Original Article

ISSN (0):2395-2822; ISSN (P):2395-2814

Variations in Iron Status Indicators in Different Phases of Menstrual Cycle - A Study of Two Age-Matched Socio-Economic Groups of 18-25 Years of Age.

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Received: November 2016 Accepted: December 2016

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ABSTRACT

Background: Iron requirements are increased in adolescent girls with growth and the onset of menarche and remain high in women until menopause. Methods: We conducted a study on two study groups of girls in age group 18-25 years belonging to different socioeconomic classes as per the Modified Kuppuswamy's Scale to study the effect of the phases of menstrual cycle & socioeconomic class on the iron status indicators (Hb, Serum Iron, TIBC, MCV & TS%). Menstrual phases were defined and blood samples were drawn from all the girls in all the 3 phases (menstrual, follicular & luteal) and Hb, Serum iron & Total iron binding capacity were assessed on a semi-auto-analyzer & MCV was estimated using a fully automated CBC machine. Transferrin Saturation was derived as Serum Iron / TIBC × 100. Results: We found significant (p< 0.01) difference in iron status indicators in both our groups, the values of iron status indicators varied significantly according to the menstrual cycle, with values highest during the luteal phase and lowest during the menstrual phase. From our observations we can conclude that the nutritional status (assessed by the height, weight & BMI) and the iron status indicators both differ significantly in the two age-matched comparable socio-economic groups. Conclusion: socio-economic class has a major determining role on an individual's health. The effect of hormonal fluctuations during the menstrual cycle also has a part to play in the variation of iron status measures, which has to be considered while measuring them in a female during her reproductive years.

Keywords: Iron status indicators(ISI), Menstrual Cycle, Socioeconomic Status(SES).

INTRODUCTION

Iron has several vital functions in the body. WHO estimated that Iron Deficiency (ID) occurs in about 66–80% of the world's population.ID has many negative effects on health, including changes in immune function, cognitive development, temperature regulation, energy metabolism and work performance.^[1] Women in reproductive age group are at risk of developing iron deficiency anemia , which can significantly effect their physical and cognitive capabilities.^[2]

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In developing nations, iron deficiency (ID)& iron deficiency anemia (IDA) occur most often in premenopausal women, mainly because of poor dietary intake and the regular loss of blood during menstruation. [3] The mean menstrual iron loss, averaged over the entire menstrual cycle of 28 days,

is about 0.56 mg/day. The marked variation of menstrual losses is a great nutritional problem. [4] In boys, body iron stores may be increased during and after their rapid growth period, whereas in girls, iron stores remain low during the whole pubertal period. The lower iron status in girls, besides growth, is also because of the iron loss with menstruation.^[5] SES and health is directly correlated across the world. Adult health outcomes follow a spectrum that closely matches social status, and this gradient of health status persists at each step on the social ladder. [6] Measurements of iron status in blood can be confounded by non-nutritional factors. The effect of such confounding factors must be considered when data are used to evaluate the iron status of population groups. In women of reproductive age, potential confounding factors include the possible fluctuations in measures of iron status associated with various phases of the menstrual cycle. Physiologic changes that occur during the menstrual cycle, such as fluctuations in fluid volume, blood loss and significant iron loss associated with it, may cause variations in iron status indicators.^[7] So, while we conduct a nation-wide survey to assess the nutritional status, it is of foremost importance to assess, keeping in mind all the factors that can alter

our results, specially menstrual cycle in females, girls at menarche, women right after child birth, women suffering from irregular/heavy menstrual cycle due to any gynecological disorder, post traumatic and post major surgical patients, people suffering from bleeding disorders. The present study was designed to study the variations in iron-status measures in two nationally representative age matched socioeconomic groups of young adult unmarried females of 18-25 years.

Aims & Objectives

- To study the iron status indicators in two age matched socioeconomic groups of 18-25 years old females.
- To determine whether normal physiological changes associated with menstrual cycle affect the iron status indicators in age matched study groups of 18-25 years age group.
- To assess differences in iron status indicators related to social class, nutrition status and body mass index (BMI).

MATERIALS AND METHODS

The study was conducted at Department of Physiology, Moti Lal Nehru Medical College, Allahabad (U.P.) between year 2011 to 2012. Approval from the institute's ethical committee was taken and written informed consent was taken from each girl participating in the study.

Inclusion criteria

Group A:- 50 Young adult unmarried females of age 18-25 years living in urban slums, Belonging to low SES, having normal menstrual cycle of 25-32 days, not on medication or hormonal supplements, not studied beyond class 8.

Group B:- 50 Young adult unmarried female students of age 18-25 years studying in M.L.N. Medical College, Allahabad, Having normal menstrual cycle of 25-32 days, Living in same hostel, eating from the same mess, Not on medication or hormonal supplements and of same educational & SES.

Exclusion Criteria

The following were excluded from the study

- 1. Non-compliant subjects
- 2. Irregular menstrual cycle
- 3. Any significant previous illness
- 4. Any bleeding disorder or hemoglobinopathy
- 5. History of major surgery, accident or blood transfusion
- 6. Suffering from or suffered from any gynecological or hormonal disorder
- 7. Family history of: Any blood related disorders/Menstrual irregularities/Infertility/Cancer of female reproductive organs/Any gynecological disorder.

Evaluation After taking the informed consent, applying exclusion and inclusion criteria the detailed

history was taken including name, age and the following:-

- a) Dietary habits-veg./non veg. with qualification and quantification of the daily intake
- b) Menstrual history with: Age at menarche & following phase definition:

Menstrual phase: day 1-5, menstruating at the time of survey

Follicular phase: 6-16 days after menstruation Luteal phase:17-30days after menstruation.

c) Detailed history regarding – Regularity/Flow/Debilitating pain/Cyclical mastalgia/Last menstrual period.

Complete general & systemic examination was carried out on each subject as per the standard guidelines. The following measurements were carried out on each subject:

Anthropometry: Height, Weight And BMI

Iron status indicators:

Serum Iron^[8]: $11.6\overline{-31.7}$ µmol/lit in males & 9.0-30.4 µmol/lit in females.

Total Iron Binding Capacity8 : $45-66 \mu mol/lit$ Hemoglobin^[9]: 13.5-18 gm/dl in males & 11.5-16 gm/dl in females.

Transferrin Saturation^[8]: 20 - 50 % in males & 15 - 50% in females.

Mean Corpuscular Volume^[9]: 74 - 95 μm3

Intra-cubital venous blood (5ml) was collected from the subjects. The blood was allowed to stand for 60 minutes in incubator at 37 °C. After that serum was obtained by centrifugation. Fresh serum was used for SI & TIBC, fresh whole blood for Hb & MCV determination.

Estimation of serum iron and TIBC was done by ferrozine method (magnesium carbonate) obtaining the absorbance using the semi-auto-analyzer by ARTOS established in the Department of Physiology M.L.N.M.C Allahabad.

Fe (II) + Ferrozine → Violet Colored Complex

Hemoglobin estimation was done by Cyan-methemoglobin method (Drabkin's reagent), obtaining the absorbance using the semi-auto-analyzer

Transferrin saturation = Serum Iron / TIBC \times 100

Estimation of MCV was done by fully automated complete blood count machine (Medonic M series) installed in the Department of Pathology, M.L.N. Medical College, Allahabad. Fresh un-clotted whole blood was used for the MCV estimation.

<u>Statistical Analysis:</u> The data obtained on both the study groups was expressed as the mean \pm standard deviation. Statistical analysis was done with the help of statistical software SPSS 17.0. Both the groups were compared using student's t test. The p value of < 0.05 was considered as statistically significant.

RESULTS

Our results show that there has been a constant significant difference in the iron status indicators among both the groups. The present results confirm the difference of the social class on the nutritional and health status of young adult females as shown by Gupta et al 2009. The significant decrease in the weight and BMI of the girls belonging to the lower SES is attributed to the poverty and the social class of the subjects, also supported by K. Prashant et al $2009^{[11]}$, Sanjeev M C et al $2008^{[12]}$ and ME Bentley et al $2003^{[13]}$.

Significant correlation was found between the iron status indicators during different phases of the menstrual cycle. Hb values were significantly associated with the menstrual phase for both the study groups. Concentrations of Hb were lowest for girls in the menstrual phase and highest for girls in

the luteal phase. Mean values of both SI and TS were also significantly associated with phases of the menstrual cycle, their values were lowest for girls in the menstrual phase and highest for girls in the luteal phase. The patterns of TIBC values were opposite to those observed for Hb, SI, and TS. Measures of TIBC were highest for girls whose blood was drawn in the menstrual phase and lowest during the luteal phase. MCV values were different only during the follicular phase, significantly lower than the values observed during the menstrual phase. MCV values were only slightly different during the luteal phase from the menstrual phase (not significant). So, we found significant differences in iron status indicators in both our groups, also the values of iron status indicators vary according to the menstrual cycle, with values highest during the luteal phase and lowest during the menstrual phase.

Table 1: Mean & standard deviation of different parameters in group 1 & 2 with their significance.

S. No.	Parameters	Group 1 (Mean ± S.D.)	Group 2 (Mean ± S.D.)	Significance
1	Age (yrs.)	18.36 ± 0.53	21.32 ± 1.95	p < 0.01
2	Height (cms)	155.90 ± 3.99	157.60 ± 6.09	p > 0.05
3	Weight(kgs)	41.08 ± 3.77	51.94 ± 5.65	p < 0.01
4	BMI(kg/m ²)	16.85 ± 1.39	20.90 ± 1.42	p < 0.01
5	SES	4.56 ± 0.49	1.58 ± 0.72	p < 0.01

Table 2: Mean & standard deviation of iron status indicators during menstrual phase in both groups.

S. No.	Parameters	Group-1 (Mean \pm S.D.)	Group-2 (Mean \pm S.D.)	Significance
1	Hemoglobin (gm/dlit)	8.44 ± 0.85	11.85 ± 1.01	p < 0.01
2	Serum Iron (µmol/lit)	11.73 ± 0.87	16.37 ± 1.46	p < 0.01
3	ΓΙΒC (μmol/lit)	68.01 ± 1.00	62.37 ± 1.96	p < 0.01
4	TS%	17.23 ± 1.49	26.28±3.11	p < 0.01
5	MCV	86.21 ± 1.96	89.51 ± 1.79	p < 0.01

Table 3: Mean & standard deviation of iron status indicators during follicular phase in both the groups.

S. No.	Parameters	Group 1 (Mean ± S.D.)	Group 2 (Mean \pm S.D.)	Significance
1	Hemoglobin (gm/dlit)	8.73 ± 0.85	12.14 ± 1.01	p < 0.01
2	Serum Iron (µmol/lit)	12.42 ± 0.89	17.01 ± 1.49	p < 0.01
3	TIBC (μmol/lit)	67.30 ± 0.98	61.71 ± 2.00	p < 0.01
4	TS%	18.44 ± 1.55	27.63 ± 3.22	p < 0.01
5	MCV	85.12 ± 1.97	88.37 ± 1.80	p < 0.01

Table 4: Mean and standard deviation of iron status indicators during luteal phase in both the group.

S. No.	Parameters	Group 1 (Mean ± S.D.)	Group 2 (Mean ± S.D.)	Significance
1	Hemoglobin (gm/dlit)	9.04 + 0.86	12.42 + 1.01	p < 0.01
2	Serum Iron (µmol/lit)	13.07 ± 0.86	17.67 ± 1.53	p < 0.01
3	TIBC (µmol/lit)	66.58 ± 0.97	61.01 ± 2.01	p < 0.01
4	TS%	19.61 ± 1.52	29.01 ± 3.37	p < 0.01
5	MCV	85.97 ± 1.97	89.23 ± 1.78	p < 0.01

DISCUSSION

Our findings suggest that females in the reproductive age group (specially, during the active menstrual phase) are more prone to lower ISI as compared to women not actively menstruating. Also, socioeconomic class has a major role to play in determining the nutritional status of these young adult girls and our findings are also confirmed by G.N. Elemo et al^[14], Mc Clung J.P et al ^[3],Leng Huat Foo et al^[15] and M Ferrari et al^[16].

The rhythms of ovarian hormones during the course of the menstrual cycle influence the secretion of hormones that control the volume and content of the vascular space. Thus changes in plasma volume and rise in plasma protein concentrations are linked to the normal course of estrogen and progesterone fluctuations during the menstrual cycle. [17] In our study we did not directly measure changes in plasma volume, but our observations are in accordance with the well-known hormonal changes of the menstrual cycle and their known influence on hemoconcentration & dilution. Hemo-concentration due to estrogen, mediated changes in plasma volume or increased synthesis or secretion of plasma proteins, might explain the mechanism for higher iron-status values during the luteal phase. An increase in plasma progesterone concentrations also occurs during the luteal phase which has a natriuretic effect due to aldosterone antagonism, resulting in plasma loss of sodium and water. Progesterone may also influence hemo-concentration during the early to mid-luteal phase when its concentrations peak. The fall in progesterone during the luteal phase is associated with a sharp rise in aldosterone activity observed few days before the onset of menses manifesting as weight increase, edema & hemodilution. Thus, the hormone-associated changes in plasma volume and plasma protein concentration occurring during course of the menstrual cycle offer a possible mechanism to explain differences in the values of iron-status indicators that we observed at different phases of the cycle.

CONCLUSION

From our observations we can conclude that the nutritional status (assessed by the height, weight & BMI) and the iron status indicators both differ

significantly in the two age matched comparable socio-economic groups. Hence, socio-economic class has a major determining role on an individual's health. The effect of hormonal fluctuations during the menstrual cycle also has a part to play in the variation of iron status measures, which has to be considered while measuring them in a female during her reproductive years, as it may alter the results depending on the phase of the menstrual cycle.

Acknowledgement

I thank all my subjects for participating in the study who so willingly volunteered, without their cooperation this study would not have been possible.

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How to cite this article: Chandra S, Bajpai M, Tiwari S, Kamal RB, Chawla K. Variations in Iron Status Indicators in Different Phases of Menstrual Cycle – A Study of Two Age-Matched Socio-Economic Groups of 18 – 25 Years of Age. Ann. Int. Med. Den. Res. 2017; 3(1):PH18-PH22.

Source of Support: Nil, Conflict of Interest: None declared