figures 1a-d, Figures 2a-d

At enrolment, the mean ± standard deviation (SD) reticulocyte count of pediatric cases of 2.44% ±0.77 was similar to controls of 2.56% ±0.81 (t-test = -0.51, P-value = 0.31) and the similarity persisted at end of study. In adult participants, the baseline reticulocyte count in the intervention group was marginally varied from that of the control group, 2.54% ±0.90 compared to 1.97% ±0.40 respectively (t-test =-1.77, P-value =0.05) but trended towards non-significance difference at the end of the study. However, the reticulocyte count of pediatric patients in the intervention group decreased significantly at the end of the study 1.54% ±1.00 (t-test = 4.19, P-value = 0.0002) indicating a 36.9% reduction. A significant reduction was also reflected in the mean reticulocyte count of pediatric patients in the control group at the end of the study 1.70% ±0.69 (t-test=3.34, P-value=0.001), albeit to a less degree (33.6% reduction). At baseline, 60.9% of pediatric patients that had reticulocyte count >2.0%, which decreased to 21.7% at the end of the study, reflecting a decrease of 38.4% compared to 32.8% in the control group. A significant difference was also noticed in the mean reticulocyte count of adult control subjects at enrollment 1.97% ±0.40 and at the end of the study 1.24% ±0.63 (t-test = 2.37, P-value = 0.02). Figure 1a and 1b illustrate the histogram and normal probability plot of reticulocyte count of pediatric cases at enrolment and the end of the study, indicating percent of values equal to, below or greater than 2%; Figure 1c and 1d show the histogram and normal probability plot of the reticulocyte count of pediatric controls at enrolment and at end of study also indicating percent of values equal to, below or greater than 2%. Figures 2a-d illustrate the histogram and normal probability plot of reticulocyte count of adult cases and controls at enrolment and at end of the study, indicating values equal to, below or greater than 2%.

3.3 Thrombocyte count: Table 2. Figures 3a-d, Figures 4a-d

Although there were no observable significant differences in the mean thrombocyte count of pediatric cases and control at enrolment 444.7± 168.8 and 442.8 ±165.4 respectively and the end of the study 414.1 ±153.7 and 428.2 ±119.2 respectively, there was a 7% reduction (444.7-414 .1) in mean thrombocyte count of patients in the intervention group compared to 3% reduction (442.8-428.2) among the controls. While there

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were no significant differences in the mean thrombocyte count of adults in the intervention and control groups at enrolment (547.0 ±271.6 versus 515.0 ±77.9 respectively; t-test = -0.35 (0.3 7) and at the end of the study (intervention group 401.4 ±179.1 versus control group 432.2 ±29.4), the mean thrombocyte count of adults in the intervention group at enrolment 515. ±77.9 was significantly higher than the end of the study (t-test ==2.63, P-value =0.02]). Figures 3a and 3b illustrate the histogram and normal probability plot of thrombocytes of pediatric cases at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L and Figures 3c and 3d elaborate on the histogram and normal probability plot of thrombocytes of pediatric controls at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L. Figures 4a-d show the histogram and normal probability plot of thrombocytes of adult cases and adult controls at enrolment and at the end of the study respectively, showing values equal to, below or greater than 400 x 10^9/L.

3.4 Hemoglobin concentration: Table 2

Astonishingly, there were no observable significant alterations in hemoglobin and in urea values of pediatric or adult cases and control throughout the study. While there was a slight insignificant reduction in the mean hemoglobin concentration of pediatric cases from 76.7 ±9.6 g/dl at enrolment to 76.2 ±9.6 at the end of the study, there were increases in the values among pediatric controls 74.6 ±10.1 g/dl at enrolment to 75.3 ±13.0 at the end of the study, among adults cases 80.2 ±15.3 g/dl at enrolment to 83.2 ±11.5 at end of the study and controls 79.1 ±11.9 at enrolment to 91.3 ±2.9 at the end of the study.

3.5 Serum Urea and Creatinine

There were no observable differences at enrolment in the mean creatinine blood level (µmol/L) of pediatric patients in the intervention 38.3 ±10.8 and control 41.5 ±10.3 (t-test =-1.01, P -value =0.16), at end of study 58.2 ±37.2 and 54.8 ±9.9 respectively (t-test =0.41, P - value =0.34) at end of study and in the adult case and control subjects. Yet, the mean creatinine blood level of pediatric patients in the intervention group at the end of the study 58.2 ±37.2 was significantly higher than levels at enrollment 38.3 ±10.8; (t-test = -2.46, P-value = 0.01). The mean creatinine level of control pediatric subjects at the end of the study 54.8 ±9.93 was even more significantly higher than levels obtained at enrollment 41.5 ±10.3 (t-test = -3.78, P-value = 0.0004).

3.6 Serum electrolytes, Sodium, Potassium, Chloride and Bicarbonate: Table 3

At the end of the study, the mean sodium concentration value of pediatric patients in the intervention group of 137.1 ±3.9 mmol/l and 136.8 ±3.3 mmol/l in the control were significantly reduced compared to their values at enrolment 140.3 ±3.8 mmol/l and 140.1 ±3.6 mmol/l respectively (t-test =2.82, P-value =0.00 4 and t-test =2.76, P-value =0.005 respectively), though all values were within normal range. The end of study mean values of serum potassium among pediatric patients in the intervention group was significantly higher 6.2 ±0.8 mmol/l compared to levels at enrolment 5.5 ±0.9 mmol/l (t-test =-2.79; P-value=0.004) and this difference was wider in pediatric patients in the control group from enrolment level of 4.9 ±0.9 mmol/l to end of study level of 6.4 ± 0.8 mmol/l (t-test =- 5. 11; P-value=0.0000001). There was no noticeable change in adult potassium levels throughout the study.

The mean serum chloride level of pediatric patients in the control group at enrolment of 103.3 ±4.9 mmol/l was notably higher than that at end of study 100.5 ±2.1 mmol/l (t-test = 2.34, P-value =0.01) and the value of adult controls at enrolment 104.7 ±3.3 mmol/l was also significantly higher than the end of study value 102.1 ±1.6 mmol/l (t-test = 2.34, P-value = 0.01). There was no momentous disparity in the values among pediatric cases or controls at enrolment or at the end of the study.

The mean serum bicarbonate levels were significantly elevated from 17.7 ±5.5 mmol/l enrolment value to 23.2 ±3.3 mmol/l end of the

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study value among pediatric cases (t-test =-4.26, P-value =0.0001), and less so from 19.5 ±5.0 mmol/l enrolment level to 22.5 ±3.4 mmol/l end of study level among pediatric control subjects (t-test =-2.11, P-value =0.02). The increase in mean serum bicarbonate concentration (mmol/l) among adult cases and among control subjects were, to a lesser extent, significantly elevated from 20.8 ±4.6 mmol/l enrolment level to 26.1 ± 2.2 mmol/l end of study level and from 23.0 ±2.9 mmol/l enrolment level to 25.7 ±2.4 mmol/l end of study level (t-test =-3.29, P-value =0.003 and t-test -1.90, P-value =0.04 respectively).

3.7 Pearson's correlation tests between thrombocyte count and reticulocyte count. Figures 5a-d, Figures 6a-d

As depicted in Figure 5a, the equation of the straight - line relating thrombocyte count (TC) and reticulocyte count (RC) of pediatric cases at enrolment was estimated as: TC = (368.4) + (31.2) RC using the 23 observations in this data set. The y-intercept, the estimated value of TC when RC was zero, was 368.4 with a standard error of 120.5. The slope, the estimated change in TC per unit change in RC, was 31.2 with a standard error (SE) of 47.1. The value of R^2, the proportion of the variation in TC that can be accounted for by variation in RC, was 0.0205. Pearson's correlation between TC and RC was 0.14 with a P-value of 0.51. As shown in Figure 5b, the equation of the straight-line relating TC and RC of pediatric cases at the end of the study was estimated as: TC = (426.7) + (-8.1) RC using the 23 observations in this data set. The y-intercept was 426.7 with an SE of 61.3, the slope was -8.1 with an SE of 33.6 and the value of R^2 was 0.0028. The correlation between TC and RC was - 0.05 with a P - value of 0.81. In Figure 5c.,the equation of the straight - line relating TC and RC of pediatric controls at enrollment was estimated as: TC = (451.4) + (-3.4) RC using the 22 observations in this data set. The y-intercept was 451.4 with an SE of 122.0. The slope was - 3.4 with an SE of 45.4. The value of R^2 was 0.0003 and the correlation between TC and RC was insignificant (P-value = 0.94) at -0.02. Figure 5d illustrates that the equation of the straight-line relating TC and RC of pediatric controls at end of the study was estimated as: TC =(511.06) +(-48.8) RC using the 13 observations in this data set. The y-intercept was 511.06 with an SE of 90.8, the slope was -48.8 with an SE of 49.7, R^2 was 0.0804 and Pearson's correlation was insignificant (P-value = 0.35) at -0.28.

In Figure 6a, the equation of the straight-line relating TC and RC of adult cases at enrolment is estimated as: thrombocytes = (316.1) + (90.9) reticulocytes using the 10 observations in this data set. The y-intercept was 316.1 with an SE of 271.6. The slope (SE) was 90.9 (101.3), R^2, was 0.0914 and the correlation between TC and RC was insignificant (P-value = 0.40) at 0.30. In Figure 6b, the equation of the straight - line relating TC and RC of adult cases at the end of the study was estimated as: TC = (244.1) + (81.9) RC using the 10 observations in this data set. The y-intercept (SE) was 244.1 (99.4), the slope (SE) was 81.9 (44.6) and C was 0.30. The correlation (P-value) between TC and RC was 0.5446 (0.10). In Figure 6c, the equation of the straight - line relating TC and RC of adult controls at enrolment was estimated as: TC = (478.8) + (18.4) RC using the 7 observations in this data set. The y - intercept (SE) was 478.8 (172.7), the slope (SE) was 18.4 (86.1) and R^2 was 0.009. The correlation (P-value) was 0.10 (0.84). Finally, in Figure 6d, the equation of the straight-line relating TC and RC of adult controls at end of the study was estimated as: TC =(407.1) + (20.3) RC using the 7 observations in this data set. The y-intercept (SE) was 407.1 (201.9), with a slope (SE) of 20.3 (143.5). The value of R^2 was 0.0040 and Pearson's correlation between TC and RC was 0.0631 (0.89).

IV. DISCUSSION

This unique study evaluated the effects of an African pharmaceutical agent (Immunozin®) on hematological and metabolic parameters in pediatric and adult patients with SCD. The study agent is approved by the National Agency for Food and Drugs Administration and Control (NAFDAC) in Nigeria and has been in production and sold as a nutritional supplement and immune and vitality booster for eight years. When taken by sickle cell sufferers, their frequency of

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vaso-occlusive crisis drastically reduces. No adverse drug effect (ADR) from the study agent has been reported, even among those who have been using it for more than seven years, though information on its mechanism of action is unavailable as no study has been done on this aspect of the study agent. Analyses suggest significant changes to hematological parameters including a global decrease in reticulocyte count with pronounced change in the intervention group and decreased thrombocyte levels specific to the pediatric cohort, whereas the adult cohort saw an increasing trend over the duration of the study. Use of this novel African pharmaceutical agent has not been previously reported with the exception of our previous study (11) in which we sought out to identify optimal management of patients with sickle cell disease in the context of affordable and available medicine to realize shorter hospital stay, lower out of pocket expenses and improved quality of life. Our previous study revealed overall pre- and post-hoc leucocytosis, thrombocytosis, hyperkalemia and significant variations in reticulocytes, monocytes, eosinophils, and some liver enzymes which may be due to the administration of the test drug. This led to the purpose of this paper to evaluate the safety and efficacy of Immunozin ® in pediatric and adult patients with SCD using hematological and metabolic surrogate markers. Hemogram or complete blood count is probably the most routinely conducted laboratory investigation at any time a SCD patient visits the hospital. However, scanty information is usually presented on serial reticulocyte count, platelet count, hemoglobin concentration and least of all serum electrolyte, urea and creatinine. The prognostic significance of these is consequent upon the therapeutic efficacy or otherwise of a drug, especially in a clinical trial. This is the case with the study drug under investigation and is the primary reason for conducting this clinical trial. This paper endeavors to originate or improve upon case-control studies of African therapeutic agents for positive impact on hematological parameters of SCD patients.

This study has some key points, one of which needs further clarification. First, there was a decrease in enrolment value of reticulocyte count among both pediatric cases (2.68 -1.44/2.68 x 100% or 46%) and control (2.56 -1.76/2.56x100% or 31%) at the end of the study, which reflects the findings of Borba et al [15], but the decrease was greater among cases than among the control subjects. There was also decrease in the enrolment value of reticulocyte count of both adult cases (2.28-1.86/2.28 x 100% or 18%) and controls (2.15-1.26/2.15 x 100% or 41%) at the end of study, though the decrease was greater in control subjects than among cases, as evidenced by the percentage of pediatrics cases with reticulocytosis (>2.0%) at enrolment (75.0%) and at end of study (18.8%), compared with controls with reticulo- cytosis at enrolment (63.2%) and at end of study (36.4%). This observation implies that the efficacy of study drug appears more pronounced among pediatric cases than among adult cases. The mechanism for this change is uncertain. Elevated reticulocyte count (>2.0%) at enrolment, as observed in many subjects in this study, is an indication of some degree of hemolysis and thus anemia. [16] In this case, a decrease in reticulocyte count may be used as an index of therapeutic effectiveness of the study drug. However, further studies are definitely needed on this point.

Surprisingly, the thrombocyte counts of pediatric cases and controls at enrollment were lower than the values at end of study while that of adult cases and controls were higher. Thrombocytosis appears to be a common phenomenon in steady-state SCD [17-20] though the prognostic implication of thrombocytosis at enrolment into study is controversial regarding its association with disease severity or complication. The prognostic implication of elevated baseline platelet count is debatable with no definitive information of its associations with disease severity or complications [18], though some authors [21, 22] associate thrombocytosis to background auto-splenectomy and hemolytic anemia.

An anticipated result was the increase in serum potassium level in both pediatric and adult cases and controls. The end of study potassium concentrations recorded in this study resonates with the findings in other studies. [23-26]

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According to Dunlop and Bennett [27], potassium fluctuation in sickle erythrocytes is related to cell dehydration and sickling. Potassium leakage into the extracellular fluid, and the consequent serum hyperkalaemia, might have resulted from cell dehydration and hypoxia often observed among sickle cell patients. One intriguing novel finding is the y-intercept interpretation of the relationship between reticulocytes and thrombocytes at enrolment and at end of study in both pediatric and adult cases and control, especially in the pediatric cases. That, prior to administration of the test drug, thrombocytes turnover per unit change in reticulocyte count was 31.2 and that at the end of the study, this value was -8.1 is a topic that needs further in-depth investigation.

V. CONCLUSION

This study evaluates the effect of the agent on hematological parameters potentially informing on safety and to an extent efficacy in patients with SCD. Efficacy studies will indicate if patients still have SCD crisis or pain, exploring need for higher levels of care, oxygen levels, fraction of sickled blood in serum etc. The evidential reduction in mean reticulocyte count of pediatric SCD patients on test drug and the difference in the slope of the equation of straight line relating thrombocyte count and reticulocyte count may reflect the therapeutic effect of the test drug among pediatric patients. It will be an advantage to the scientific world if this study is carried further in respect to the mechanism of action of the study pharmaceutical agent.

Conflict of interest statement

Competing Interests: The authors have no conflicts of interest to disclose.

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Table 1: Demographic characteristics of study participants

Variable		Statistics	Case (n=33, 53.2%)		Control (n=29, 46.8%)		Pediatric (Case-Control)		Adult (Case-Control)	
			Pediatric	Adults	Pediatric	Adults	t-test	P-value	t-test	P-value
Age		Freq. (%)	23 (69.7)	10 (30.3)	22 (75.9)	7 (24.1)	0.77	0.22	0.60	0.28
		Mean (±sd)	10.2 (3.2)	21.2 (5.0)	9.5 (2.9)	20.0 (3.2)				
		Std. Err.	0.7	1.6	0.6	1.2				
		95% CL Mean	8.9 - 11.6	17.6 - 24.8	8.2 - 10.8	17.1 - 22.9				
		Median	11.2	21.5	10.0	21.0				
		Min. – Max.	5.2 - 15.0	15.0 - 27.0	5.0 - 15.0	16.0 - 25.0				
BMI		Freq. (%)	23	10	22	7	0.00	1.00	-0.35	0.36
		Mean (±sd)	15.3 (1.7)	18.4 (3.2)	15.3 (1.7)	18.9 (2.6)				
		Std. Err.	0.4	1.0	0.4	1.0				
		95% CL Mean	14.5 - 16.0	16.1 - 20.7	14.6 - 16.1	16.5 - 21.3				
		Median	15.1	17.8	15.2	18.5				
		Min. – Max.	12.6 - 19.6	16.0 - 26.9	11.7 - 18.1	15.2 - 22.0				
Sex	Male	Freq. (%)	10 (43.5)	5 (50.0)	10 (45.5)	2 (28.6)	-		-	
	Female		13 (56.5)	5 (50.0)	12 (54.5)	5 (71.4)				

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Table 2: Mean distribution of some hematological parameters among case and control study subjects at enrolment (1st visit) and at end of study (6th visit) post administration of test medication

Hematological variable	Statistics	At enrolment (1st visit)				ttest (Pvalue)		Post administration of Study drug (6th visit; 6th month)			
		Case		Control				Case			
		Pediatric	Adult	Pediatric	Adult	Ped	Adult	Pediatric	Adult	Pediatric	
Reticulocytes (%)	n	23	10	22	7	- 0.51 (0.31)	1.77 (0.05)	23	10	13	
	Mean (±sd)	2.44 (0.77)!	2.54 (0.90) *	2.56 (0.81) ^	1.97 (0.40) #			1.54 (1.00)!	1.92 (1.19)*	1.70 (0.69)^	
	Median	2.10	2.50	2.50	2.0			1.30	1.65	1.6	
	Min. – Max.	1.7 – 4.1	1.2 – 4.1	1.6 – 4.3	1.4-2.4			0.4-3.9	0.4 – 4.0	0.6 – 3.0	
	No. (%) >2.0%	14 (60.9%)	7 (70.0%)	14 (63.6%)	3 (42.9)			5 (21.7)	3 (30.0)	4 (30.8)	
	Normality test@	0.82 (0.00) R	0.99 (0.99) CR	0.91 (0.04) R	0.90 (0.35) CR			0.89 (0.01) R	0.90 (0.23) CR	0.99 (1.00) CR	
Thrombocytes ($x10^9$/L)	Mean (±sd)	444.7 (168.8)!!	547.0 (271.6)**	442.8 (165.4)^^^	515.0 (77.9)##	0.28 (0.39)	- 0.35 (0.37)	414.1 (153.7)!!	401.4 (179.1)**	428.2 (119.2)^^^	0.17 (0.447)
	Median	429.0	510.0	447.5	490.0			407.0	399.5	415.0	
	Min. – Max.	149.0-924.0	158.0-928.0	193.0-916.0	428.0-637.0			141.0-702.0	127.0-720.0	230.0- 666.0	
	No. (%) >400	13 (56.5%)	6 (60.0)	14 (63.6)	7 (100.0)			12 (52.2)	5 (50.0)	8 (61.5)	
	Normality test@	0.96 (0.406.) CR	0.94 (0.56) CR	0.92 (0.06) CR	0.94 (0.61) CR			0.98 (0.82) CR	0.97 (0.91) CR	0.97 (0.91) CR	
Hemoglobin (g/dl)	Mean (±sd)	76.7 (9.6)!!!	80.2 (15.3)***	74.6 (10.1)^^^	79.1 (11.9)###	0.71 (0.24)	0.17 (0.447)	76.2 (9.6)!!!	83.2 (11.5)***	75.3 (13.0)^^^	
	Median	77.0	78.0	74.5	83.0			74.0	82.0	71.0	
	Min. – Max.	59.0-96.0	60.0-103.0	59.0-105.0	57.0-92.0			60.0-94.0	61.0-103.0	59.0-110.0	
	Normality test@	0.97 (0.73) CR	0.94 (0.56) CR	0.93 (0.10) CR	0.91 (0.41) CR			0.97 (0.61) CR	0.97 (0.87) CR	0.86 (0.04) R	
Urea (mmol/L)	Mean (±sd)	2.1 (0.9)$	2.2 (1.07)$$	2.2 (0.8)&	2.3 (0.6)&&	- 0.39 (0.35)	- 0.25 (0.40)	3.04 (2.9)$	2.05 (0.50)$$	2.3 (0.8)&	0.86 (0.04) R
	Median	1.9	1.9	2.1	2.3			2.3	1.95	2.2	
	Min. – Max.	1.1 – 4.6	1.0-4.7	1.1-4.4	1.5-3.1			1.5-16.2	1.4-2.9	1.2-4.3	
	No. (%) >9.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (4.3)	0 (0.0)	0 (0.0)	
	Normality test@	0.80 (0.0004) R	0.85 (0.05) CR	0.92 (0.08) CR	0.91 (0.39) CR			0.41 (0.000000001) R	0.93 (0.45) CR	0.86 (0.04) R	
Creatinine (µmol/L)	Mean (±sd)	38.3 (10.8)€	46.1 (11.3) €€	41.5 (10.3)£	48.0 (9.6) ££	- 1.01 (0.16)	- 0.37 (0.36)	58.2 (37.2) €	55.0 (8.2) €€	54.8 (9.9) £	0.94 (0.50) CR
	Median	39.0	46.5	44.0	47.0			50.0	55.5	51.0	
	Min. – Max.	14.0-56.0	29.0-65.0	16.0-61.0	33.0-63.0			33.0-221.0	45.0 - 72.0	42.0-73.0	
	No. (%) > 133	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (4.3)	0 (0.0)	0 (0.0)	
	Normality test@	0.96 (0.41) CR	0.92 (0.40) CR	0.92 (0.07) CR	0.98 (0.95) CR			0.49 (0.000000001) R	0.94 (0.50) CR	0.90 (0.13) CR	

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	Control		t-test (P-value)	
	Adult		Ped.	Adult
	7		- 0.57 (0.29)	1.47 (0.08)
	1.24 (0.71)#			
	1.4			
	0.3 – 2.1			
	1(14.3%)			
	0.92 (0.47) CR			
	432.3 (29.4)##		- 0.31 (0.38)	- 0.54 (0.30)
	360.0			
	211.0-876.0			
	3 (42.9)			
	0.88 (0.24) CR			
	81.3 (2.9)###		0.22 (0.41)	0.50 (0.31)
	82.0			
	78.0-84.0			
	0.79 (0.04) R			
	2.8 (1.1)&&		1.15 (0.13)	- 10.69 (0.07)
	2.4			
	1.6-4.5			
	0 (0.0)			
	0.91 (0.41) CR			
	53.4 (14.9) ££		0.41 (0.34)	0.26 (0.40)
	54.0			
	24.0-72.0			
	0 (0.0)			
	0.86 (0.16) CR			

Reticulocyte t-test (P-value): !=4.19 (0.0002); = 1.13 (0.10); 3.34 (0.001); #= 2.37 (0.02): *Thrombocyte t-test (P-value):* !!= 0.64 (0.26); **= 1.42 (0.09); ^^- 0.30 (0.38); ##= 2.63 (0.02): *Hemoglobin t-test (P-value):* !!!= - 0.18 (0.43); ***= -0.50 (0.31); ^^^= - 0.17 (0.43); ###= - 0.48 (0.32); *Urea t-test (P-value):* $= - 1.48 (0.07); $$= 0.40 (0.35); &= -0.36 (0.36); &&= - 1.06 (0.16); *Creatinine t-test (P-value):* €= - 2.46 (0.01); €€= -2.01 (0.03); ££= - 3.78 (0.0004); @=Shapiro Wilk W for normality test (P-value); R=reject normality; CR=Cannot reject normality; Ped.=Pediatric.

Table 3: Mean distribution of serum electrolytes among case and control study subjects at enrolment (1st visit) and at end of study (6th visit)

		At enrolment (1st visit)						t-test (P-value)	
		Case		Control					
		Pediatric	Adult	Pediatric (Ped.)	Adult			Ped.	Adult
Sodium (mmol/l)	n	23	10	22	7			0.18 (0.43)	- 0.05 (0.48)
	Mean (±sd)	140.3 (3.8)!	137.6 (5.6) *	140.1(3.6) ^	136.7 (1.50) #				
	Median	140.0	138.0	139.0	137.04				
	Min. – Max.	130.0-146.0	122.0-145.0	134.0-148.0	135.0-139.0				
	No. (%) > 145.0	1(4.3)	0 (0.0)	1(4.5)	0 (0.0)				
	Normality test@	0.94 (0.19) CR	0.89 (0.04) R	0.96 (0.51) CR	0.93 (0.59) CR				
Potassium (mmol/l)	Mean (±sd)	5.5 (0.9)!!	5.5 (1.0) **	4.9 (0.9) ^^	6.5 (3.6) ##			2.24 (0.015)	- 1.23 (0.11)
	Median	5.2	5.2	5.1	5.3				
	Min. – Max.	3.9-7.4	4.0-7.0	2.9-6.2	4.5-14.7				
	No. (%) > 5.2	11 (47.8)	4 (40.0)	9 (40.9)	4 (57.1)				
	Normality test@	0.93 (0.13) CR	0.91 (0.10) CR	0.93 (0.22) 3CR	0.55 (0.000006) R				
Chloride (mmol/l)	Mean (±sd)	101.6 (3.7)!!!	99.1(6.1) ***	103.3 (4.9) ^^^	104.7 (3.3) ###			-1.31 (0.10)	- 0.32 (0.38)
	Median	103.0	100.0	102.0	106.0				
	Min. – Max.	93.0-108.0	82.0-108.0	97.0-115.0	99.0-108.0				
	No. (%) > 108.0	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)				
	Normality test@	0.91 (0.04) R	0.90 (0.08) CR	0.91 (0.09) CR	0.90 (0.32) CR				
Bicarbonate (mmol/l)	Mean (±sd)	17.7 (5.5) $	20.8 (4.6) $$	19.5 (5.0) &	23.0 (2.9) &&			- 1.15 (0.13)	- 1.37 (0.09)
	Median	17.0	19.0	19.0	23.0				
	Min. – Max.	8.0-30.0	15.0-29.0	13.0-29.0	19.0-27.0				
	No. (%) > 32.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
	Normality test@	0.98 (0.93) CR	0.92 (0.14) CR	0.94 (0.23) CR	0.95 (0.81) CR				

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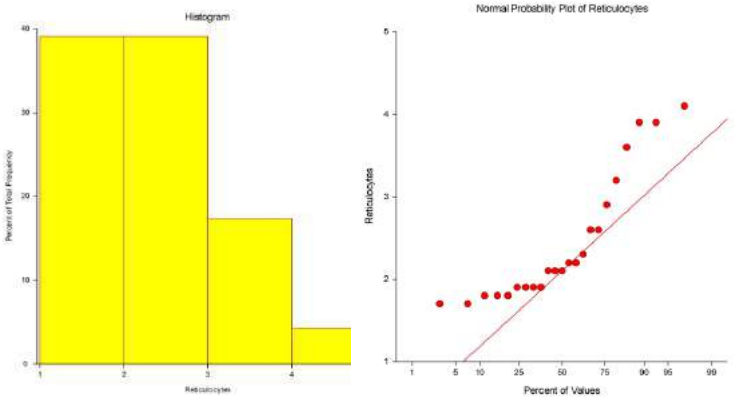
Post administration of Study drug (6th visit; 6th month)						
Case		Control		t-test (P-value)	
Pediatric	Adult	Pediatric (Ped.)	Adult	Ped	Adult
23	10	13	7		
137.1 (3.9)!	139.9 (2.7) *	136.8 (3.3) ^	138.9 (2.4) #		
137.0	139.5	137.0	139.0		
127.0-144.0	136.0-144.0	130.0-142.0	135.0-142.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.93 (0.12) CR	0.94 (0.52) CR	0.95 (0.53) CR	0.98 (0.98) CR	0..25 (0.40)	0.80 (0.22)
6.2 (0.8)!!	5.1 (0.9) **	6.4 (0.8) ^^	6.4 (0.9) ##		
6.2	5.0	6.4	6.1		
4.4-7.6	4.0-7.2	4.4-7.4	5.3-8.0		
13 (56.5)	4 (40.0)	9 (69.2)	7 (100.0)		
0.95 (0.35) CR	0.91 (0.27) CR	0.88 (0.07) CR	0.94 (0.60) CR	- 0.72 (0.24)	- 2.93 (0.006)
100.7 (3.8)!!!	100.7 (2.0) ***	100.5 (2.1) ^^^	102.1 (1.6) ###		
102.0	101.0	101.0	102.0		
89.0-105.0	96.0-103.0	96.0-104.0	100.0-104.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.84 (0.002) R	0.85 (0.07) CR	0.97 (0.87) CR	0.91 (0.42) CR	- 0.20 (0.42)	- 1.60 (0.07)
23.2 (3.3) $	26.1 (2.2) $$	22.5 (3.4) &	25.7 (2.4) &&		
24.0	26.0	23.0	26.0		
14.0-27.0	22.0-29.0	15.0-27.0	22.0-29.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.88 (0.009) R	0.94 (0.53) CR	0.94 (0.50) CR	0.98 (0.4) CR	- 0.60 (0.28)	0.35 (0.37)

Sodium t-test (P-value): !=2.82 (0.004); *= -1.17 (0.13); ^2.76 (0.005); #= - 0.25 (0.40): *Potassium t-test (P-value):* !!= - 2.79 (0.004); **= 0.94 (0.18); ^^- 5.11 (0.0000001); ##= 0.07 (0.47): *Chloride t-test (P-value):* !!!= 0.81 (0.21); ***= -0.79 (0.22); ^^^= 2.34 (0.01); ###=1.88 (0.04); *Bicarbonate t-test (P-value):* $= - 4.26 (0.0001); $$= -3.29 (0.003); &= - 2.11 (0.02); &&= - 1.90 (0.04); @=Shapiro Wilk W (P-value) for normality test; R=reject normality; CR=Cannot reject normality.



a. Pediatric Cases: Reticulocyte at enrolment (1st visit)

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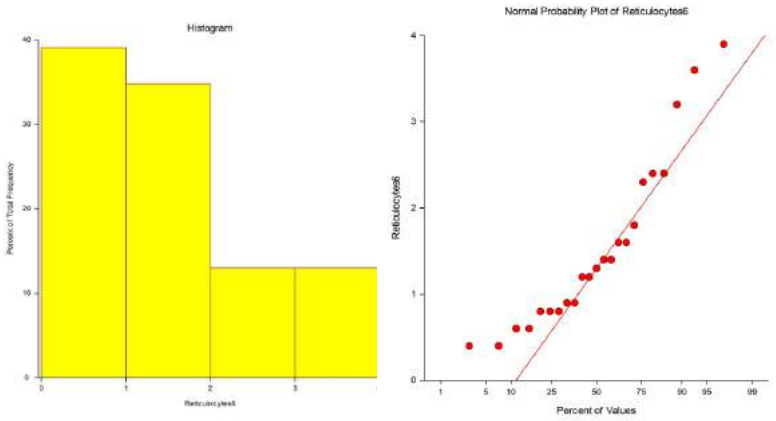
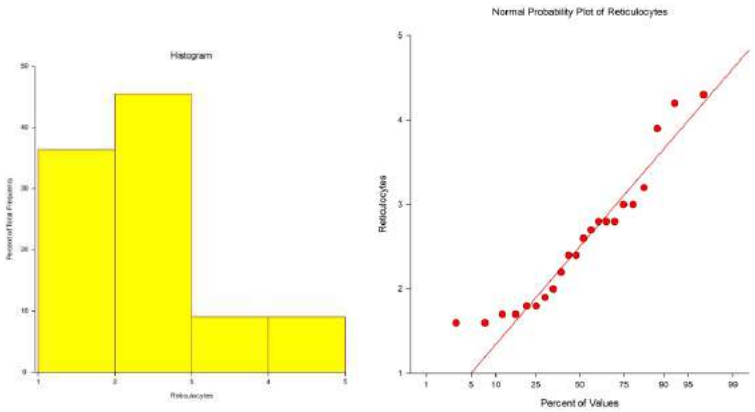
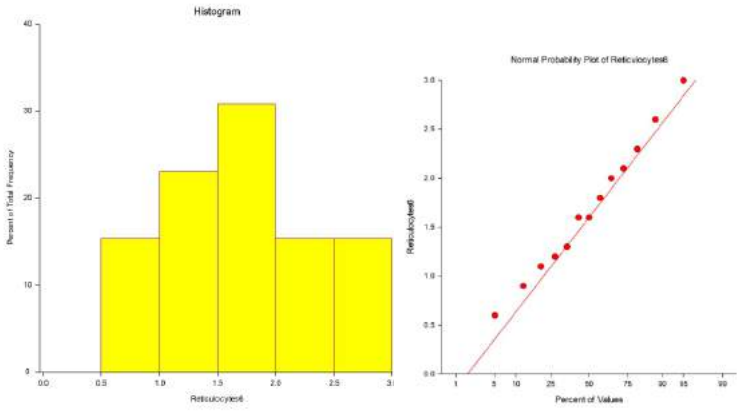


b. Pediatric cases: Reticulocytes count at end of study (6th visit)



c. Pediatric Controls: Reticulocyte at enrolment (1st visit)



d. Pediatric control: Reticulocyte count at end of study (6th visit)

Figure 1 a-d: Reticulocyte count of pediatric cases at enrolment (a), at end of study (b) and pediatric controls at enrolment (c) and at end of study (d)

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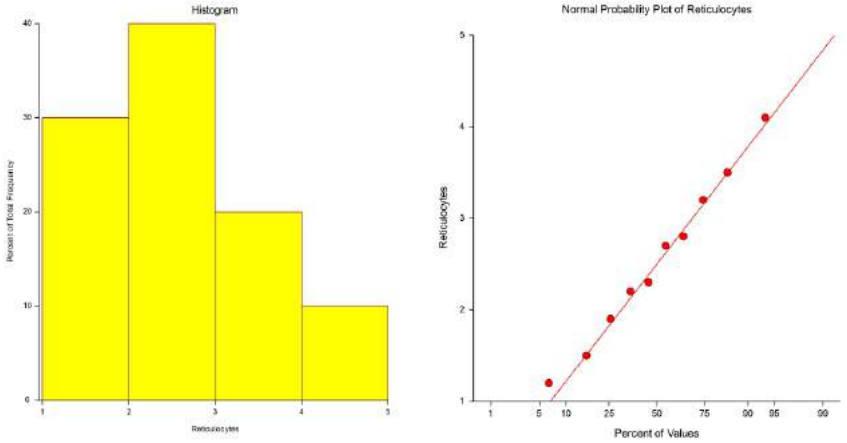
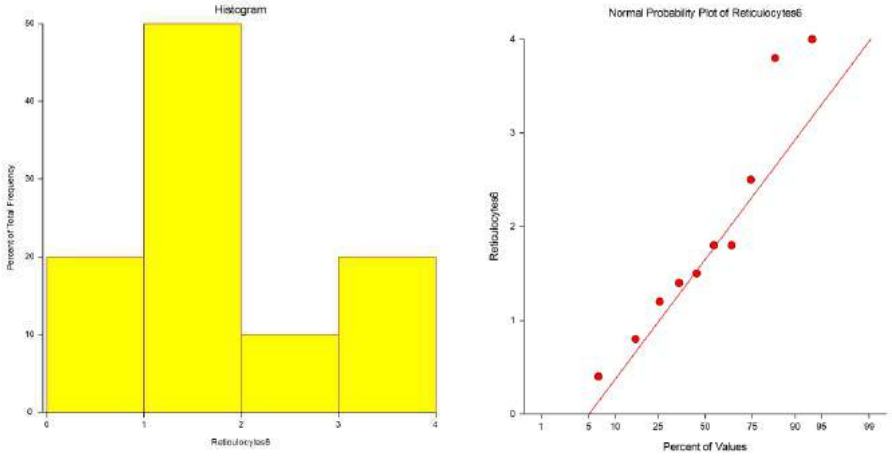
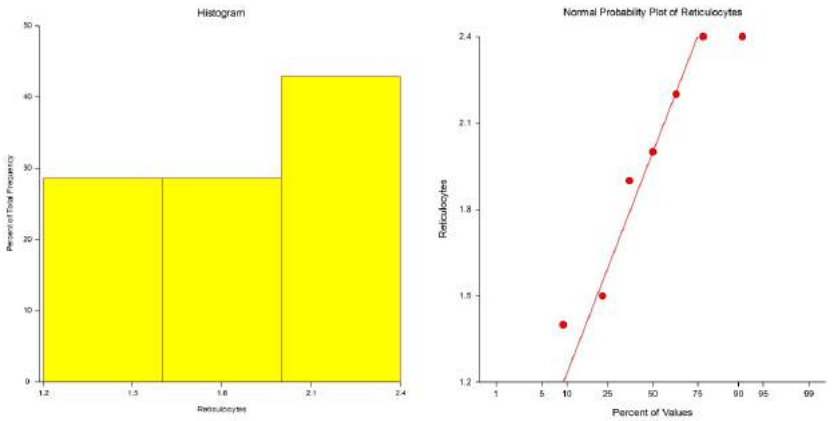


a. Adult Cases: Reticulocyte Count at enrolment (1st visit)



b. Adult Cases: Reticulocyte count at end of study (6th visit)



c. Adult Controls: Reticulocyte count at enrolment (1st visit)

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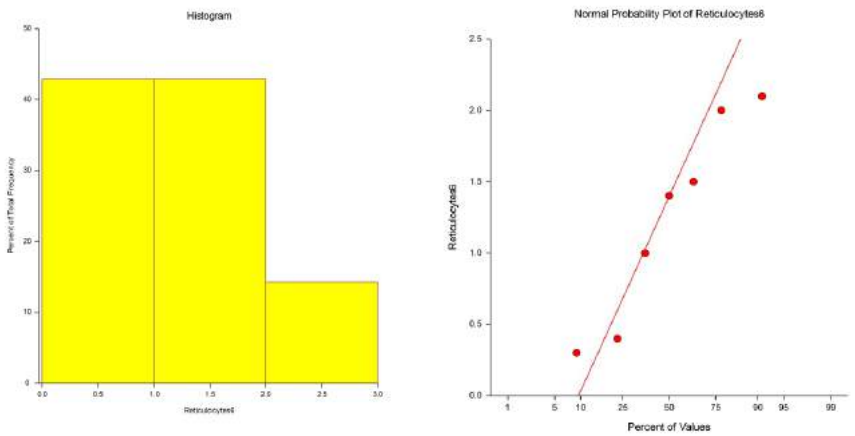
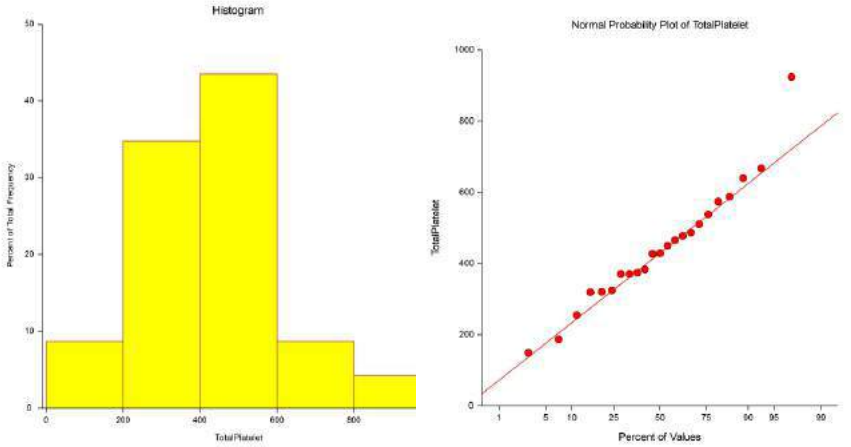


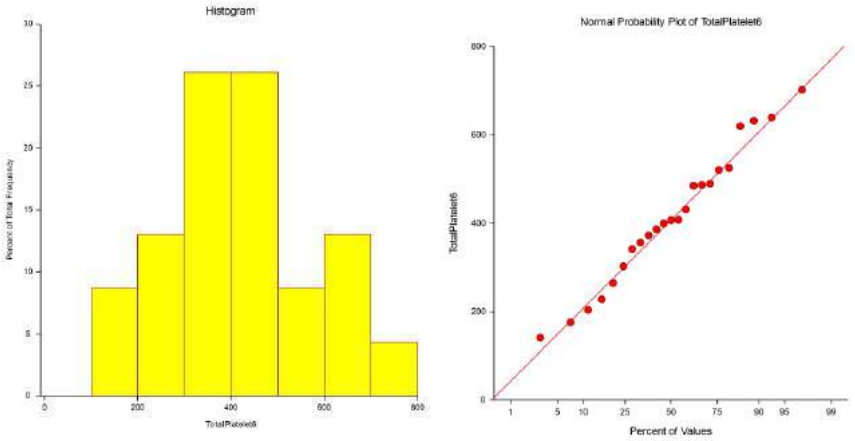
d. Adult Controls: Reticulocyte count at end of study (6th visit)

Figure 2 a-d: Reticulocyte count of adult cases at enrolment (a), at end of study (b) and of adult controls at enrolment (c) and end of study (d)

Figures 2a-d illustrate the histogram and normal probability plot of reticulocyte count of adult cases and controls at enrolment and at end of the study, indicating values equal to, below or greater than 2%



a. Pediatric Cases: Platelets count at enrolment (1st visit)



b. Pediatric Cases: Platelets count at end of study (6th visit)

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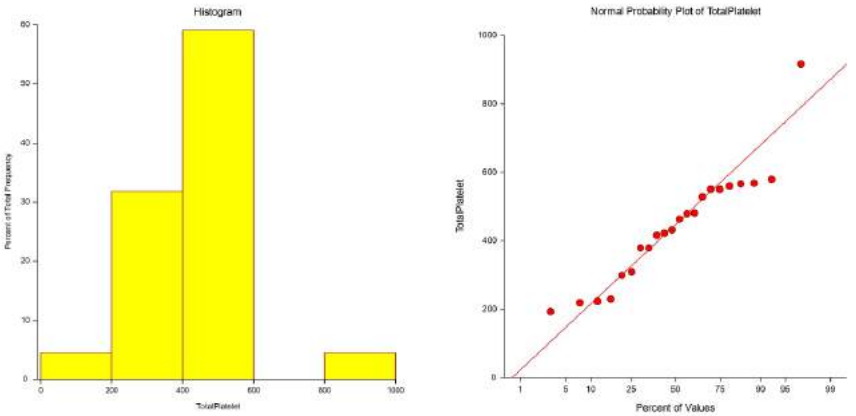
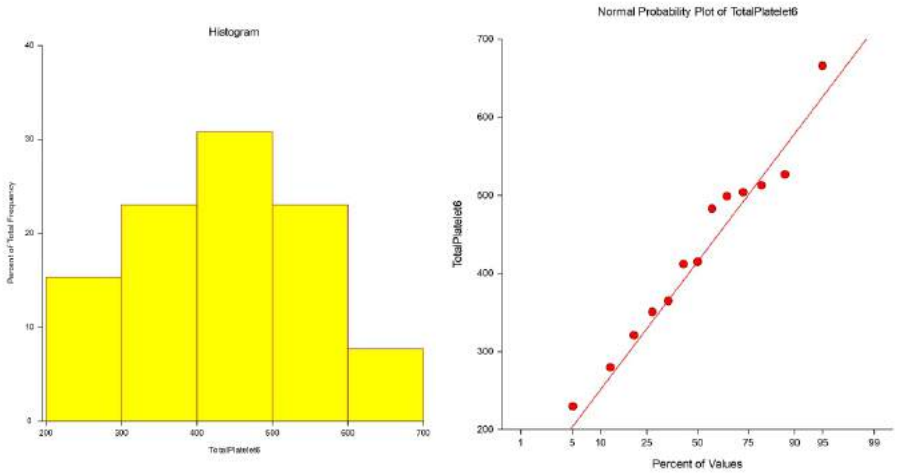


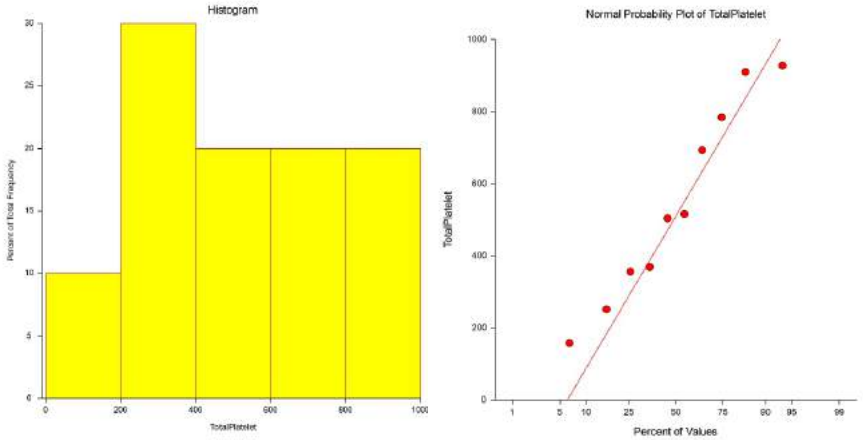
c. Pediatric Controls: Platelets count at enrolment (1st visit)



d. Pediatric Controls: Platelet count at end of study (6th visit)

Figure 3 a-d: Platelet count of pediatric cases at enrolment (a), at end of study (b) and pediatric controls at enrolment (c) and end of study (d)

Figures 3a and 3b illustrate the histogram and normal probability plot of thrombocytes of pediatric cases at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L and Figures 3c and 3d elaborate on the histogram and normal probability plot of thrombocytes of pediatric controls at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L.



a. Adult Case: Platelets count at enrolment (1st visit)

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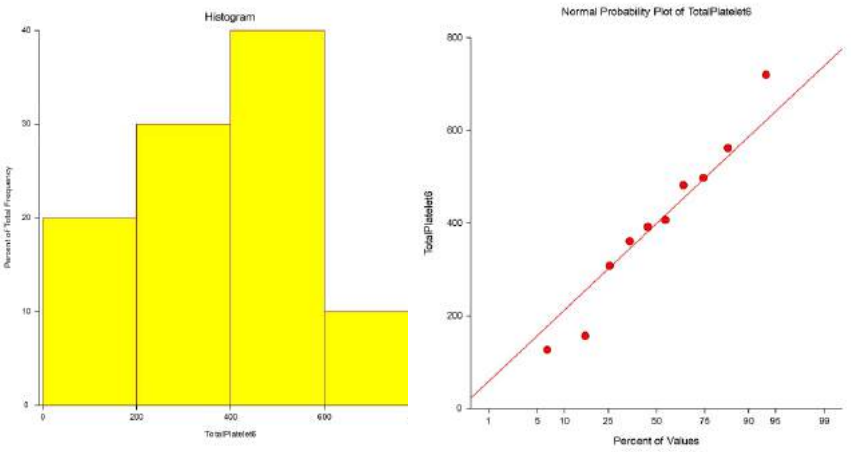
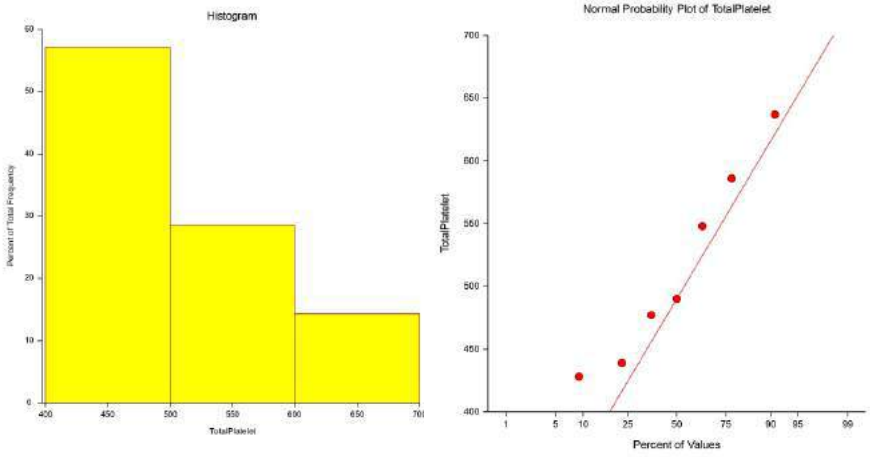
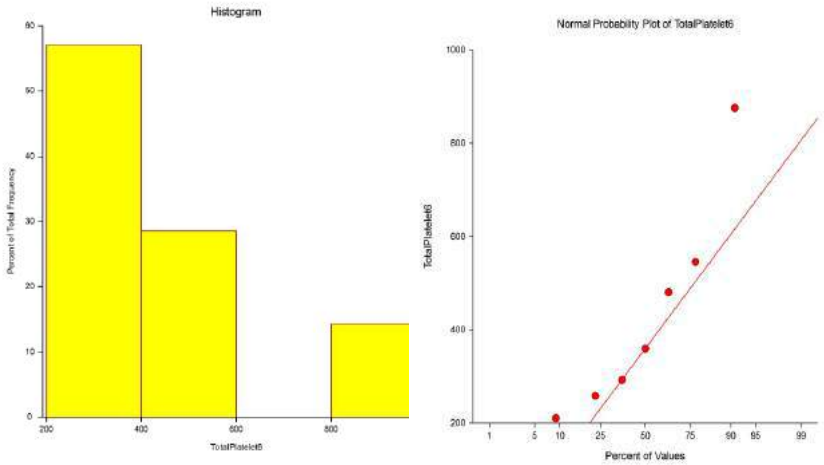


b. Adult Cases: Platelets count at end of study (6th visit)



c. Adult Controls: Platelet count at enrolment (1st visit)



d. Adult Controls: Platelet count at end of study (6th visit)

Figure 4 a-d: Platelet count of adult cases at enrolment (a), at end of study (b) and adult controls at enrolment (c) and end of study (d)

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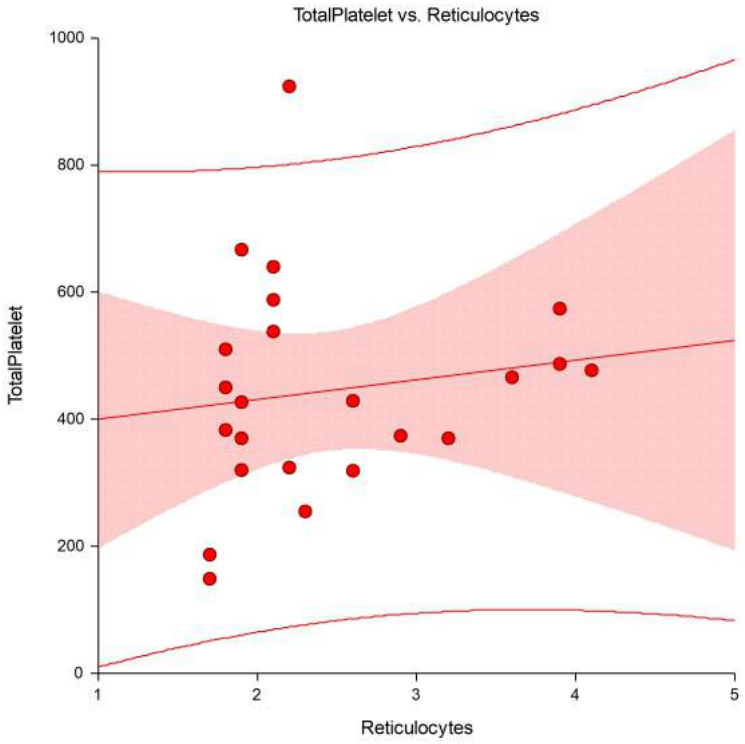
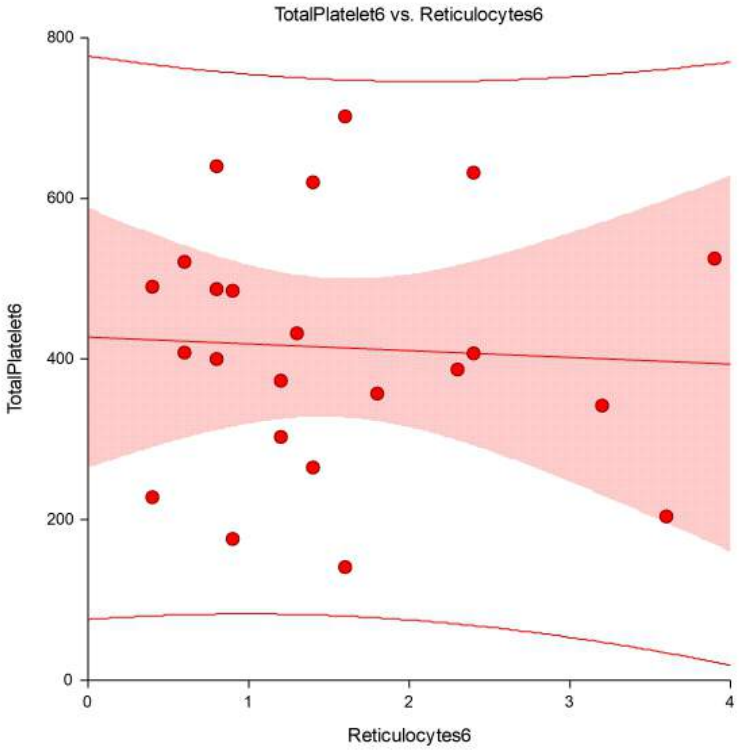


a



b

Figure 5 a,b: Simple linear regression (scatter plot) of pediatric cases reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

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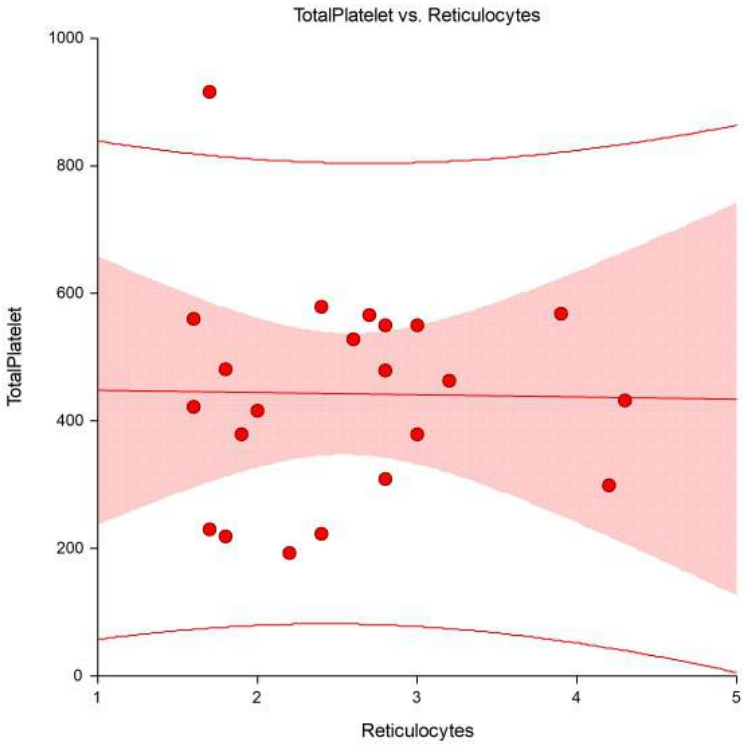
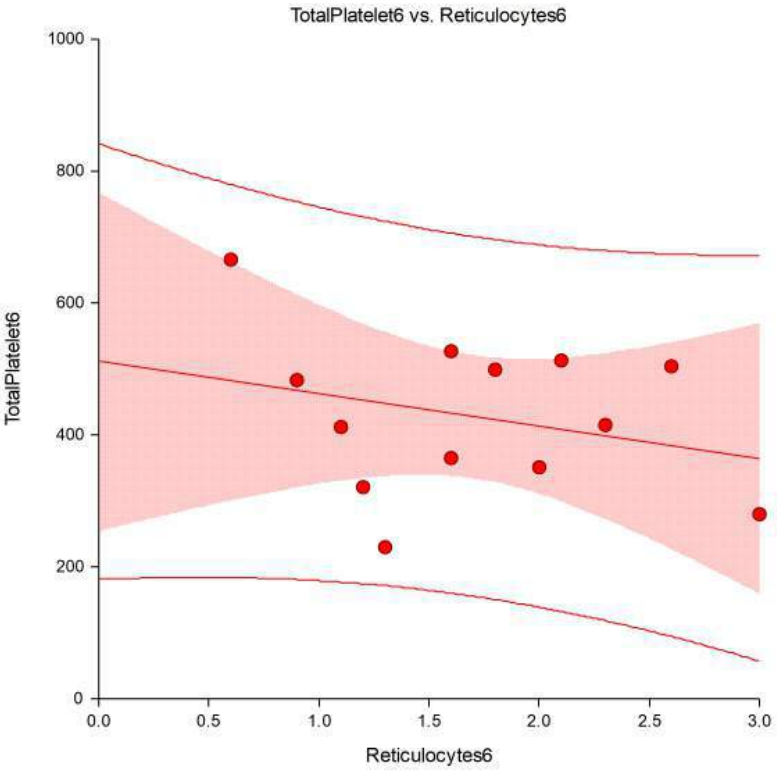


c



d

Figure 5 c,d: Simple linear regression between pediatric control reticulocyte and platelet counts at enrolment 1st visit (c) and at end of the study 6th visit (d)

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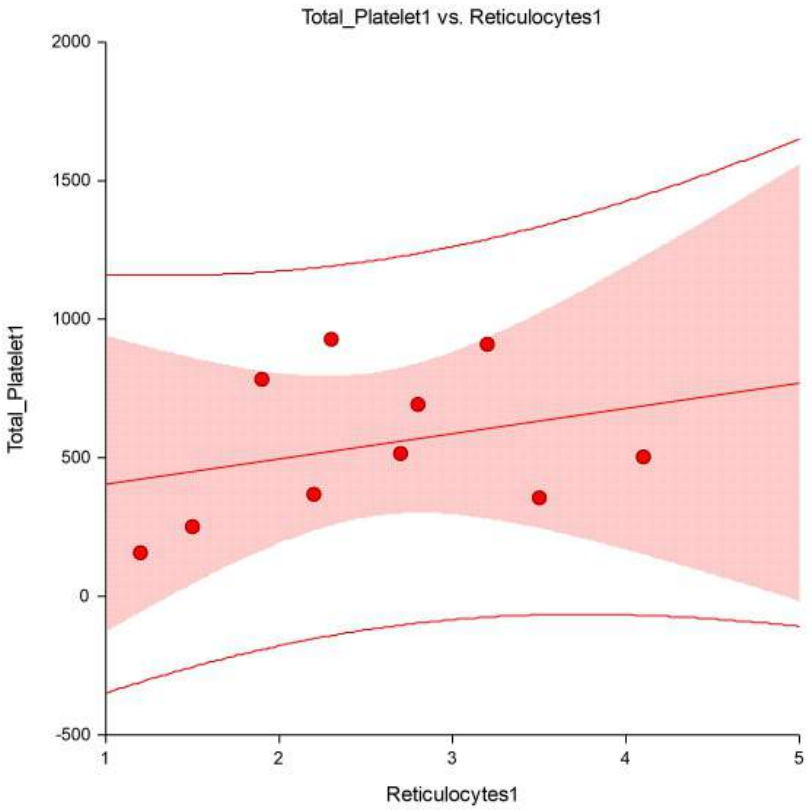
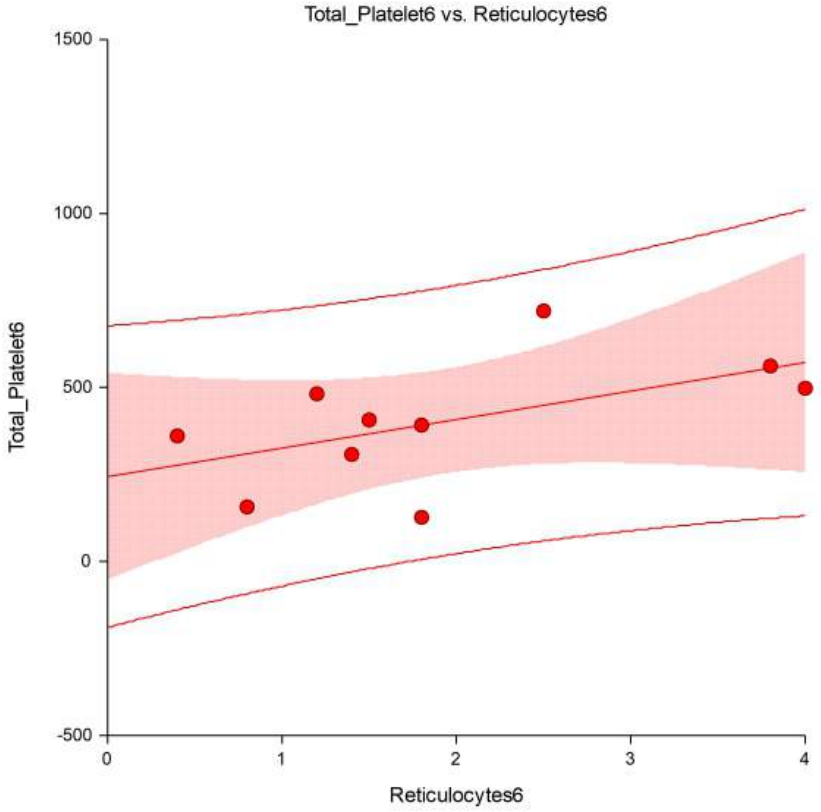


a



b

Figure 6 a,b: Simple linear regression (scatter plot) of adult cases reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

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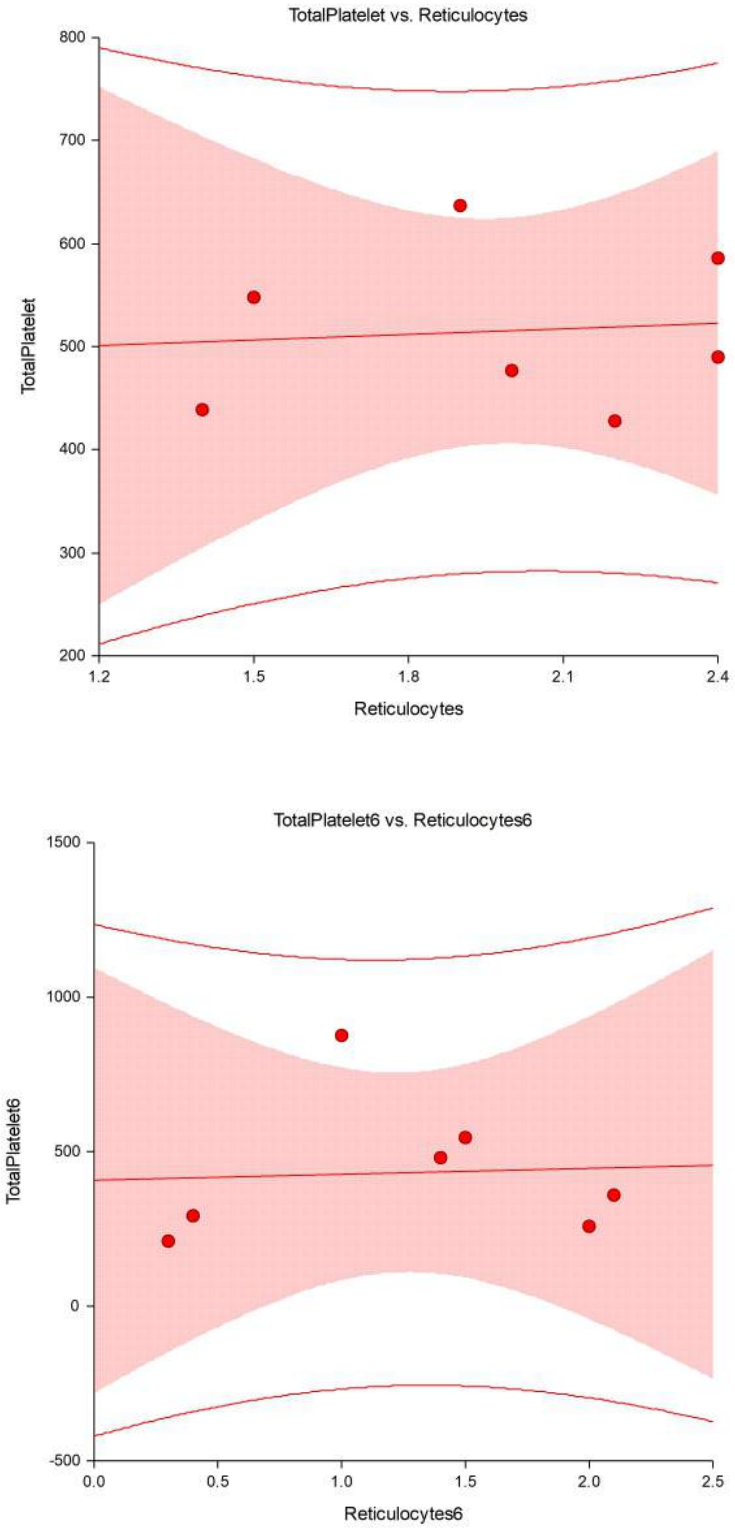

Figure 6 c,d: Simple linear regression (scatter plot) of adult control reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

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