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ASSESSMENT OF ANAEMIA INDUCE SPLENOMEGALY IN PREGNANT WOMEN USING RADIO-HAEMATOLOGICAL INDICES IN A TROPICAL AFRICAN COMMUNITY

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ABSTRACT

Background: Anaemia affects about 50% of pregnant women worldwide and 75% in tropical African regions. Splenomegaly is incidentally diagnosed among pregnant women on routine antenatal ultrasonography with a suspected cause of anaemia.

Objective: To assess the nexus between anaemia and splenomegaly among pregnant women in a tropical African region.

Methodology: Prospective longitudinal research employing an ultrasound scan (using a 3.5 MHz convex transducer) on 120 pregnant women was conducted to assess foetal biometry and maternal spleen. Laboratory tests consisting of packed cell volume (PCV), Reticulocyte counts, Erythrocyte Sedimentation Rate, and Malaria parasite were conducted to assess anaemia, bone marrow response, presence of infectious disease, and malaria parasite, respectively. Patients' age, weight, and height were recorded, and Body Mass Index (BMI) was calculated.

Results: Patients' ages ranged between 17 and 56 years with mean age and standard deviation (SD) of 26.12 ±3.7 with 66% in their third trimester. The mean and SD of the weight, height and BMI are 67.00 ±11.0, 1.57 ±0.16 and 24.02 ±1.50 respectively. Spleen length ranges from 12.1 cm to 18.5 cm with a mean of 14.88 ±2.0. PCV levels were found to be lower than the normal range with a mean % of 29.29 ±2.0.

Conclusion: The findings of this study, indicate a relationship between splenomegaly and anaemia among pregnant women without underlying health conditions or previous history of organomegaly. Wide-ranging research is recommended to gather additional information that can help reduce the incidence of anaemia and, consequently, maternal death in expectant mothers in low-income communities.

Keywords: Splenomegaly, Ultrasonography, Anaemia, Pregnant women, Packed cells volume

Introduction

According to the World Health Organization (WHO), anaemia which is defined as a reduction in the total red blood cell (RBC) count affects about 35% of the global population[1,3], 50% to 80% of pregnant women in developing countries[4]. The most common type of anaemia in pregnant women worldwide is iron deficiency anaemia (IDA)[4,5]. It occurs due to

insufficient iron needed to produce enough haemoglobin in the body, which is responsible for carrying oxygen to tissues. The developing foetus increases the placenta's demand for iron-rich blood which causes the body to require more iron during pregnancy. Pregnancy-related IDAs can have detrimental effects on both the growing foetus and the mother. Supplementation in most healthcare centres is commonly via prescription of prenatal vitamins containing iron. During the early stages of embryonic development, the spleen, the largest organ involved in immune processes, performs certain haematological tasks[4] this function disappears post-partum and in some pathological contexts (e.g., a severe hematopoietic disorder), the spleen can be prompted to restore the function which is often accompanied by spleen enlargement[6]. Splenomegaly is a clinical presentation of a systemic disease(s) however, it has been discovered that splenomegaly exists in some IDA patients concomitantly which may result from an immunocompromised state of pregnancy and placental sequestration of infected erythrocytes[7,8]. Ultrasound scan is one of the most common medical imaging tools for assessing the spleen, such as size, because of its wide availability, non-invasiveness, and safety, and it does not use ionising radiation[9-11].

Anaemia in pregnancy is a global health concern[6], its prevalence, however, varies across different geographical regions of the world. It is generally more common in places with lower socioeconomic levels, less access to healthcare, and poor diet. The following are some of the areas where IDA in pregnancy is commoner.

- Countries with Low and Intermediate Incomes: Many countries in the regions with low- and middle-income sources such as the sub-Saharan Africa regions and southern regions of Asia have higher rates of anaemia among pregnant women and some parts of southeastern Asia. This may be due to some disposing factors like malnutrition, poor access to prenatal care, and burdens of infectious diseases that can contribute to anaemia.
- Rural Areas: Within countries, rural areas often have higher rates of anaemia in pregnancy compared to urban areas. Contributing factors to higher prevalence include limited access to healthcare facilities, poor sanitation, and agricultural-based economies with seasonal food insecurity.
- Conflict Affected and Refugee areas: Areas affected by conflict, humanitarian crises, or displacement, can face challenges in accessing healthcare services and maintaining adequate nutrition, leading to higher rates of anaemia among pregnant women.
- Indigenous and Marginalized Populations: Indigenous communities and marginalised populations within countries may experience disparities in healthcare access and nutrition, contributing to higher rates of iron deficiency anaemia in pregnancy.

Iron is a mineral that plays a vital role in health and overall well-being that helps reproduce RBCs that maintain a good immune system, it is therefore, essential that pregnant women get sufficient iron. A comprehensive strategy that integrates clinical evaluation, imaging techniques, and laboratory investigations is required to assess tropical splenomegaly induced by anaemia. This study conducted a simple and cost-effective assessment of anaemia-induced splenomegaly using the least hazardous radiographic assessment tool, abdominal ultrasound (US) and cost-effective laboratory investigations among pregnant women in an intermediate-income and partially conflict-affected sub-Saharan African region to evaluate the nexus between anaemia and spleen enlargement. Spleen enlargement was recurrently observed among pregnant women with clinical suspicion of anaemia presenting for an obstetric ultrasound scan for foetal biometry by the authors of this research which prompted the research question.

MATERIALS AND METHOD

Research design.

A prospective longitudinal study was performed in a higher institutional hospital in Northeastern Nigeria over a six (6) months period consisting of two groups; an experimental group of one hundred (100) pregnant

women who presented for antenatal care and were diagnosed with spleen enlargement without any previous history of organomegaly or any underline disease condition and another group of gender- and age-matched healthy but pregnant women were recruited as controls. Consents were obtained for both groups before enrolment in the study. Subjects were identified during antenatal obstetrics ultrasound scanning, first, the procedure is explained to the participants for consent agreements. Depending on the condition of the maternal spleen, the participants are partitioned into either the experimental group or the control group according to the subject selection criteria. The participants' demographic information and maternal spleen lengths were collected and recorded as per the data collection statement. The next and final stage was the blood sample collection, which was performed by a dedicated licensed medical laboratory scientist. As an incentive, the laboratory investigation results were shared with the participants.

Subjects Inclusion and Exclusion Criteria:

The experimental group subject's inclusion was based on the sonographic findings of the spleen length of 12.1 cm and above with no other organ enlargement in pregnant women, while subjects were excluded based on; normal size spleen length (≤ 12 cm), previous history of sickle cell disease, splenic trauma, liver disease, and those whose spleen length cannot be properly evaluated. The selection criteria for participants in the control group were based on pregnant women with normal spleen lengths without any previous history of organomegaly or any underlying disease condition presented for antenatal care.

Data collection

Demographic data including patients' age, height and weight were recorded using the standard anthropometric technique[10]. The measured height was expressed in meters (m), and weight in kilograms (Kg). Using both weight and height, a standard formula was used to calculate the body mass index (BMI) as shown in Equation 1.

$$BMI = \frac{Weight}{Height^2}\left(\frac{kg}{m^2}\right) \quad (1)$$

Splenic length was measured and recorded for each subject (anterolateral length). The mean value length was obtained for the accuracy of the result by repeated measurements up to three times. After scanning, subjects that fall within the inclusion criteria were asked to present themselves for blood sample collection at a mini-side lab for subsequent laboratory investigations that include PCV, ESR, Retics count and MP microscopy. PCV was performed by centrifuging the blood sample, ESR was using the Westergren method while Retics and MP were performed using the microscopic method.

Scanning procedure:

Examinations were performed with a real-time commercially available ultrasound scan machine (Sonoscape, S11 PLUS) with a 3.5 MHz transducer by two authors experienced Sonographers in the abdominal US. Scan images in real time were best obtained by maintaining the subject in a supine position or slightly adjusted to the right lateral decubitus position and in suspended deep inspiration. The longest spleen length (in cm) is measured on images with the transducer on the upper left quadrant through the splenic hilum per the guidelines recommended by the American Institute of Ultrasound in Medicine and as previously explained by Lamb et al.[12]. The accurate length of the spleen has been described as the maximum length between the inferior margin of the spleen and the most superior margin on a longitudinal coronal sonogram.

Ethical consideration

Research approval was obtained from the Committee on Scientific Research and Ethics of the Federal Medical Centre Azare, Nigeria with reference number 1125/21. Before the examination, the researchers ensured that all subjects consented to take part in the study after a detailed explanation of the reason for the study, benefits, possible effects, and stages of the examination. The study was performed under the

ethical standards and its later amendments of the 1964 World Declaration of Helsinki.

Data analysis

The analysis was performed with the Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, Illinois, USA) where descriptive statistics such as tables and charts were generated. Pearson's correlation was calculated, and a t-test was performed to compare group differences. $P < 0.001$ was regarded as a statistically significant value.

RESULTS

Demographic characteristics

One hundred (100) pregnant women with Sono-graphically enlarged spleen length in one group and twenty (20) healthy pregnant women in another group were involved in this study. The population of the study has an age range of between 17 years and 56 years with a Mean Age of 26.12 and a Standard Deviation (SD) of ±3.7. The age group of 17 to 25 years have the highest frequency consisting of about 60% of the population. The Weight (in Kg) of the population ranges from 41–90 Kg with a Mean weight of 67.00 and SD of ±11.0. while the height in meters ranges from 1.41-1.72 meters with Mean = 1.57 and SD = ±0.16. The BMI of the population with splenomegaly ranges from 16.65-34.95 with Mean = 24.02 and SD = ±0.15 as shown in Table 1.

Table 1: Means and standard deviations of the population

Demography	Mean	Min	Max	SD
Age	26.12	17	57	±3.70
Weight	67.00	41	90	±11.00
Height	1.57	1.41	1.72	±0.16
BMI	24.02	16.65	34.95	±1.50

The BMI of patients with splenomegaly which is also according to WHO a measure for indicating nutritional status in adults is presented in Table 2 showing the classification of the BMI findings in this research.

Table 2: Classification of BMI of patients with splenomegaly according to the World Health Organization

Range	Implication	Freq.	%age
Less than 18.5	Underweight	10	10.0
18.6-25.0	Normal	60	60.0
25.0-30.0	Overweight	20	20.0
Above 30	Obesity	10	10.0
Total		**100**	**100.00**

Patients (pregnant women with enlarged spleen) were classified according to the period of their pregnancy on a trimester basis. A larger percentage of the patients were in their third trimester (Figure 1).

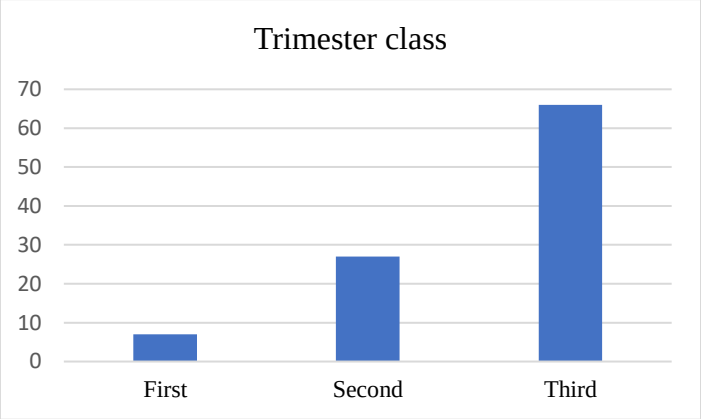

Figure 1: Bar chart showing the classification of the pregnancy period (in a trimester) of the population

The findings of the means and SD of the control group (n=20) were presented in Table 3, and all mean values were found to be within the normal range, except for ESR with a slight rise in sedimentation rate however, still considered insignificant compared to the cohort with splenomegaly.

Table 3: Means and SD of control sample population.

Test	Mean	SD
Spleen length	11.11	±0.20
BMI	23.55	±0.01
PCV	32.00	±0.00
Retics count	2.30	±0.01
ESR	81.00	±3.0

Prevalence of anemia-induced tropical splenomegaly.

Spleen enlargement as a clinical suspicion of anemia was considered to qualify patients for hematological investigations leading to the confirmation of anemia. The measurements of the spleen length in the experimental study range from 12.1 cm to 18.5 cm with a Mean length of 14.88 and SD = ±2.0. Blood sample findings of packed cell volume (PCV) showed a slightly low percentage of haemoglobin % levels with a mean and SD of 29.29 ±2.0. Reticulocyte count (Retics) was conducted to evaluate the bone marrow's capacity to boost the production of RBCs in response to different types of anemias. However, the results are within normal values and range from 1.5 to 3.2 with a Mean and SD of 2.14 ±0.62. (Table 4). Also, to assess other possible causes of asymptomatic underlying diseases like inflammation, an erythrocyte sedimentation rate (ESR) test was performed, and the result (in mm/hr) range from 41-160 with Mean and SD of 105.10 ±27.7 as presented in Table 4.

Table 4: Distribution of spleen length, PCV range, Reticulocyte count and ESR range

Range	Frequency	Percentage
Spleen length (cm)		
12.1-13.9	49	49.0
14.0-15.9	35	35.0
16.0-18.5	16	16.0
Total	100	100.00
PCV (%)		
14-19	19	19.00
20-25	49	49.00
26-32	32	32.00
Total	100	100.00
Retics count (%)		
1.5-2.5	85	85.0
2.6-3.5	15	15.0
Total	100	100.00
ESR (mm/hr)		
41-60	8	8.00
61-80	11	11.0
81-100	20	20.0
101-120	33	33.0
121-140	17	17.0
141-160	11	11.0
Total	100	100.0

Microscopic malaria parasite test showed that only 21% of the population had positive results, indicating the presence of plasmodium as shown in Figure 2

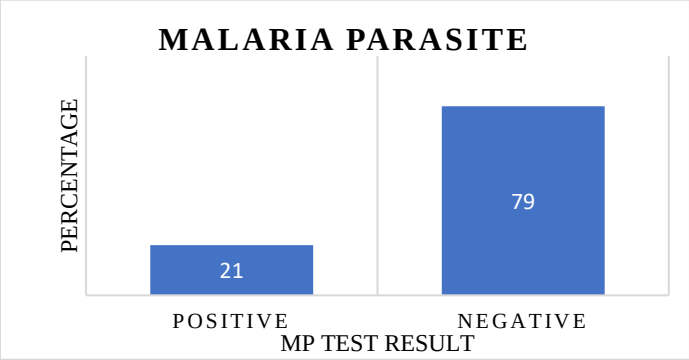

Figure 2: Bar chart showing the % of MP test results of the population

DISCUSSION

Although there is no statistically fixed and comprehensive data on the normal values for abdominal organ lengths, especially across different geographical, racial, and socio-economic classes, however, a spleen length value ranging between 10 and 12 cm according to [13] has been cited in various works of literature as normal. It has been accepted in clinical practice, especially in areas of the current study. The study has found a mean spleen length of 11.11±0.20 in the control cohort which is in tandem with studies by Mustapha et al.[13] and Ehimwenma and Tagbo[14] conducted within the region of the present study. Other laboratory examinations in the control group also revealed normal ranged PCV values affirming the absence of anaemia. The measurements of the spleen length in the experimental study group consisting of 100 pregnant women ranged from 12.1 cm to 18.5 cm with a mean length of 14.88±2.0. The spleen enlargement is a clinical suspicion of anaemia, and subsequent haematological investigations revealed elevated levels of PCV values which serves as a common tool for confirmation of anaemia in the study area. The indices of this comparison have matched the raised research question about splenomegaly being induced by anaemia in pregnancy.

Malaria parasite (MP) infection has been a major public health issue and was regarded as a national health concern in Nigeria[15]. Therefore, to exclude MP as a cause of anaemia, a general microscopic MP test on all

subjects revealed that about 80% of the research population is negative. This is indicative of the use of preventable measures such as the use of mosquito nets and other measures in line with [15].

A study by Akinbami et al.[1] in Lagos Nigeria reveals that the mean ± SD value of PCV in the 1st, 2nd and 3rd trimesters was 32.07% ± 6.80%, 29.76% ± 5.21%, and 33.04% ± 3.88% respectively, in addition, Lara[16] reported that PCV drops to roughly 34% and 30% in late single and multifetal pregnancies from between 38% and 45% respectively in healthy non-pregnant women. The WHO criteria state that a PCV of <30% is considered anaemic[3]. Also, Olatumbosun et al.[17] reported the mean PCV from their research on pregnant women in Uyo, Nigeria was 31.8% ±3.2 and 54.5% of the women were anaemic. All patients with splenomegaly in this study showed a lower percentage of PCV compared to Akinbami et al.[1] and Olatumbosun et al.[17] as well as the control PCV result of this study which showed a mean PCV of 32%. From the results of this research, 66% of the population are in their third trimester while 27% and 7% are in their second and third trimesters respectively. This suggests that the reduced PCV and the enlargement of the spleen signal anaemia in pregnancy in agreement with Khaled and Seifeldein[6], however, this study cannot tell the degree as further laboratory investigations might be required to that effect, whereas a study by Wei et al.[4] concludes that, this phenomenon is considerably caused by extramedullary haematopoiesis.

The Westergren-based ESR test has been in clinical use for a long time as an indicator of sickness. This could be due to its reproducibility and low cost[17]. Although, it is not specific to any single disease. Previous studies[18] have reported normal ESR values as ≤30 mm/hr in adults. Normal subjects from the current study showed a mean ESR value of 81 mm/hr with an SD of ±3.0 indicating an increase, however, according to Tishkowski and Gupta[18], a slight elevation can occur due to laboratory errors, ageing, menstruation or pregnancy. This may explain the slight rise in ESR values of healthy pregnant women. There is, however, extreme elevation (above 100 mm/hr) on subjects with splenomegaly with a mean of 105.10 and SD = ±27.7. Although increased ESR values should only raise a suspicion index that there is a potential basal illness underlying like anemia, infection, arthritis etc. Studies suggest that, if the level is highly elevated (greater than 100 mm/hr), usually, there is an apparent cause.

Reticulocyte counts in this study are used as a sign of anemia and a response of bone marrow to anemia. A healthy person's normal reticulocyte count is between 0.5% to 2.5% [1]. In this study, the mean reticulocyte counts were 2.30±0.01 in the research control group with approximately 85% of results within the normal range in the experimental group. The remaining 15% falls between 2.6% and 3.6% and could be due to Haemolytic anaemias where the bone marrow is yet operating and responding to anaemia by increasing the production of reticulocytes.

The BMI findings of the group showed the classification according to the WHO definition where only 10% are in the obesity class and 10% are underweight in contrast to 80% that are within normal (20% normal and 60% overweight). Although in some advanced regions hospital practice protocols for the management and care of people with pregnancy and women planning to have pregnancy with obesity are implemented[19-21], the current study indicates that the BMI of most subjects (80%) in this part of tropical Africa are of the normal range suggesting a balanced nutritional status in line with WHO recommendation.

In general terms, the sonographic and laboratory findings of this study suggest a nexus between splenomegaly and anemia in pregnant women within the study area. To the best of the researchers' knowledge, this is the first study to investigate the relationship between splenomegaly and anemia among pregnant women. Amplification of this research with larger cohorts and wide-ranging areas is recommended.

Limitations of the study

The study faced recruitment challenges from participants with normal spleen lengths (control group) who were unwilling to join the research. Additionally, some participants withdrew during the second phase of the study which involves invasive procedures. This

resulted in a small number of the research control group.

CONCLUSION

Findings of this study, indicate that there is a nexus between spleen enlargement and anemia among pregnant women in the study area without any underlying health condition or previous history of organomegaly. Wide-ranging research is recommended to gather additional information that can help reduce the incidence of IDA and, consequently, maternal death in expectant mothers.

STATEMENTS AND DECLARATIONS

Competing interest

The authors of this research have no competing interests relevant to this article's content.

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Ethics approval

The study was conducted according to the guidelines of the Declaration of Helsinki. The Committee on Ethics of the Federal Medical Centre Azare approved the research with reference number 1125/21.

Author contributions

All the authors of the research contributed to the study's conceptual and design framework. Preparation of materials, data collection and analysis were performed by [BSY], [HS], [AA] [MMS] [NY] and [UA]. The first draft of the manuscript was written by [BSY]. Comments by all authors on previous versions of the manuscript were amended and all authors read and approved the submission of the final manuscript for publication.

Data Availability

The data collated during the current study will always be available for any justifiable request made through the corresponding author.

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