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Changes in Biochemical and Hematological Indices during Menstruation

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ABSTRACT

Biochemical and hematological changes may show insight into metabolic events of menstruation. However, there appears to be paucity of information with regards to protein pattern in menstruation among the Igbo community especially since these factors are known indices in monitoring health and disease. Therefore, this work was designed to explore the changes in some biochemical and hematological indices during menstruation in young women of Igbo ethnic group in Nigeria. A cross sectional study involving 140 young Igbo women aged between 18 and 36 y was recruited in Enugu Nigeria and willingly participated in the study on the second day after the onset of menstruation and one week after. Blood samples were collected from all subjects for the measurement of both biochemical (total protein, albumin, globulin, phosphate, calcium) and hematological indices (hematocrit, hemoglobin and reticulocyte). Standard routine methods were employed for all determinations. The results showed significant difference ($P<0.001$) in serum globulin concentration during menstruation (2.6 ± 0.52 g/dl) and one week after menstruation (3.6 ± 0.80 g/dl). Other measured parameters: Total protein, albumin, calcium, phospholipids, hematocrit, hemoglobin, reticulocyte showed no significant difference ($P>0.001$) when compared. The same pattern of result was obtained when were compared on the basis of age, difference in menstrual cycle and number of bleeding days. The results suggest that menstruation affects globulin concentration in this ethnic group of Nigerians and this may have direct link and help in the explanation of some aspects of osteoporosis in women.

Keywords: Menstruation, Ethnicity, Nigeria, Globulin, Igbo

INTRODUCTION

Menstruation is the cyclical and rhythmic change that occurs in the reproductive life of a sexual mature female [1]. It occurs when there is a fertilization failure and involves the dismantling and shedding from endometrium elaborately prepared for the growth of a fertilized ovum. Menstrual bleeding also referred to as ovarian cycle is the rhythmic outflow of blood from the vagina as a result of release of the uterine endometrial lining and occurs monthly in a mature female when fertilization does not occur. The duration of the cycle averages 28 days or as long as 45 days in normal women. This process is under the influence of estrogen which promotes the follicular phase and progesterone which acts on the uterus to prepare it for implantation of the fertilized ovum. When fertilization does not occur, these hormones decline and the uterine lining then sloughs off producing menstrual flow [2].

Though menstruation is primarily physiological, it still constitutes a challenge to both biochemical and hematological processes that prevent undue loss of blood [3,4]. Proteins are the basic component of clotting factors, hormones and enzymes. These proteins can be estimated as albumin and globulin. Low levels of hormones have been linked to post-menopausal osteoporosis [5]. Also decrease in progesterone at menstruation can lead to inflammatory processes which can affect prostaglandin metabolism and hence leads to decrease reactive oxygen species [6]. Furthermore, activation of certain latent enzymes may be a feature of the menstrual endothelium and hence, tissue degradation [7].

The cyclical nature of menstrual bleeding may affect both biochemical and hematological profiles which are non-specific indices of health and disease. Consequently, this may be valuable in both diagnosis and management of some diseases [8,9]. Also, the investigation of biochemical and hematological profiles in young women especially of African origin may give more insight into menstrual disorder and may have some usefulness in diagnosis. This is what informs this study.

MATERIALS AND METHODS

Subjects

A total one hundred and forty apparently healthy test subjects with age range of 18-36 years were recruited for the study using simple random sampling.

Study subjects (n=40) were drawn from apparently health young women of Igbo ethnic group with menstrual cycles of 21-26 days and menstrual bleeding between 3-7 days. Exclusion criteria included those with irregular cycles, diagnosed clinically with menstrual disorders and on those fertility drugs. All participants that met the inclusion criteria gave written informed consents before the commencement of the work. Also a proforma based report was used to record participants' demographics.

Sample collection

Blood sample was collected from each participant during and one week after menstruation from the ante-cubital vein with sterile instrument. Six milliliters of blood was withdrawn: 3 ml into Ethylenediaminetetraacetic Acid (EDTA) anticoagulant bottle for hemoglobin, hematocrit (packed cell volume) determinations and reticulocyte count while the remainder was added into plain tube, allowed to clot and serum extracted for biochemical profile: proteins (total and differential), calcium and phosphate determination. The blood samples during menstruation were collected on the second day after the onset of menstrual bleeding.

Total protein

Principle

Cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a purple coloured complex whose absorbance is measured calorimetrically at 540 nm. The absorbance is proportional to the amount of protein present.

Procedure

In a test tube, 0.05 ml of sample or standard was added together with 2 ml of biuret reagent. After thorough mixing, it was incubated at 25°C for 30 min and the absorbance read with a colorimeter at 540 nm as described by Kingsley [10].

Albumin

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'- Tetrabromo-m cresol-sulphonephthalein (Bromocresol Green (BCG)). The albumin–BCG complex absorbs maximally at 578 nm. The absorbance is directly proportional to the concentration of albumin in the sample. Distilled water was used as blank.

Procedure

Fresh distilled water was used to perform a new gain calibration in cuvette mode. Albumin was selected in the run test screen and water blank was used as instructed. Three µl of sample or standard was added into a cuvette together with 1000 µl of the reagent. It was mixed and incubated for 10 min at 37°C. The mixture was inserted into RX Monza flow cell holder and read within 60 min. Globulin was calculated from total serum protein minus serum albumin=Serum globulin.

Calcium

The classic method for the determination of calcium is titration with EDTA.

Principle

A diluted serum sample is titrated in the presence of calcium in an alkaline pH. The initial yellow green fluorescence caused by the calcium-calcein complex changes to a non-fluorescence salmon pink colour when all calcium present has been cheated by EDTA.

Procedure

Test tubes were labeled for test, standard and blank, 0.5 ml of sample was added to the tube labeled test, and 0.5 ml of standard was added to the standard test tube while 0.5 ml of water was added into the tube for blank. 0.25 ml of the indicator was put into all test tubes. Titration was done using EDTA solution in a 1 ml graduated pipette. Similarly, titration with 0.5 ml of calcium standard for standard and 0.5 ml of deionized water for blank was also done. The calculation for calcium was done as test result minus blank divided by standard minus blank result.

Phosphate

Principle

When a protein-free filtrate obtained with Trichloroacetic acid (TCA) is treated with an acid phosphomolybdate reagent, it reacts with inorganic phosphate to form Phosphomolybdic acid. This acid is reduced by the colour reagent metol to form a blue complex. The intensity of the colour is read colorimetrically at 680 nm (red filter).

Procedure

0.8 ml of serum was added to 7.2 ml TCA. It was then mixed well and centrifuged. Three tubes were set up and labeled test, standard and blank. Into the tube labeled test, 5.0 ml of protein free filtrate of serum was added. 0.5 ml of working standard phosphate was added into the tube labeled standard. 4.5 ml and 5.0 ml TCA were added into the tube labeled standard and blank respectively. 1.0 ml of ammonium molybdate and 1.0 ml of Metol ((p-methyl aminophenol sulphate) reagent were added into all the labeled three tubes. It was allowed to stand for 30 min and the absorbance as read at 680 nm (Red filter) against the blank.

Reticulocyte count

Principle

Reticulocytes contain remnants of ribosomal ribonucleic acid (RNA). Ribosomes have the property of reacting with certain dyes such as brilliant cresyl blue or new methylene blue to form a blue precipitate of filaments.

Procedure

Two drops of the filtered dye New Methylene blue and test sample were allowed into a 75 × 10 mm test tube. The mixture was incubated at 37°C for 20 min.

The cells were resuspended by gentle mixing and a drop of the stained blood placed on a glass slide and was used to make a thin film. When it was dry, the film was examined under the microscope with the oil immersion lens. The counting procedure of at least 100 reticulocytes was followed according to method as described by Bain et al.

Haemoglobin and packed cell volume were analyzed using auto haematology analyzer with Mindray BC 2800.

The Biuret method of Kingsley was adopted for total and albumin while globulin was calculated from protein and albumin. Calcium was determined by EDTA titration method inorganic phosphate was measured by the method of Goldberg and Fernandez [10-13].

Ethical approval

The study was approved by the ethical committee and review board of the University of Nigeria Teaching Hospital, Ituku, Ozalla, and Enugu, Nigeria. All participants were handled according to the Helsinki declaration. Informed consent was obtained from all participants before the commencement of the work.

Data analysis

The results were compared according to age, menstrual bleeding and menstrual cycle using IBM Statistical Package for Social Science version 20. Values that have $P<0.05$ were considered statistically significant with 95% Confidence. Statistical techniques used to analyze mean differences during and after menstruation included student t test, analysis of variance followed by Tukey post hoc analysis. Association was sought using Pearson's correlation.

RESULTS

The results obtained in this study showed a significant difference ($P<0.05$) in the mean concentration of globulin value during menstruation when compared to that obtained one week after menstruation (Table 1). However, albumin, hemoglobin value, calcium, phosphate, hematocrit and reticulocyte count showed a non-significant difference ($P>0.05$) (Table 2). The same pattern was obtained when the results were compared on the basis of age, menstrual cycle and bleeding. The globulin value was found to be significantly increased ($P<0.05$) across all measured criteria but other parameters did not show any significant mean difference.

The mean values of total protein, albumin, hemoglobin, hematocrit, calcium, phosphate and reticulocyte count were not significant different ($P>0.05$) and maintained the same pattern irrespective of age, menstrual cycle and bleeding (Tables 3 and 4).

Table 1: Laboratory analysis during and one week after menstruation

Subjects N=140	Total proteins (g/dI)	Albumin (g/dI)	Globulin (g/dI)	Calcium (mmol/I)	Phosphate (mmol)	Hb (g/dI)	PCV (I/I)	Retics (%)
During menstruation	7.38 ± 0.83	4.5 ± 0.83	2.6 ± 0.52	2.6 ± 0.20	13.0 ± 10	12.1 ± 1.0	0.37 ± 0.63	0.65 ± 0.21
One week after menstruation	7.74 ± 1.35	3.33 ± 0.80	3.6 ± 0.80	2.5 ± 0.80	1.2 ± 0.80	12.4 ± 0.2	0.35 ± 0.05	0.71 ± 0.30

Data presented as mean ± standard deviation. Hb-haemoglobin level, PCV-packed cell volume, Retics- reticulocyte count

Table 2: Biochemical and hematological indices analyzed on the basis of age ranges (Y)

Subjects N=140	Total proteins (g/dI)	Albumin (g/dI)	Globulin (g/dI)	Calcium (mmol/I)	Phosphate (mmol/l)	Hb (g/dI)	PCV (I/I)	Retics (%)
During menstruation 18-25 (n=25)	7.38 ± 1.35	4.5 ± 0.83	2.83 ± 0.52	2.6 ± 0.20	1.3 ± 0.10	12.1 ± 1.0	0.37 ± 0.06	0.5 ± 0.12
One week after Menstruation	8.1 ± 02.25	4.59 ± 1.2	3.51 ± 0.95	2.8 ± 0.22	1.8 ± 0.11	12.70 ± 1.3	0.68 ± 0.04	0.58 ± 0.10
During menstruation 25-36 (n=15)	7.20 ± 1.1	4.3 ± 1.0	2.90 ± 1.2	2.5 ± 0.20	1.4 ± 0.11	12.1 ± 1.10	0.35 ± 0.03	0.60 ± 0.04
One week after Menstruation	17.1 ± 0.12	3.50 ± 1.0	3.60 ± 1.0	2.6 ±0.51	1.5 ± 0.15	11.50 ± 1.0	0.34 ± 0.04	0.52 ± 0.03

Data is expressed as mean±standard deviation. Hb- haemoglobin level, PCV- packed cell volume, Retics- reticulocyte count

Table 3: Analysis based on the days of menstrual bleeding (Days)

Subjects N=140	Total proteins (g/dI)	Albumin (g/dI)	Globulin (g/dI)	Calcium (mmol/I)	Phosphate (mmol)	Hb (g/dI)	PCV (I/I)	Reticulocyte (%)
During menstruation (2-4)	7.70 ± 2.0	4.60 ± S 25	2.90 ± 0.82	2.76 ± 0.22	21.36 ± 0.11	12.4 ± 1.0	0.37 ± 0.10	0.6 ± 1.0
One week after menstruation	8.0 ± 1.4	4.35 ± 1.20	3.85 ± 0.90	2.61 ± 0.23	1.40 ± 0.13	12.90 ± 1.2	0.39 ± 0.09	0.70 ± 0.09
4-6 days (during menstruation)	7.80 ± 2.20	4.45 ± 1.0	3.15 ± **0**.93	2.5 ± 0.25	1.42 ± 0.15	11.8 ± 1. 0	0.3 ± 0.07	0.6 ± 0.06
One week after	8.05 ± 2.10	4.31 ± 1.1	3.9 ± 0.85	2.61 ± 0.26	1.45 ± 0.16	12.50 ± 1.3	0.3 ± 0.06	0.7 ± 0.05

Data presented as mean ± standard deviation. Hb-haemoglobin level, PCV-packed cell volume, Retics- reticulocyte count

Table 4: Biochemical and hematological analysis on the basis of menstrual cycle (days)

Menstrual cycle	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Calcium (mmol/l)	Phosphate (mmol/l)	Hb (g/dl)	PCV ±SD(l/l)	Reticulocytes ±SD(%)
21-18 days (during menstruation)	7.73 ± 1.3	4.70 ± 0.85	3.02 ± 0.21	2.5 ± 0.21	1.4 ± 0.10	12.0 ± 1.1	0.37 ± 0.03	0.72 ± 0.03
One week after menstruation	7.81 ± 1.4	4.50 ± 0.85	3.81 ± 0.85	2.4 ± 0.30	15.40 ± 11	12.40 ± 1.2	0.38 ± 0.02	0.63 ± 0.030
24-36 cycle (during menstruation)	7.5 ± 15	4.30 ± 0.90	3.20 ± 0.06	2.30 ± 0.5	1.2 ± 0.09	11.50 ± 1.6	0.34 ± 0.05	0.63 ± 0.04
One week after	7.7 ± 1.5	3.50 ± 0.85	4.20 ± 1.0	2.35 ± 0.87	1.15 ± 0.1	11.9 ± 1.10	0.35 ± 0.04	0.59 ± 0.04

Data is expressed as mean±standard deviation. Hb- haemoglobin level, PCV- packed cell volume, Reticulocytes- reticulocyte count

DISCUSSION

Biochemical and hematological values vary in diseases and in some physiological conditions. Menstruation though a physiological process is associated with changes in intermediary metabolism [14]. For example, changes in blood concentration of steroid hormones during menstruation have been variously reported [15,16]. In addition circadian rhythm of plasma melatonin has being reported during menstrual cycle and in amenorrhea women [17]. The results obtained for globulin concentration appear to suggest that during menstruation, the globulin concentration in plasma is lower when compared with values obtained one week after menstruation. The implication may be that globulin may directly or indirectly be involved in events of menstruation. There appears to be paucity of information with regards to protein pattern and immunoglobulin levels in menstruation. A fall in platelet count has been documented as a feature of cellular immunity [18]. Although immunoglobulin levels and types were not studied in the work, it is probable that the significant increase in globulin fraction of total protein may be a part of immune response in view of the leukocytosis already documented [19]. This also agrees with the work of Evan and Salamonsen who reported that decreased progesterone at menstruation may lead to decrease in reactive oxygen species and this may cause an increase in the synthesis of proinflammatory prostaglandin and thus to leukocyte recruitment [21-23].

Furthermore, when the results were compared on the basis of age, length of menstrual cycle and bleeding the same pattern was observed. These suggest that the significant increase in globulin one week after menstruation is directly related to events of menstruation. This pattern may probably suggest that globulin level may be reduced during menstruation but returns to basal level one week after menstruation (though the total protein was unaffected). The paucity of data on works that studied plasma protein during menstruation did not facilitate comparison. However, hormonal changes throughout the reproductive cycle, has been documented [24] and it is possible that this might have influenced the finding in this study. Binding of globulin to some sex hormones may further be the reason for this decrease. Researchers have suggested that reduced sex hormone binding globulin explains subsequent post-menopausal increased osteoporosis [24].

The other profiles; total protein, calcium, phosphate, albumin, hematocrit and hemoglobin that were measured showed no significant difference in mean value when compared on the basis of age, menstrual cycle and bleeding maintained same pattern. The inference is that these metabolites are unaffected in menstruation. However, of particular interest are hematocrit and reticulocyte which constitute indices of effective erythropoiesis. The results showed that they were not significantly reduced during menstruation. This is in support by the work of Rajnee et al. who demonstrated that there was no change between hemoglobin and hematocrit during menstruation and during the follicular phase of the menstrual cycle [25]. There has been a long lasting debate whether there is decrease in red cell indices during menstruation [26]. Experience in the blood bank has shown that women are averse to blood donation, a critical arm of blood transfusion because of the monthly loss of blood during menstruation. This phobia does not appear to be based on potent scientific data as shown by the result of this study. It can be concluded that menstruation may be associated with decreased globulin level. Decrease globulin level may affect the elucidation of some sex hormone and may predict increased osteoporosis.

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