STUDY OF ERYTHROCYTE SEDIMENTATION RATE, SERUM TOTAL PROTEIN, SERUM ALBUMIN, SERUM GLOBULIN AND RED BLOOD CELL COUNT IN DIFFERENT PHASES OF MENSTRUAL CYCLE
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ABSTRACT: BACKGROUND: Menstruation is a phenomenon unique to females and nearly universal experience in women’s lives and is poorly understood. It is characterized by co-ordinate sequence of hormonal changes but the changes that occur in the distribution of hematological parameters, biochemical parameters have not been clearly established. AIMS: To compare Erythrocyte Sedimentation Rate (ESR), Serum total protein, Serum albumin, Serum globulin and Red Blood Cell count (RBC) in different phases of menstrual cycle. MATERIALS & METHODS: The present study was carried out on 100 healthy female medical students in the age group of 18 to 23 years with normal menstrual cycle. E.S.R, Serum total protein, Serum albumin, Serum globulin and RBC count were studied on 2nd day MP(Menstrual Phase), 11th day PP(Proliferative Phase), 22nd day SP(Secretory Phase) of menstrual cycle using Wintergreen’s method, semi auto analyzer Erba chem-7 (BCG Dye method) for serum proteins, Hemo Auto analyzer SYSMEX KX-21 respectively. Statistical analysis was done using SPSS 17.0 Software. To compare means of two independent groups, student’s t-test for independent samples was used. RESULTS: The study revealed that ESR was significantly (P<0.01) higher in menstrual phase compared to proliferative phase. Serum total protein was significantly increased in Secretory Phase (SP) compared to Menstrual Phase (MP) (P<0.05). Serum albumin was significantly (P<0.05) increased in proliferative phase compared to secretory phase and menstrual phase. Serum globulin was significantly increased in SP compared to PP (P<0.05). Red blood cell count showed no significant changes during various phases of menstrual cycle. CONCLUSION: In the present study we found significantly low level of serum albumin and decreased RBC count though not significant increases rouleaux formation thus increasing ESR in the menstrual phase but serum globulin and serum total protein showed increase in secretory phase which does not explain ESR changes.

KEYWORDS: Menstrual cycle; Erythrocyte Sedimentation Rate, Serum total protein, Serum albumin, Serum globulin and Red Blood Cell count.

INTRODUCTION: Females have higher age-standardized rates of acute conditions, chronic conditions, and disability due to acute conditions, compared to males according to National Health Interview Survey for 1957-72.1 Excess female morbidity is primarily due to social and psychological factors. Menstrual dysfunction, like other aspects of sexual and reproductive health, is not included in the Global Burden of Disease estimates2, 3 and, even as reproductive health programs expand their focus to address gynecologic morbidity, the utility of evaluating and treating menstrual problems is not generally considered. Available data from developing countries on the frequency of menstrual disorders and their impact on women’s health status, quality of life and social integration suggest that...
evaluation and treatment of menstrual complaints should be given a higher priority in primary care programs.\(^4\)

The menstrual cycle is a window into the general health and well-being of women, and not just a reproductive event. The hormonal changes occurring during menstrual cycle not only affect oocyte maturation and the endometrial and vaginal environment but can also have an effect on a number of other physiological and biochemical phenomena.\(^5\) Apart from it being physiological there are various disorders associated with menstrual cycle which cause morbidity & mortality.

The menstrual cycle is characterized by cyclical fluctuations in the levels of FSH, LH, Estrogen and Progesterone. These hormones are known to have an effect on Hematological and Biochemical parameters. In developing countries, abnormal uterine bleeding appears to affect about 5–15% of women of reproductive age. It is a major cause of gynecological morbidity, affecting up to one in five women some point during their reproductive life span.\(^6\)

Nine to fourteen percent of reproductive-aged women have blood loss that exceeds 80ml \(^7\) and prolonged and excessive bleeding may provoke or exacerbate anemia and in a certain percentage of cases, may eventually be life threatening if left untreated, thus there arises a need to estimate Red Blood Cell(RBC) count and ESR during the menstrual cycle. The lack of awareness about the potential importance of reducing menstrual flow when women are anemic and lack of knowledge among women about treatment alternatives is of some concern.

Serum proteins bind sex steroids and regulate activity of menstrual cycle. The sex steroids have an anabolic effect. Estrogen causes positive nitrogen balance due to growth promoting effect which causes slight increase in the total body proteins.\(^8\) Progesterone exerts anabolic effect & this partly accounts for some of the weight gain.\(^9\) Hence there is need to study the changes in serum proteins in different phases of menstrual cycle.

Numerous scientific studies have been undertaken on hematological, biochemical parameters during menstrual cycle but the results are often contradictory & variable. So, the present study was undertaken to study relation of ESR, RBC Count and serum proteins in menstrual cycle and thus provide a screening tool to avoid related morbidity and mortality.

**MATERIALS AND METHODS:** The study protocol was approved by the institutional ethical committee in Mahadevappa Rampure Medical College, Gulbarga. Hundred subjects were recruited after the informed and written consent. In the present study, apparently healthy hundred female medical students aged between 18-23 years and Normal regular menstrual cycles of 27-33 days with ovulatory cycles were included.

Subjects below 18yrs and above 23yrs of age, Subjects with endocrinal & gynecological disorders, chronic diseases, allergic conditions, presence of infection at the time of sampling, subjects with diabetes, pregnancy, subjects with irregular menstrual cycle, subjects taking drugs which affect menstrual cycle and subjects performing regular exercise were excluded.

Venous blood sample was collected from the antecubital vein (2 ml) in a disposable syringe between 1- 2 pm to avoid diurnal variation and counting was done within half an hour to avoid variations due to storage. Blood was taken in EDTA bulb and mixed well and RBC Count was performed using Hemo auto analyzer-SYSMEX KX-21. ESR estimation was performed by Westergren’s method using 3.8% sodium citrate and reading was taken at the end of 1st hour.
Venous blood sample was collected from the antecubital vein (2 ml) in a disposable syringe between 1-2 pm to avoid diurnal variation and analysis was done within half an hour to avoid variations due to storage. Serum total protein and serum albumin estimation was performed using automated analyzer Erba chem-7. The blood sample was centrifuged using R-8C centrifuge machine and serum was separated. E.S.R, Serum total protein, Serum albumin, Serum globulin and RBC count were studied on 2nd day (Menstrual Phase), 11th day (Proliferative Phase), 22nd day (Secretory Phase) of menstrual cycle.

**Serum Total protien** estimation was done by Biuret method: The peptide bonds of protein react with the copper II ions in alkaline solution to form blue–violet complex (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as a stabilizer whilst Iodide is used to prevent autoreduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520-560nm).

**Serum Total albumin** estimation was done by BCG Dye method: Albumin binds with bromocresol green (BCG) at Ph 4.2 causing a shift in absorbance of the yellow BCG dye. The blue green colour formed is proportional to the concentration of albumin present, when measured photometrically between 580-630nm with maximum absorbance at 625nm.

**Serum Globulin estimation was done by calculation:**

- Serum Globulin (g/dl) = Serum Total protein (g/dl) - Serum Albumin (g/dl).
- Statistical Analysis: Data was expressed as Mean ± S.D. and was analysed for statistical analysis using SPSS 17.0 Software. To compare means of two independent groups, student's t-test was used.

**RESULTS:** In the present study, E.S.R, Serum total protein, Serum albumin, Serum globulin and RBC count were studied to investigate the modulation of these parameters in different phases of menstrual cycle. Erythrocyte Sedimentation Rate (mm/hr) in MP, PP, SP were (23.83±11.57), (15.67±9.71), (18.50±11.31) respectively. It was significantly increased in MP compared to PP (P<0.01). Serum total protein (g/dl) in MP, PP, SP were (7.61±1.42), (8.01±0.74), (8.35±1.12) respectively. It was significantly increased in SP compared to MP (P<0.05). Serum Albumin (g/dl) in MP, PP, SP were (3.80±0.64), (4.09±0.58), (3.81±0.44) respectively. It was significantly increased in PP compared to SP (P<0.05). Serum globulin (g/dl) in MP, PP, SP were (3.81±1.59), (3.92±0.95), (4.47±1.39) respectively. It was significantly increased in SP compared to PP (P<0.05). Red Blood Cell Count (cells/cumm) in MP, PP, SP were (4.34±0.37), (4.37±0.45), (4.44±0.47) respectively. It was decreased in MP compared to other phases but statistically significant values were not noted.

**DISCUSSION:** E.S.R, Serum total protein, Serum albumin, Serum globulin and RBC count are indicators of the health and nutritional status of females, which in turn affects its reproductive capability. In the present study, Erythrocyte Sedimentation Rate(mm/hr) was significantly increased in MP compared to PP. Fibrinogen being an acute phase protein could rise during MP due to necrosis of the uterine endometrium because of vasospasm of its blood vessels and inflammatory cell infiltration of the uterine endometrium due to menses and these changes are due to sudden loss of...
estrogen and progesterone support of the endometrium leading to desquamation. In our study we found significantly low level of serum albumin due to sudden loss of hormones which have anabolic effect, hence rouleaux formation which is directly proportional to fibrinogen and indirectly proportional to albumin increase ESR by increasing density and decreasing surface area of RBCs.

Since in the present study, the RBC count was decreased in MP thus there was decrease in the ratio of RBCs to plasma and hence there was increase in rouleaux formation and ESR in MP. Several studies showed no significant changes of ESR in different phases of menstrual cycle. Some study showed significant increase in fibrinogen where as significant decrease in haematocrit & Erythrocyte Sedimentation Rate (ESR) in the menstrual phase. Serum globulin is not correlating with the ESR in the present study.

In the present study, Serum total protein (g/dl) was significantly increased in SP compared to MP. Secretory phase increase in serum protein may be an influence of progesterone as a protein anabolic effect on synthetic mechanism in liver but interestingly significant increase in serum albumin at PP & total protein in SP in our study remained unanswerable. In other study total protein was significantly increased in the luteal compared to the follicular phase. In a study of oestrous cycle in goats there was significant positive correlation between total protein & globulin. In some study the total protein level did not show statistical significance but was high during luteal phase.

In the present study, Serum Albumin (g/dl) was significantly increased in PP compared to SP. Other study showed no significant changes. In some study albumin concentration was significantly increased in the luteal compared to the follicular phase. Albumin did not significantly alter after estrogen and progestogen treatment in other study. The Serum albumin concentrations are sometimes used to indicate the degree of hemodilution.

In the present study, Serum globulin(g/dl) was significantly increased in SP compared to PP due to sex steroid anabolic effect. In some study the distribution of α2-PEG(pregnancy associated endometrial α2-globulin), a human analogue of β2-lactoglobulin, in endometrium at different phases of the cycle was determined and α2-PEG was first found in mid luteal phase and maximum in late luteal phase which is hormonally regulated. Raised alpha(2)-macroglobulin, IgG and transferrin levels were found after estrogen treatment. In the progestogen-treated group IgG, alpha(2)-macroglobulin and transferrin levels remained unchanged. In a study of oestrous cycle in goats there was significant negative correlation between progesterone and globulin.

In the present study Red Blood Cell Count increased from Menstrual Phase(MP) to Secretory Phase(SP) but was not statistically significant. Hemoglobin concentration may increase from menstrual phase to secretory phase due to increased erythropoiesis to compensate for the blood loss during menses. Progesterone has a natriuretic effect attributed to aldosterone antagonism, which results in plasma loss of sodium and water and hemoconcentration and thus increase RBC count in Secretory Phase.

The menstrual cycle is also affected by so many various factors--e.g. stress and changes in diet and iron. Several other studies showed no significant changes during various phases of menstrual cycle. In other study there was a pronounced tendency towards an increase in RBC Count from the early menstrual phase until the post-ovulatory period, with a subsequent decrease towards the end of the cycle. The cyclic variations act as indicators of iron status and are a potential source of error when iron status is assessed in large population surveys that include women of reproductive age.
Some studies showed that hemoglobin concentrations were significantly lower in follicular phase than in the luteal phase.\textsuperscript{25,31,39,40} The natural fluctuations in ovarian hormones during the course of the menstrual cycle influence the secretion of hormones that control the volume and content of the vascular space. Further study has to be conducted with fibrogen levels and gamma globulins and study has to be conducted with wide range of reproductive age group with large sample size.

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| PARAMETER                        | MP       | PP       | SP       | MP Vs PP  | PP Vs SP |
|----------------------------------|----------|----------|----------|-----------|----------|
| Erythrocyte sedimentation rate   | 23.83±11.57 | 15.67±9.71 | 18.50±11.31 | 2.96  P=0.01* | 1.805 P=0.06 |
| Serum total protein (g/dl)       | 7.61±1.42 | 8.01±0.74 | 8.35±1.12 | 1.394 P=0.23 | 2.242 P=0.04* |
| Serum albumin (g/dl)             | 3.80±0.64 | 4.10±0.58 | 3.81±0.44 | 1.877 P=0.42 | 0.092 P=0.99 |
| Serum globulin (g/dl)            | 3.81±1.59 | 3.92±0.95 | 4.47±1.39 | 0.328 P=0.74 | 1.711 P=0.09 |
| Red blood cell count (cells/cumm)| 4.34±0.37 | 4.37±0.45 | 4.44±0.47 | 0.294 P=0.84 | 0.942 P=0.50 |

*P<0.05 - Significant
MP, Menstrual Phase; PP, Proliferative Phase; SP, Secretory Phase.

Table 1
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