Hemoglobin and Serum Iron Concentrations in Menstruating Nulliparous Women in Jos, Nigeria

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ABSTRACT

Background: Low hemoglobin (Hb) and iron deficiency among child bearing females have been linked to decreased immune system function, impaired cognitive functioning and complications in pregnancy.

Methods: A total of 106 blood samples from apparently healthy nulliparous female students were assayed for Hb and serum iron concentrations using the cyanmethemoglobin and bathophenanthroline methods, respectively, to evaluate changes that may occur in these parameters at different phases of the reproductive cycle.

Results: The mean (SD) Hb values during the ovulatory, menstrual, and follicular phases were 13.27 (1.14) g/dL, 12.05 (1.31) g/dL, and 12.23 (1. 56) g/dL, respectively. The prevalence of anemia (Hb<12 g/dL) was reported among 21 (19.8%) subjects, and 31 subjects declined to complete their samples collection. The mean serum iron concentrations during the 3 phases were 92.98 (18.25) µg/dL, 79.90 (13.14) µg/dL and 70.85 (18.65) µg/dL, respectively. A total of 28 (26.4%) study participants showed iron deficiency (serum level, <65 µg/dL). These variations in the values of Hb and serum iron concentrations were statistically significant in the 3 phases. However, no significant difference was observed in Hb concentrations between the menstrual and follicular phases. Of interest, a positive correlation was observed between the hemoglobin and serum iron concentrations within the phases, with the exception of a few cases that showed negative correlations.

Conclusion: Menstruation has been shown to be the major cause of anemia and iron deficiency in nulliparous women. A prophylactic dose of iron and folate supplements may be indicated for menstruating females to cushion the adverse effects of menstruation on hematologic status.

Keywords: menstruation, anemia, hemoglobin, iron, nulliparous

Studies have shown that approximately 10% of women lose more than 1.4 mg of iron per day through menstrual bleeding.[1,2] Li et al[3] estimated the average menstrual blood loss by weighing menstrual pads before and after use by their subjects (mean [SD], 59.3 [25.1] g/dL). They also estimated the average content of serum ferritin, free protoporphyrin, and hemoglobin as mean [SD] values of 25.13 [14.33] ng/mL, 0.06 [0.01] µg/mL, and 131.61 [9.76] g/L, respectively.[3] The total amount of blood loss during menstruation ranges from 30 to 180 ml, with an average of 80 ml per menstrual period.[4] Also, it has been shown[5] that blood loss of 40 ml during menstruation yields an average loss of 1.6 mg of iron. Further, consecutive blood loss of more than 60 ml per menstrual period will deplete the body's iron stores; loss of more than 80 ml may indicate clinical anemia.[7] Of importance, iron deficiency affects approximately 20% to 25% of the world's population, predominantly children and women. It has been domonstrated[8] that iron deficiency is more likely in women of reproductive age because of menstrual blood loss.

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Abbreviations

Hb, hemoglobin; EDTA, ethylene diamine tetraacetic acid

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Approximately 65% of iron is stored in hemoglobin (Hb). Iron is involved in energy metabolism, cell growth, oxygen binding, enzyme reactions, and synthesis of neurotransmitters.[9] It has been reported[10] that 3 to 24 mg of iron is lost during each menstrual cycle. Emerging evidence suggests that iron deficiency without anemia can have negative consequences in adults, particularly neurocognitive disorders.[8] In addition, the implications of iron deficiency may include decreased work capacity,

decreased immune system functioning, impaired cognitive functioning and memory and complications in pregnancy.[9] Conversely, Ba et al[11] stated that iron overload is a significant risk factor in the development of hepathocellular carcinoma, the third-most-common cause of cancer-related death. A study[12] carried out with baboons showed that hemoglobin, red blood cells and white blood cell counts were low during the menstrual phase and high in the follicular phase.[12]

A substantial proportion of women in developing countries such as Nigeria have been reported to enter pregnancy with inadequate iron stores, predisposing them to iron deficiency anemia.[13] However, heavy menstrual blood loss is the most important factor contributing to iron-deficiency anemia in women.[14]

Recent evidence[15] suggests that poor fetal growth is associated with preconception anemia. As a precautionary measure, iron and folate supplements taken before conception may help ameliorate pre-existing anemia caused by menstruation.[15] Also, the use of oral contraceptives reduces the volume of blood loss during menstruation and thereby increases iron stores.[16] Our study was designed to asses hemoglobin and serum iron status in nulliparous women at various phases of the reproductive cycle with a view toward identifying changes that may occur in hemoglobin and serum iron concentrations that result from menstruation.

Materials and Methods

Study Participants

Forty-six nulliparous female students from the Federal School of Medical Laboratory Science and the School of Nursing and Midwifery in Jos, Nigeria, consented to participate in the study. Study participants were between the ages of 19 and 30 years. The average menstrual cycle of the participants was 27 days, with 4 days of menstrual bleeding. Women with heavy menstrual bleeding or a history of amenorrhea were excluded from the study.

Sample Size/Study Design

A total of 106 blood samples were collected for the study. Venous blood samples were collected from the study participants at the 3 major reproductive phases: the ovulatory phase (2 weeks before menstruation), the menstrual phase (1 day after the onset of menstruation), and the follicular phase (1 day after cessation of menstruation).

Sample Collection

A sample of 9.0 ml of venous blood was collected by venipuncture from each subject at the 3 different phases of the menstrual cycle, between the hours of 8:00 am and 12:00 pm. Six ml of the sample were each dispensed into a Z/10 Sterilin plastic container (ThermoFisher Scientific Inc, Waltham, MA). The bottles were each labeled with the name and the menstrual phase of the subject. The samples were allowed to clot for 10 minutes at room temperature. The was centrifuged at 3000 revolution per minutes for 10 minutes, and the serum was transferred into a Z/5 Sterilin plastic container (ThermoFisher Scientific Inc). The serum specimens were analyzed for iron using the bathophenanthroline method.[17] Three ml of blood sample was transferred into a labeled specimen container that included ethylenediaminetetraacetic acid (EDTA), and the contents of the bottle were thoroughly mixed. The specimens were analyzed for hemoglobin using the cyanmethemoglobin method.[18]

Determination of Iron Concentration Using the Bathophenanthroline Method

We used a method reported by Peters et al.[17] Iron was released from transferrin by acidification with hydrochloric acid, the specimens were deproteinized with trichloroacetic acid, centrifuged, and ferric ions in the supernatant were reduced to ferrous ions using thioglycolic acid. Bathophenanthroline was added and absorbance was measured colorimetrically at 540-nm.

Hemoglobin Estimation Using Cyanmethemoglobin Method

Whole blood was diluted with Drabkin's solution, (buffered potassium ferricyanide and potassium cyanide). Potassium ferricyanide converts hemoglobin to methemoglobin, which is converted to cyanmethemoglobin by the action of potassium cyanide. The absorbance was measured colorimetrically at 540 nm.

Data Management and Statistical Applications

The study data were analyzed using SPSS, version 15.0 (IBM Corporation, Armonk, NY). The mean values of hemoglobin and serum iron concentrations, standard deviations, percentage, and Pearson correlation were determined. Relevant hypotheses were tested using the *t* test and χ^2 test, as appropriate.

Results

The mean (SD) hemoglobin values at the ovulatory, menstrual, and follicular phases were 13.27 (1.14) g/dL, 12.05 (1.31) g/dL, and 12.23 (1 56) g/dL, respectively (**Table 1**). Statistical comparison indicated a significant reduction in hemoglobin concentration between the ovulatory and menstrual phases. Significant changes also were observed between the ovulatory and follicular phases. No significant differences were observed between the menstrual and follicular phases (**Table 1**). Anemia (Hb<12 g/dL) was revealed in 21 (19.8%) subjects: 2 (9.5%) in the ovulatory phase, 7 (33.3%) in the menstrual phase, and 12 (57.1%) in the follicular phase.

The mean (SD) serum iron concentrations during the ovulatory, menstrual, and follicular phases were 92.98 (18.25) µg/dL, 79.90 (13.14) µg/dL, and 70.85 (18.65) µg/dL, respectively (Table 2). Twenty-eight study participants (26.4%) were iron deficit (serum level, <65 µg/dL), of whom 4 (14.3%) were in the ovulatory phase, 4 (14.3%) were in the menstrual phase, and 20 (71.4%) were in the follicular phase. Significant differences were found in serum iron concentrations between the ovulatory and menstrual phases, the menstrual and follicular phases, and the ovulatory and follicular phases (**Table 2**).

Pearson correlation coefficients for hemoglobin versus serum iron concentrations during the 3 phases of the reproductive cycle are shown in **Table 3**. Positive correlations were found in all 3 phases, although there were 3 comparisons that showed a negative correlation. Hemoglobin and serum iron mean (SD) concentrations were decreased during menstruation, and in the follicular phase, whereas hemoglobin increased in the follicular phase.

Discussion

The results of this study showed that menstruation affects the hemoglobin and iron concentrations in nulliparous women. Despite the finding of a significant reduction in hemoglobin concentrations between the ovulatory and menstrual phases and the ovulatory and follicular phases, no significant decrease was observed in the Hb values between the menstrual and follicular phases. The lowest mean (SD) Hb value was recorded in the menstrual phase of the reproductive cycle; by implication, this means that the menstrual phase could be a potential cause of anemia

Table 1. Hemoglobin Concentrations During the 3 Phases of the Reproductive Cycle Among Nulliparous Women Aged 19 to 30 Years

Phase	Subjects, No.	Mean (SD)[a]	*t* Test	*P* Value
Ovulatory	31	13.27 (1.14)	3.734	<.001
Menstrual	25	12.05 (1.31)		
Ovulatory	31	13.27 (1.14)	3.157	.002
Follicular	44	12.23 (1.56)		
Menstrual	25	12.05 (1.31)	–0.498	.62
Follicular	44	12.23 (1.56)		

[a]*Measured in g/dL.*

Table 2. Serum Iron Concentrations During the 3 Phases of the Reproductive Cycle Among Nulliparous Women Aged 19 to 30 Years

Phase	Subjects, No.	Mean (SD)[a]	*t* Test	*P* Value
Ovulatory	33	92.98 (18.25)	3.117	<.001
Menstrual	27	79.90 (13.14)		
Ovulatory	33	92.98 (18.28)	5.248	<.001
Follicular	46	70.85 (18.65)		
Menstrual	27	79.90 (13.14)	2.229	.03
Follicular	46	70.85 (18.65)		

[a]*Measured in µg/dL.*

Table 3. Pearson Correlation of the Mean (SD) Values of Hemoglobin and Serum Iron Concentrations During the 3 Phases of the Reproductive Cycle Among Nulliparous Women Aged 19 to 30 Years

	Serum Iron		
Hemoglobin	**Ovulatory**	**Menstrual**	**Follicular**
Ovulatory			
Pearson correlation	–0.014	0.049	–0.041
P Value	.94	.82	.84
Subjects, No.	31	25	26
Menstrual			
Pearson correlation	–0.039	0.038	0.069
P Value	.85	.86	.74
Subjects, No.	25	25	25
Follicular			
Pearson correlation	0.298	0.301	0.046
P Value	.148	.144	.769
Subjects, No.	25	25	44

among nulliparous women. Anemia was identified in 21 (19.8%) subjects, of whom 2 (9.5%) were in the ovulatory phase, 7 (33.3%) in the menstrual phase, and 12 (57.1%) in the follicular phase. By comparison, a case study carried out on baboons[12] revealed that nulliparous females in the study had decreased hemoglobin concentration during the menstrual phase and increased serum iron concentration levels in the follicular phase.

The mean (SD) serum iron concentrations were 92.98 (18.25) µg/dL, 79.90 (13.14) µg/dL, and 70.85 (18.65) µg/dL in the ovulatory, menstrual, and follicular phases, respectively. Significant reduction in serum iron was observed in the 3 phases (**Table 2**). Iron deficiency was discovered in 28 (26.4%) women, of whom 4 (14.3%) were in the ovulatory phase, 4 (14.3%) in the menstrual phase, and 20 (71.4%) in the follicular phase. Mean hemoglobin and iron concentrations were decreased during menstruation and serum iron further decreased in the follicular phase, whereas hemoglobin increased in the follicular phase.

Conclusion

Menstruation has been shown to be a major cause of anemia and iron deficit in nulliparous women. Reduction in serum iron has been reported to adversely affect cognitive and memory capacity. The adverse effects of menstruation could be linked to complications associated with pregnancy and anemia. A prophylactic dose of hematinics or iron and folate supplements may be indicated for menstruating women to offset the effects of menstruation on Hb and serum iron levels. LM

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Author Contributions

Mary-Jane N. Ofojekwu conceived and designed the experiments, carried out the laboratory analysis, and drafted the manuscript; Ikechukwu O. U. Isiguzoro and Lolade A. Odewumi analyzed and interpreted the data. Ogbonnaya U. Nnanna, Charles E. Okolie, and Moses D. Lugos interpreted data and revised the manuscript for intellectual content. All authors read and approved the final manuscript for publication.

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