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# Establishment of a New early Diagnostic criteria for Dilutional Anemia using Maternal Total Plasma Proteins levels and Hemoglobin Concentrations Among Pregnant Women Attending Antenatal Clinic at University of Calabar Teaching Hospital, Calabar

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# Abstract:

# Background: Dilutional anaemia has been hypothesized to be a common complication in pregnancy usually characterized by decreased hemoglobin concentration due to increased blood volume and changes in maternal total plasma protein level. However, accurate measurement of combined hemoglobin and total plasma protein levels are yet to be recognized by researchers as early new diagnostic criteria parameters for its, management and differentiation from other types of gestational anemias

# Objective: The index study was aimed at establishing a new early criterion for the diagnosis of dilutional anemia using maternal total plasma protein levels and maternal hemoglobin concentration estimated using five different types of hemoglobinometers during the trimesters of pregnancy among pregnant women attending the Antenatal clinic at University of Calabar Teaching Hospital (UCTH) Calabar compare to non-pregnant women.

# Methods: This cross-sectional study involved 400 participants comprising of (n=200 registered pregnant women (50%) and n=200 non-pregnant women (50%) with ages from 18 years and above , who had been previously counseled and had completed an opened-ended, semi-structured self-administered questionnaire form after providing informed / written consent. All blood samples collected by standard methods, were analyzed for total plasma proteins levels using Biuret’s spectrophotometric method, while hemoglobin concentration was measured using HemoCue rapid diagnostic test, micro-hematocrit /Packed cell volume (PCV), Copper sulphate (CuSO4) gravimetric method, Tallquist’s hemoglobin scale and Sahli’s method (also called as acid hematin method which is the visual comparator method for the estimation of hemoglobin).

# Key findings: This study highlights the importance of accurate hemoglobin concentration measurement throughout the three trimesters of pregnancy using reliable five different types of point of care testing rapid diagnostic hemoglobinometers. The combined hemoglobin concentration and total plasma protein levels suggests additional potential utility in diagnosing dilutional anemia among pregnant women compared to non-pregnant women.

**Results:** The Mean age ± SD (years) for pregnant women was 24.99±1.01 compared to nonpregnant women 29.95±7.85 with statistically significant difference between ages (P<0.05, t=7.2822, P=0.0001).The mean value plus one standard deviation (X±SD) of total plasma protein (TPP) levels in g/dl for first ,second, third trimesters and total for pregnant women at which dilution anemia occurred were 6.47 ± 0.88, 5.7± 0.76, 5.6 ± 0.67 and 5.92 ± 0.77 respectively compared to 7.48±.79 for non-pregnant women. The mean value plus one standard deviation of hemoglobin concentration in g/dl per trimester using the five different type of hemoglobinometers at which dilutional anemia occurred were 12.77±.52,11.4±.23,10.9±.89 and 10.98±.36 respectively for pregnant women compared to 12.84±1.7 for nonpregnant women .The prevalence of dilutional anemia was significantly higher among pregnant women (03.5%) compared to non-pregnant women (1.5%).

# Keywords: new criteria, diagnosis of dilutional anemia, hemoglobin concentration, total plasma protein, pregnant women, non-pregnant women , five hemoglobinometers types

1. **INTRODUCTION**

Pregnancy is a state of having implanted products of conception located either in the uterus or elsewhere in the body and usually ends through either spontaneous or elective abortion or delivery [1]. Since 1965, the American College of Obstetricians and Gynecologists (ACOG) have defined pregnancy as beginning when an embryo implants in the uterine wall and this definition have been accepted by many researchers worldwide [2,3,4,5,6,7]. During the duration of period of pregnancy, the mother’s body goes through immense changes involving all organ systems to sustain the growing fetus [8]. All medical providers must be aware of these alterations present in pregnancy to be able to provide the best possible care for both mother and fetus [9]. Pregnancy usually lasts about 40 weeks, or just over 9 months, as measured from the last menstrual period to delivery. Health care providers refer to three segments of pregnancy as “trimesters” [10]. The events of the first trimesters starts from one week to week 12 and lead to pregnancy beginning with conception or fertilization in which a sperm penetrates an egg. The fertilized egg (called a zygote) then travels through the woman's fallopian tube to the uterus, where it implants itself in the uterine wall. The zygote is made up of a cluster of cells that later form the fetus and the placenta. The placenta connects the mother to the fetus and provides nutrients and oxygen to the fetus [11]. The second trimester covers week 13 to week 28. Between 18 and 20 weeks, represent the typical timing for ultrasound to look for congenital anomalies and also to find out the sex of the index baby. At 20 weeks, a woman may begin to feel fetal movements. At 24 weeks, footprints and fingerprints have formed and the fetus sleeps and wakes regularly. According to research from the NICHD Neonatal Research Network, the survival rate for babies born at 28 weeks was 92%, although those born at this time will likely still experience serious health complications, including respiratory and neurologic problems [12]. The third trimester starts from week 29 to week 40. At 32 weeks, the bones are soft and yet almost fully formed, and the eyes can open and close. Infants born before 37 weeks are considered preterm. These children are at increased risk for problems such as developmental delays, vision and hearing problems, and cerebral palsy. Infants born between 34 and 36 weeks of pregnancy are considered to be "late preterm"[13]. Infants born in the 37th and 38th weeks of pregnancy—previously considered “term”—are now considered "early term." These infants face more health risks than infants who are born at 39th weeks or later 40thweeks, is now considered “full term” [14]. Full-term infants have better health outcomes than do infants born earlier or, in some cases, later than this period. Therefore, if there is no medical reason to deliver earlier, it is best to deliver at or after 39 weeks to give the infant's lungs, brain, and liver time to fully develop [15,16]. Infants born at 41 weeks through 41 weeks and 6 days are considered “late term”, while those infants born at 42 weeks and beyond are considered “post-term”[17].

Epidemiologically, about 213 million pregnancies occurred in 2012, of which, 190 million (89%) were in the less developing countries of the world and 23 million (11%) were in the developed countries of the world. The number of pregnancies in women with ages between 15 and 44 were 133 per 1,000 women [18]. According to recent study an estimated number of pregnancies worldwide each year stands at 208 million, with 46% of them being unintended. According to another study published in 2023, reported that the global adolescent birth rate (ABR) was 41.3% births per 1,000 women aged between 15–19 years and this was lower from 64.5% in 2000 [19]. Similarly , the number of births in the United States was 3,596,017, which recoded 2% decrease from 2022 and the general fertility rate for the United States in 2023 was 54.5% births per 1,000 females ages between 15–44 years, which also recorded a 3% decrease from 2022 [20]. About 10% to 15% of recognized pregnancies end in [miscarriage](https://en.wikipedia.org/wiki/Miscarriage) [21]. In 2016, complication of pregnancy resulted in 230,600 maternal death, down from 377,000 deaths in 1990. Common causes include different types of anemias that resulted from bleeding , [infections](https://en.wikipedia.org/wiki/Postpartum_infections), hypertensive diseases in pregnancy, obstructed labor, miscarriage, abortion, or ectopic pregnancy [22]. Globally, 44% of pregnancies are unplanned and more than half (56%) of unplanned pregnancies are aborted [23]. Among unintended pregnancies in the United States, 60% of the women used birth control to some extent during the month pregnancy began [24]. This is in sharp contrast to Nigeria pregnancy data , that recorded a total of 10,500,000 pregnancies annually in 2015 to 2019, and with 2,990,000 being unintended and 1,430,000 ended in abortion [25].

**Statement of the problem**

Anemia has been considered as one of the most frequent complications related to pregnancy [26]. Normal physiologic changes in pregnancy affect the hemoglobin (Hb) and studies have shown that there is a relative or absolute reduction in Hb concentration. The most common true anemias during pregnancy is gestational anaemia, defined as hemoglobin level below 11g/dl, which is caused by iron deficiency anemia (approximately 75%) and folate deficiency megaloblastic anemia, which are more common in women who have inadequate diets and who are not receiving prenatal iron and folate supplements. It is associated with severe anemia which may have adverse effects on the mother and the fetus. Anemia with hemoglobin levels less than 6 g/dl is associated with poor pregnancy outcome such as prematurity, spontaneous abortions, low birth weight, and fetal deaths are complications of severe maternal anemia. Nevertheless, a mild to moderate iron deficiency does not appear to cause a significant effect on fetal hemoglobin concentration. An Hb level of 11 g/dl in the late first trimester and also of 10 g/dl in the second and third trimesters are suggested as lower limits for Hb concentration. In an iron-deficient state, iron supplementation must be given and follow-up is indicated to diagnose iron-unresponsive anemias [27]. Dilutional anaemia also known as physiologic anemia of pregnancy is one of the most common cause of anemia in pregnancy and may be caused by other pathological conditions which are well contrasted and distinguished from gestational anaemia. It may be cause by chronic inflammatory conditions such as infectious disease, autoimmune diseases, chronic kidney diseases and malignancies, thus leading to increased plasma volume expansion [28,29].During pregnancy, the RBC volume increases by 20% to 30%, while the plasma volume increases by 45 to 55%. This disproportionate volume increase leads to dilutional anemia with decreased hematocrit. WBC count increases to 6 to 16 million/mL and can be as high as 20 million/mL during and shortly after labor. Platelet concentration decreases slightly due to the increased plasma volume but typically stays within normal limits. A small proportion of women (5 to 10%) will have platelet levels between 100 and 150 billion/L without any pathology present. Fibrinogen and factors VII – X levels increase, but the clotting and bleeding times remain unchanged. However, increased venous stasis and damaged vessel endothelium result in higher rates of thromboembolic events during pregnancy. The increase in the risk of thromboembolic events starts in the first trimester and continues at least 12 weeks postpartum [28,29].Assessment of hemoglobin is one of the most reliable indicators for anemia, and is widely used to screen for anemia among pregnant women and others pathological condition involving shortages of blood. The HemoCue, Packed cell volume (PCV) micro-hematocrit, Copper sulphate (CuSO4) gravimetric method, Tallquist’s hemoglobin scale and Sahli’s method had been widely used as a point-of-care devices for hemoglobin estimation in some health facilities that are considered as resource-poor setting where there is no availability of power supply or electricity. In recent time high or low levels of total plasma proteins have been hypothesized to have significance effects on the accuracy of the results from these instruments [30].

**Justification and rational of the study**

During normal pregnancy, there is a greater expansion of the plasma volume relative to the increase in the red cell mass, which results in dilutional anemia, termed as the physiologic anemia of pregnancy. Although this type of anemia is mild (hemoglobin of 10 to 11 g/dl), there is no specific hemoglobin or hematocrit value that can distinguish physiologic anemia from other causes of anemia in pregnancy such as gestational anaemia [31,32].More so there is paucity of knowledge on the relationship between dilutional anemia, maternal total plasma protein levels and maternal hemoglobin concentration estimated using different types of hemoglobinometers during the trimesters of pregnancy among pregnant women compared to non-pregnant women in the study area .

**Research question**

1. What is the new criteria for the diagnostic dilutional anemia during the trimesters among pregnant women attending antenatal clinic at University of Calabar Teaching Hospital Calabar compared to non-pregnant women ?

2. what is the levels of maternal total plasma protein levels and maternal hemoglobin concentration during the trimesters of pregnancy among pregnant women attending antenatal clinic in University of Calabar Teaching Hospital ,Calabar compare to nonpregnant women?

3. what is the hemoglobin concentration estimated by the five different hemoglobinometers during the trimesters of pregnancy among pregnant women attending antenatal clinic at University of Calabar Teaching Hospital Calabar compare to nonpregnant women .

**General objective**

The current study was aimed at establishing a new criteria for diagnostic of dilutional anemia using maternal total plasma protein levels and maternal hemoglobin concentration estimated using five different types of hemoglobinometers during the trimesters of pregnancy among pregnant attending the Antenatal clinic at University of Calabar Teaching Hospital (UCTH) Calabar compare to non-pregnant women

**Specific objective** **of the study**

1. Determine the maternal total plasma protein levels during the three trimesters of pregnancy among pregnant women attending antenatal clinic at University of Calabar Teaching Hospital Calabar compare to non-pregnant women .
2. Determine the maternal hemoglobin concentration estimated by five different types hemoglobinometers during the trimesters of pregnancy in pregnant women attending antenatal clinic at University of Calabar Teaching Hospital Calabar compare to nonpregnant women
3. Determine prevalence of dilutional anemia during the trimesters among pregnant women attending antenatal clinic in University of Calabar Teaching Hospital Calabar compare to non-pregnant women ?

**3) METHODOLOGY**

**3.1) Study Setting**

**Study area :** The study area of this index study was carried out in the Antenatal clinic of the Department of obstetrics and gynecology, at University of Calabar Teaching Hospital (UCTH) which is a public hospital located in Calabar, Cross River State, Nigeria. UCTH came into existence in 1979 following the need for a Tertiary Health Institution that will render clinical services at a level that meets the requirements for the training of medical students at the College of Medical Sciences of the University of Calabar, resident doctors for the different postgraduates as well as other healthcare providers in different specialties of medicine and its allied professions. The UCTH offers tertiary health care to the residents of Cross River State, surrounding states and counties like southern Cameroon and Equatorial Guinea. [33,34,35] University of Calabar Teaching Hospital (UCTH) is under Calabar municipality and Calabar is the present capital of Cross River State in the south eastern part of the Federal Republic of Nigeria [36] Geographically, Calabar has a total land area surface of 142 km² while the total local government area population is estimated to be 320,826 of which 166,203 are males and 154,659 females [37]. The inhabitants are mainly of the Efiks, Quas, Ejagham, Efut, Ibibio, Annang by tribe and others– include the migrant workers and mixed multitudes. They are mainly civil servants, subsistence farmers, traders and fishermen. There are many important, primary, secondary, tertiary health facilities and educational centers belonging to either federal or state government in Calabar municipality [38].

**3.2 Sites for Participant Recruitment and Pre-Counseling for Samples Collection**

The participants of the current study were made up of apparently healthy individual who presented to the blood donors department of UCTH Calabar for eligibility assessment and screening profile for blood donation.

**3.3 Sampling Techniques**

This study utilizes the convenient and random sampling method in the selection and enrolment of participants who were found to serve as eligible voluntary apparently healthy prospective blood donors and who gave their written/ informed consent. This study adopted a cross-sectional approach which was conducted within a year period (2021 to 2022).

**3.4 Study participants**

The participants were enrolled at the University of Calabar Teaching Hospital, Calabar, Cross River State. The documentation of the study participant’s demographics, blood transfusion history, risky behavioral conduct, number of sexual relationships, drug injection history and clinical background was done using the semi close- ended research questionnaire prepared, verified and adopted for this study.

**3.5 Study Design**

Experimental and analytical designed were adopted in this study and all collected samples for estimation of hemoglobin concentration and total plasma protein concentration was carried out in the Department of Hematology & Blood Transfusion Sciences and total plasma proteins levels from the Department of Chemical Pathology,Faculty of Medical Laboratory Science, University of Calabar, Cross Rivers State ,Nigeria.

**3.6 Calculation of Sample Size**

The Formula of Cochran, 1977, for calculating the sample size (S) was adopted in current study and is denoted by formula viz: [38]**: S= t2 p (1-p)/ ҽ2** , *Where* t= t value (The alpha level used in determining sample size in most educational research studies is either 0.05 % or 5% **.** In Cochran’s formula, t-value for alpha level of .05 is 1.96 for 95% confidence level for sample sizes above 120*.***P=** prevalence rate in percentage (%) from previous study of estimation of hemoglobin concentration in non-Caucasian population in Calabar and in this case it is taken to be 0.5 or 50% since someone had never worked on this population [39,40] While **ҽ =** tolerance error or confidence interval expressed as decimal and it is taken to be 0.05.Therefore **S =** (1.962)2 (.5(1-0.5)/ (0.05)2, S = (1.962)2 (0.5)2/ (0.05)2 = 384.16 ,hence S = ~ 400 subjects were used in cases of any loss data or specimen during the study or in cases of non-respondent individuals .

Correction for a small/finite population below 10,000 the formula viz: **n=no/1+(no-1)/N** was used.

where n is the corrected sample size, n0 is the calculated sample size and N is the population size. Therefore n=384/1+(384-1)/5650=360= minimum sample size needed.

Non- respondent rate =384/1-0.1=384/.9=426 samples

Approximately 430 as maximum samples were collected by convenient sampling techniques after correction for missing or spoiled samples [41,42,43,44].

**3.7 Inclusive and Exclusive Criteria for Selection of Participants**

A total of 400 apparently healthy pregnant women, and non-pregnant aged from 18years and above and who were randomly recruited attending the antenatal clinic at University of Calabar Teaching Hospital Calabar, Cross River State, Nigeria. The participants were divided into two study groups according to their ages and pregnant status and a questionnaire form designed and prepared for this purposed, was used for both inclusive and exclusive criteria.

**3.10 Administration of Questionnaire**

The harmless nature and advantage of the research was also explained to each participant in the form of pre-counselling in which the prepared questionnaire forms were administered on each of the participants to obtain more medical information about the clinical history and pregnancy history . After the Previous counselling, informed consent forms were filled and signed by these participants for research to start **.**

**3.11 Study Population**

A total of 400 apparently healthy pregnant women and non-pregnant women aged from 18 years and above were recruited from the antenatal clinic at University of Calabar Teaching Hospital Calabar. Recruited participants were previously counseled and screened in accordance with the Questionnaire form designed, validated and prepared to be adopted for this purpose.

**3.12 Method for Collection and Treatment of Blood Samples**

About five milliliters of venous blood samples was withdrawn from the antecubital vein of the arms of previously counseled and screened apparently healthy pregnant women and non-pregnant . By a mean of a disposable plastic five milliliters syringe fitted with 19 SWG needle. The area of venipuncture was first of all cleaned with methylated spirit (70%) alcohol and allowed to dry. A tourniquet was tied just for a short time. The withdrawn samples were put into sample bottles containing 4mg of potassiumethylene dimethylamine tetra acetic acid (K2 EDTA) and thoroughly mixed immediately. The samples were used for determination of hemoglobin concentrations and those samples that were not analyzed immediately within 2 hours of collection were stored at 4o C – 6oC. The samples were usually spun at 4000 rpm for 10 minutes to harvest plasma which were used for estimation of total plasma protein stored at -20oC and the screening was done within 7 days. Collection and preparation of blood samples were done from Monday to Friday of each week and between the hours of 7.00am and 5.00 pm.

**3.13 Laboratory Methodology for Analyzing Various Parameters**

**1)HemoCue** : The HemoCue is a point-of-care device that uses spectroscopy to measure hemoglobin concentration

**Principle:** The HemoCue uses a specific wavelength of light to measure the absorbance of hemoglobin in a blood sample)The color intensity of this mixture is measured in a colorimeter at a wavelength of 540nm or using a yellow green filter. The absorbance of the solution is proportional to be concentration of hemoglobin in the whole blood sample. All forms of hemoglobin are measured with this method, except sulfhaemoglobin

**Procedure**: - Obtain a blood sample from the patient, - Place the blood sample on the HemoCue device, - Close the device and wait for the measurement to be taken, - Read the hemoglobin result on the device [45,46].

**Method and procedure for microhematocrit or PCV (Packed Cell Volume)**: The microhaematocrit is a laboratory test used to measure the packed cell volume (PCV) of blood.

**Principle**: The microhaematocrit method uses centrifugation to separate the blood cells from the plasma Whole blood is centrifuged for maximum red blood cell packing. The space occupied by the red cells in measured and expressed as percent of whole volume [45,47].

**Procedure:**- Obtain a blood sample from the patient**,** - Place the blood sample in a microhaematocrit tube**,** - Centrifuge the tube for 5 minutes**,** - Read the packed cell volume (PCV) by measuring the height of the packed cells in the tube [47]

**Copper sulphate solution method:** The copper sulfate solution method is a laboratory test used to measure the specific gravity of blood **.**

**Principle**: The copper sulfate solution method uses the principle of specific gravity to measure the density of the blood The principle of the copper sulphate solution method is based on the fact that, when whole blood is dropped into a solution of CuSO4, the CuSO4 reacts with the protein at the periphery of the drop to form copper proteinate which acts as a protective membrane. Thus, preventing the dispersion of the drop. Whether or not the drop will float or sink is dependent on the hemoglobin concentration in it. (Phillips et al, 1950, and Henry et al, 1974)[9, 31,32].

**Procedure:** - Obtain a blood sample from the patient**,** - Mix the blood sample with a copper sulfate solution**,** - Centrifuge the mixture for 5 minutes, - Read the specific gravity of the resulting solution using a hydrometer [48]

4)**Sahli device**: The Sahli device is a manual device used to measure hemoglobin.

**Principle**: The Sahli device uses the principle of colorimetry to measure hemoglobin.

**Procedure**:-Obtain a blood sample from the patient, - Mix the blood sample with a solution of potassium ferricyanide and potassium cyanide, - Place the mixture in the Sahli device, - Compare the color of the mixture to a standard color chart,- Read the hemoglobin result from the chart[49]

5)**Tallquist**:The Tallquist method is a manual method used to measure hemoglobin.

**Principle:** The Tallquist method uses the principle of acid hematin formation to measure hemoglobin.

**Procedure:** - Obtain a blood sample from the patient**,** - Mix the blood sample with a solution of hydrochloric acid

- Place the mixture in a spectrophotometer, - Measure the absorbance of the mixture at a specific wavelength, - Read the hemoglobin result from a standard curve [50]

**6) Method and procedure for biuret’s method:** The principle of Biuret method or Biuret reaction is based, on the fact all proteins contain a large number of peptide bonds. When a solution of protein is treated with Cu2+ in a moderately alkaline medium, a violet color chelating-complex is formed between the Cu2+ and the carbonyl (=COOH) and amino (=N-H) groups of the peptide bonds, the intensity of the color changed produced is proportional to the number of peptide bonds presence or (undergoing in the reaction), when measured calorimetrically at 540 nm [45]

**3.14) Method of Data Collection and Statistical Tools for Data Analysis**

After codification and collation of the raw data for both sexes of the results were entered and subjected to statistical analysis using Statistical Package for Social Students software version 26 (SPSS Incorporation, Chicago, United State America). Data were represented with frequency and percentages while continuous data were expressed as mean plus or minus standard deviations (X±SD). One sample Kolmogorov-Smirnov test was used to assess the normality of the data. All data were normally distributed; hence, parametric procedure was used for the statistical analysis of the data. The prevalence rate formulae were used to calculate the prevalence rate. A two tailed p-value of <0.05 was considered indicative of a statistically significant difference. Comparison of the parameters and variables between the samples were performed using independent t-test while comparison among various age groups were analyzed using ANOVA. Association between variables was analyzed using Chi Square and Fischer exact test. Alpha value of 0.5 was used. Coefficient of Variation (CV) Formula given by CV=σ/μ, where: σ=standard deviation and μ=mean was used to calculate the coefficient of variation of the desire variables.

**3.15) Calculation of the prevalence of dilutional anemia during pregnancy from hemoglobin concentration, using the using the five different types of hemoglobinometers during the three trimesters of pregnancy involved the following steps**:

**Step 1**: Define the criteria for dilutional anemia, Dilutional anemia is typically defined as a hemoglobin concentration below 11.0 g/dl in the first trimester, below 10.5 g/dl in the second trimester and below 9.5 g/dl in the third trimester [51].

**Step 2**: Collect the data on hemoglobin concentration from the pregnant women throughout the trimesters of pregnancy among the index study population while noting the total protein levels .

**Step 3**: Categorize the data based on trimesters of pregnancy (first, second, or third and total compared to non-pregnant ).

**Step 4:** Calculate the prevalence of dilutional anemia by dividing the number of women with hemoglobin concentration below the cutoff value for each trimester by the total number of women in that trimester.

The formula for calculating prevalence is:

Prevalence = (Number of women with dilutional anemia / Total number of women) x 100

**Step 5**: Report the results

Report the results as the prevalence of dilutional anemia in each trimester, along with the corresponding 95% confidence intervals.

**4) RESULTS**

The results obtained for the current study are shown in the Tables 1, 2 and 3.Table1 shows the results of the frequency distribution by demographic parameters and Age range of participants recruited within UCTH Calabar, Cross River State, Nigeria . A total of 400 blood samples were collected using standard procedures from apparently healthy individuals comprising of pregnant women] n=200 (50%) ] and non-pregnant women [n= 200 (50%)] and with ages from 18 years and above, All apparently healthy individuals recruited have been consented and previously counselled before recruitment into the study. The Mean age ± SD (years) for pregnant women was 24.99±1.01 compared to nonpregnant women 29.95±7.85 with statistically significant difference between ages (P<0.05, t=7.2822, P=0.0001).

**Table 1.** **Distribution by demographic parameters and Age range of pregnant women and non-pregnant women attending Antenatal clinic at UCTH Calabar, Cross River State, Nigeria**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No of  Group | Age Range  (Year) | Pregnant women  Frequency (%) | Non- pregnant women  Frequency (%) | Total  Women  Frequency (%) | t-value  *P*-value remarks |
| 1 | 18-20 | 20 (5) | 25 (6.25) | 45 (11.25) | 7.2822 0.0001 p<0.05 S\* |
| 2 | 21 -25 | 50 (12.5) | 35 (8.25) | 85 (21.25) |  |
| 3 | 26-30 | 46 (11.5) | 50 (12.5) | 96 (24) |  |
| 4 | 31-35 | 45 (11.25) | 55 (13.75) | 100 (25) |  |
| 5 | 36 -40 | 34 (8.5) | 25 (6.25) | 59 (14.75) |  |
| 6 | 41 -45 | 5 (1.25) | 10(2.5) | 15 (3.73) |  |
| Totalsample |  | 200 (50) | 200 (50%) | 400 (100%) |  |

S \* denotes statistically significant difference

**Table 2. Results of Means Values of Parameters done during first, second and third trimesters of pregnancy among pregnant women and non-pregnant women attending Antenatal clinic in UCTH Calabar, Cross River State, Nigeria**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter done |  | | Mean Values in first trimester of Pregnancy (X±SD) | Mean Values in second trimester of Pregnancy(X±SD) | Mean Values in third trimester  of Pregnancy  (X±SD) | Means Values  Total trimesters  of pregnancy  (X±SD) | Means Values  for Non-  pregnant  Women (X±SD) | p-values | Remarks |
| Total Plasma Protein | TPP | (g/dl) | 6.47 ± 0.88 | 5.7± 0.76 | 5.6 ± 0.67 | 5.92 ± 0.77 | 7.48 ± .79 | P<0.001 | (P>0.05) NS\* |
| Hb. Concentration | (HEMOCUE) | g/dl | 12.43 ± 1.92 | 11.6 ±0.14 | 11.4 ± 0.41 | 11.81 ±.82 | 12.77 ± 2.20 | P<0.001 | (P>0.05 ) NS\* |
| Microhaematocrit | (PCV) | % | 40.51 ± 1.7 | 31 .10 ± 2.7 | 29 .11 ± 3.1 | 33.57 ± 2.5 | 39.16 ± 8.24 | P<0.001 | (P>0.05)NS\* |
| (Hb. Equivalent in |  | (g/dl) | 13.50 ± .60 | 10.4±0.9 | 9.70±1.0 | 11.2 ± .80 | 13.05 ± 2.75 | P<0.001 | (P>0.05)NS\* |
| Specific gravity of copper sulphate | CuSO4 (S.G.) | Spg | 1.058± 0.006 | 1.050 ±.001 | 1.045±.002 | 1.051 ± 0.003 | 1.055 ± 0.009 | P<0.001 | (P>0.05)NS\* |
| (Hb. Equivalent In |  | g/dl) | 13.60 ± 0.07 | 11.5± .13 | 10.5 ±.14 | 11.86 ± 0.11 | 12.5 ± 0.11 | P<0.001 | (P>0.05)NS\* |
| Sahli device | SHD | g/dl | 12.11± 0.02 | 11.9±.10 | 11.5±.11 | 11.50± 0.07 | 13.4± 0.06 | P<0.001 | (P>0.05)NS\* |
| Tallquist paper | TQP | % | 80 | 75 | 70 | 75 | 80 | P<0.001 | (P>0.05)NS\* |
|  |  | g/dl | 12.2±.12 | 11.6±.11 | 11.4±.10 | 11.7±.03 | 12.5±.14 |  |  |
| Total (X±SD) |  |  | **12.77±.52** | **11.4±.23** | **10.9±.89** | **10.98±.36** | **12.84**±**1.7** |  |  |

*NS \* denotes no statistically significant difference. There was no statistically significant difference between the results of total mean values and that of the male and female genders respectively (p >0.05). Using ANOVA, the association between rows (groups) and columns (outcomes) were not statistically significant (p >0.05).*

In **Table 2** the results of means values of parameters investigated during the three trimesters of pregnancy among pregnant women and non -pregnant women attending Antenatal clinic in UCTH Calabar, Cross River State, Nigeria are shown. The mean value plus one standard deviation (X±SD) of total plasma protein (TPP) levels in g/dl for first ,second, third trimesters and total for pregnant women were 6.47 ± 0.88, 5.7± 0.76,5.6 ± 0.67 and 5.92 ± 0.77 respectively compared to 7.48±.79 for non-pregnant women .The mean value plus one standard deviation of hemoglobin concentration using HemoCue in g/dl for first ,second , third trimesters and total for pregnant women were 12.43 ± 1.92, 11.6 ±0.14, 11.4 ± 0.41 and 11.81 ±.82 respectively compared to 12.77 ± 2.20 for non-pregnant women. The mean value plus one standard deviation of hemoglobin concentration using Microhematocrit (PCV) in % for first, second ,third trimesters and total for pregnant women were 40.51 ± 1.7 ,31 .10 ± 2.7, 29 .11 ± 3.1, 33.57 ± 2.5 respectively compared to 39.16 ± 8.24 for non-pregnant women. The hemoglobin equivalence in g/dl in the first,second, third trimesters and total for pregnant women were 13.50 ± .60, 10.4±0.9,9.70±1.0, and 11.2 ± .80, respectively compared to 13.05 ± 2.75 for nonpregnant .The mean value plus one standard deviation of hemoglobin concentration using Copper sulphate solution gave a specific gravity of 1.058± 0.006, 1.050 ±.001, 1.045±.002, 1.051 ± 0.003, respectively for first, second, third trimesters and total for pregnant women compared to 1.055 ± 0.009 for non-pregnant women.This gave hemoglobin concentration equivalence in g/dl of 13.60 ± 0.07,11.5± .13,10.5 ±.14 and 11.86 ± 0.11for pregnant women respectively compared to 12.5 ± 0.11for nonpregnant women. The mean value plus one standard deviation of hemoglobin concentration in g/dl using Sahli device for first, second , third trimesters and total were 12.11± 0.02,11.9±.10 ,11.5±.11 and 11.50± 0.07 for pregnant women compared to 13.4± 0.06 for non-pregnant women with corresponding hemoglobin concentration equivalence in g/dl of 12.2, 11.6, 11.4 and 11.7 respectively for pregnant women compared to 12.5 for nonpregnant women

The mean value plus one standard deviation of hemoglobin concentration using Tallquist for first, second ,third trimesters and total for pregnant women were 12.11± 0.02,11.9±.10,11.5±.11 and 11.50± 0.07 respectively compared to 13.4± 0.06 for non-pregnant and with corresponding equivalence hemoglobin concentration in g/dl of 12.2, 11.6, 11.4 and 11.7 respectively compared to 12.5 for non-pregnant women. The mean value plus one standard deviation of hemoglobin concentration in g/dl per trimester per five type of hemoglobinometers were 12.77±.52,11.4±.23,10.9±.89,10.98±.36, 12.84±1.7 respectively.

In **Table 3** the Comparative results of coefficient of variance CV (%) of parameters among apparently healthy pregnant women and non-pregnant women attending UCTH Calabar, Cross River State, Nigeria are shown. The coefficient of variance CV (%) of total plasma protein (PTP) was 15.4 %, Hemoglobin concentration (HemoCue) was 6.94 %, that of Microhaematocrit (PCV) was 7.14 % and that of Copper sulphate (CuSO4 method) was 0.93% , that of Sahli device was 6.08% and that of Tallquist paper was 0.26 %

**Table 3. Comparative result of coefficient of variance cv (%) of each parameter among pregnant and non-pregnant women attending antenatal clinic at UCTH Calabar , Cross River State, Nigeria.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters Investigated | | | Coefficient Variation | |
|  | Code | Unit | (%) |  |
| Total plasma protein | TPP | g/dl | 15.4 |  |
| Haemoglobin conc. | (HemoCue) | g/dl | 6.94 |  |
| Microhaematocrit | PCV | % | 7.14 |  |
| Copper sulphate solution | CuOS4 | % | 0.93 |  |
| Sahli device | SHD | g/dl | 6.08 |  |
| Tallquist paper | TQP | % | 0.26 |  |

**Key findings :** This study highlights the importance of accurate hemoglobin measurement throughout the three trimesters of pregnancy using reliable five different types of point of care testing rapid diagnostic hemoglobinometers. The combination of hemoglobin concentration and total plasma protein levels suggests additional potential utility in diagnosing dilutional anemia among pregnant women compared to non-pregnant women.

**5. DISCUSSION**

Dilutional anaemia has been hypothesized to be a common complication in the three trimesters of pregnancy, which is usually characterized by decreased hemoglobin concentration caused by increased blood volume and physiological changes in maternal total plasma proteins levels. Therefore, accurate measurement of maternal hemoglobin concentrations and total plasma protein levels remained the only crucial biomarkers for its diagnosis and management and differentiation from other complications of pregnancy. Studies have shown that over the years that pregnant women hemoglobin (Hb) concentration estimation is an important test that is performed during antenatal visit of pregnant women. This is because it plays the double role of protecting pregnant women health against anemia development, diagnosis and at the same time ensuring safety and healthy growth of the fetus [52].

Due to the fact that diverse cutoff criteria have been used for hemoglobinometry worldwide depending on the population characteristics, however, no testing methodology and sample requirement have been specified for hemoglobin screening. This is why the British Committee for Standards in Hematology (BCSH) [53] and the International Committee for Standardization in Hematology (ICSH) and the European Society of Hematology (ESH) [54] have been instituted. Besides the technique, there are several physiological and methodological factors that can affect accuracy, precaution, reproducibility and reliability of hemoglobin estimation such as the effects of the reference values and numerical ratios of total plasma protein (TPP) and hemoglobin concentrations (estimated using different types of hemoglobinometers). The objective of the index study was to evaluate the correlative relationship between dilutional anemia, maternal total plasma protein levels, and maternal hemoglobin concentration estimated using five different types of point of care testing rapid diagnostic hemoglobinometers during the three trimesters of pregnancy among pregnant and non-pregnant women in University of Calabar Teaching Hospital Calabar, Cross Rivers state Nigeria.

**Table 1** shows the results of the frequency distribution by demographic parameters and age range of participants recruited within Antenatal clinic, UCTH Calabar, Cross Rivers State, Nigeria. A total of 400 blood samples comprising of and from pregnant [n=200 (50%)] and non- pregnant women [n=200 (50%)] respectively and with ages between 18 to 60 years were collected using standard procedures. All a participant recruited within Antenatal clinic, UCTH Calabar were consented and counselled before recruitment into the study. The Mean age ± SD (years) for pregnant women were 24.99 ±1.01 and nonpregnant women were 29.95±7.85 respectively and with statistically significant difference between ages (P<0.05, t=7.2822, P=0.0001). From the results in Table 1 it is clearly seen that there was turn out and response rate difference between pregnant women and nonpregnant. There were more pregnant women than nonpregnant women who responded positively and turned out for the study. Therefore, using Chi Squared (*X*2) statistical stool there was a statistically significant differences between the response rate in the results. This is in line with previous findings that have been published by others authors who said that pregnant women may be more motivated to participate in studies due to several factors like personal investment reported by [54,55], health concerns report [56,57], sense of community reported by [58,59] ,incentives reported [60] and awareness of health risks reported by [61,62].

In **Table 2** the results of means values of parameters investigated during the three trimesters of pregnancy among pregnant women and non -pregnant women attending Antenatal clinic in UCTH Calabar, Cross River State, Nigeria are shown. The mean value plus one standard deviation (X±SD) of total plasma protein (TPP) levels in g/dl for first ,second , third trimesters and total for pregnant women 6.47 ± 0.88, 5.7± 0.76,5.6 ± 0.67, 5.92 ± 0.77 and compared to non-pregnant women who had 7.48±.79 respectively .These findings are in line with those published by [63].The mean value plus one standard deviation of hemoglobin concentration using HemoCue in g/dl for first ,second ,third trimesters and total for pregnant women 12.43 ± 1.92, 11.6 ±0.14, 11.4 ± 0.41,11.81 ±.82 respectively compared to non-pregnant women who had 12.77 ± 2.20. These findings were in line with those published by [64] .The mean value plus one standard deviation of hemoglobin concentration using Microhematocrit (PCV) in % for first, second, third trimesters, total for pregnant women 40.51 ± 1.7 ,31 .10 ± 2.7, 29 .11 ± 3.1, 33.57 ± 2.5 compared to non-pregnant women who had 39.16 ± 8.24 respectively. The hemoglobin equivalence in g/dl were 13.50 ± .60, 10.4±0.9,9.70±1.0,11.2 ± .80 compared to non-pregnant women who had 13.05 ± 2.75 respectively. These results were in line those published by [65,66] .The mean value plus one standard deviation of hemoglobin concentration using Copper sulphate solution gave a specific gravity of 1.058± 0.006, 1.050 ±.001, 1.045±.002and 1.051 ± 0.003, respectively for first, second, third trimesters and total for pregnant women compared to 1.055 ± 0.009 for nonpregnant. This gave a corresponding hemoglobin concentration equivalence in g/dl of 12.11± 0.02, 11.9±.10,11.5±.11 and 11.50± 0.07 respectively for first ,second, third and total for pregnant women compared to 13.4± 0.06 for non-pregnant women. These results were in line with those published by [67] .The mean value plus one standard deviation of hemoglobin concentration in g/dl using Sahli device for first, second and third trimesters and total for pregnant women were 12.11± 0.02,11.9±.10,11.5±.11 and 11.50± 0.07 respectively compared to 13.4± 0.06 for non-pregnant. These results were in line with those by [68]. The mean value plus one standard deviation of hemoglobin concentration in g/dl using Tallquist for first, second, third trimesters and total for pregnant women were 12.2, 11.6, 11.4 and 11.7 respectively compared to respected 12.5 in nonpregnant women . These results were in line with those published by [69] . The mean value plus one standard deviation of hemoglobin concentration in g/dl per first, second ,third trimester and total using the five type of hemoglobinometers combined were 12.77±.52,11.4±.23,10.9±.89, and 10.98±.36 respectively compared to 12.84±1.7 for nonpregnant .

The clinical importance of these results are pointing to the fact that Pregnancy is a unique physiological state that results in many changes in bodily function, including cellular, metabolic, and hormonal changes [70]. These changes can have a significant impact on Total plasma protein and haemoglobin concentractions and the way small-molecule drugs and monoclonal antibodies (biologics) function and are metabolized, including efficacy, safety, potency, and adverse effects on the feotal and maternal outcomes [ 71]. .The plasma volume and total body water also increases during pregnancy and this in turn can lower the plasma concentration of hydrophilic medicines as shown by the report of [73] .The results of the index study have demonstrated that plasma protein concentrations also fall during pregnancy, which can result in decreased plasma protein binding of substances. as already reported. by [74 ].The total plasma concentration of highly protein bound medicines may decrease in parallel with albumin levels. Physiological changes during pregnancy can also affect the absorption, distribution, metabolism and elimination of medicines.Plasma volume expansion is an important physiologic change across gestation.

In **Table 3** the Comparative results of coefficient of variance CV (%) of parameters among apparently healthy pregnant women and non-pregnant women attending UCTH Calabar, Cross River State, Nigeria are shown. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. Coefficient of Variation (CV) formula used in the index study was given by CV=σμ where: σ=standard deviation and μ=mean [64]. The higher the coefficient of variation, the greater the level of dispersion around the mean. It is generally expressed as a percentage and without units, it allows for comparison between distribution of values whose scales of measurements are not comparable.The coefficient of variance CV (%) of total plasma protein (PTP) was 15.4 %, Hemoglobin concentration (HemoCue) was 6.94 %, that of Microhaematocrit (PCV) was 7.14 % and that of Copper sulphate (CuSO4 method) was 0.93% , that of Sahli device was 6.08% and that of Tallquist paper was 0.26 % .These results are in line with those published by [75 ,76,77].

**6. CONCLUSI****ON :**

The current study has established new early diagnostic criteria for dilutional anemia using combined parameters of maternal total plasma protein levels and hemoglobin concentration (estimated using five different types of points of care testing rapid hemoglobinometers) throughout the trimesters of pregnancy . The findings of this study have significant implications for the diagnosis and management of dilutional anemia throughout the trimesters of pregnancy among the two study populations . The use of maternal total plasma protein levels and hemoglobin concentration as early new diagnostic criteria will enable healthcare providers to accurately diagnose dilutional anemia, thus preventing unnecessary blood transfusions and ensuring proper management and differentiation from others types of gestational anaemia in pregnancy trimesters. This study has contributed to the existing body of knowledge on dilutional anemia in the trimesters of pregnancy thus providing a foundation for future research. The findings of this study also have the potential to improve maternal and fetal outcomes, particularly in low-resource settings where access to blood transfusions may be limited.

**7. RECOMMENDATIONS:**

Based on the findings of this study, the following recommendations are made:

1. Healthcare providers should use maternal total plasma protein and hemoglobin concentration as diagnostic criteria for dilutional anemia in through out the trimesters of pregnancy.

2. Pregnant women should be screened for dilutional anemia at each antenatal visit through out the trimesters of pregnancy.

3. Healthcare providers should provide education to pregnant women on the importance of maintaining a healthy diet and lifestyle to prevent dilutional anemia.

4. Future research should focus on validating the diagnostic criteria established in this study and exploring the use of other biomarkers for diagnosing dilutional anemia.

**LIMITATIONS :**

This study had several limitations. Firstly, the study was conducted in a single tertiary hospital, which may not be representative of all pregnant women. Secondly, the study did not control for other factors that may affect hemoglobin concentration, such as iron deficiency anemia.Future Research Directions, Future research should focus on validating the diagnostic criteria established in this study and exploring the use of other biomarkers for diagnosing dilutional anemia. Additionally, studies should investigate the effectiveness of interventions aimed at preventing dilutional anemia in pregnancy.

**Ethical Approvals:**

These were sought and obtained from the university of Calabar teaching hospital Research Ethical Committee, Centre for Clinical Governance, Research & Training Ministry of Health Calabar, Cross Rivers State, Nigeria.

**Consent:**

These were also sought and obtained from these subjects before inclusion in the study.

Disclaimer (Artificial intelligence)

Option 1:

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

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