

Original Research Article

Impact of menstrual cycle phases on C-reactive protein concentrations

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ABSTRACT

Background: C-reactive protein (CRP) is one of the most commonly employed indicator of acute phase reaction and predictors of cardiovascular disease in healthy women; although, only a sparse information on its variations during a regular menstrual cycle is currently available. Our objective was to see whether CRP levels are affected during different phases of menstrual cycle.

Methods: Females aged 15-45 years with regular menstrual cycle in terms of length, flow and variation from cycle to cycle were followed for one menstrual cycle. Serum levels of C-reactive protein were measured in the Follicular (10th day) and in the Luteal (22nd day) phase of the menstrual cycle.

Results: C-reactive protein levels were observed to vary in response to the phases of menstrual cycle. The concentrations of C-reactive protein levels tend to be highest during the Follicular phase and it declined significantly in the Luteal phase.

Conclusions: Identifying the fluctuations in C-reactive protein levels during the menstrual cycle is essential as there may be clinical implication of the suitable timing of assessment while framing and concluding studies in women of reproductive age.

Keywords: C-reactive protein, Menstrual cycle, Predictor

INTRODUCTION

CRP is a established independent predictor of future cardiovascular disease.¹ CRP is an acute phase reactant produced in liver in response to interleukin-6, interleukin-1, and tumour necrosis factor α . It has a molecular weight of approximately 11,800 Da.² Besides liver, coronary-artery smooth-muscle cells, inflamed kidneys, human neurons, alveolar macrophages, and adipose tissue also produce CRP.³ CRP is normally present in very small amount in serum and it levels rises quickly and significantly in various infectious and inflammatory conditions. It is an indicator for low level of persistent inflammation.⁴ Although CRP was initially believed to be only an indicator of vascular inflammation, latest studies show that it also actively participates in atherogenesis by increasing the formation of plaque on interior walls of

blood vessel, decreasing the vessel ability to heal itself, causing proliferation of immune cells that help to create blockage on the inside of a blood vessel, reducing the vessels ability to create new healthy cells to counteract the injury.^{5,6} In addition khreiss et al have proposed that loss of the pentameric symmetry of CRP can bring about an altered or monomeric CRP, which might be the significant CRP promoter of the pro-inflammatory reaction in the coronary arteries.⁷

CRP is noticeable in the early stages of plaque development and is supposed to be involved during the atherogenic progression.⁸ In healthy women, raised levels of CRP is one of the most significant predictor of cardiovascular disease. A latest investigation from the Women's Health Study reported that CRP increases prognostic value beyond that identified by standard lipid

estimations, even after adjusting for age, obesity, smoking, blood pressure and diabetes mellitus.⁹ Principal female sex hormone estrogen, apart from being important in reproduction also plays an important role in regulating inflammation. Estrogen is mainly synthesized by ovaries, and then it is released in the blood circulation where it plays a major anti-inflammatory role by generating vasodilator substance nitric oxide, decreasing white blood cell and tumor necrosis factor- α (a pro-inflammatory cytokine) recruitment,¹⁰ searching and destroying free radicals and hence increasing the existence of the cell.^{10,11} Studies of the effect of exogenous estrogen on CRP levels have shown consistent results. Intake of oral contraceptive and orally administered hormone replacement therapy constantly increase CRP, whether the preparation incorporates estrogen and progesterone or estrogen alone.^{12,13} Though, dosage and delivery means appear to intervene exogenous estrogen's impact on levels of CRP.¹⁴ While the outcome of some studies exploring the effects of endogenous hormones on CRP concentrations seems to be not in agreement with the above patterns. As such, impact of the menstrual cycle related hormones changes in various phases across the normal menstrual cycle on the concentrations of CRP needs exploration. Hence this study was proposed.

METHODS

The study was accomplished on 111 normal healthy and regularly menstruating female subjects selected from the Gian Sagar Medical College and Hospitals, Rajpura. Permission from the institutional ethical committee and an informed written consent from all the subjects were obtained. One menstrual cycle of each subject was included in the analysis.

Inclusion criteria

The International consensus conference proposed terms for the most important features of menstrual bleeding was followed. Females aged 15-45 years having menstrual cycle length of 24-38 days with flow of 4-8 days and variation of 0-20 days from cycle to cycle were considered as regularly menstruating women.¹⁵

Exclusion criteria

- Women using oral contraceptives during the last 3 months.
- Current use of supplements or prescription medications.
- Pregnancy or breastfeeding in the past 6 months.
- Diagnosis of polycystic ovary syndrome.
- Recent history of infections or diagnosis of chronic medical conditions.
- Having autoimmune disease or thyroid disorder.
- Having a history of coronary vascular disease.

Measures

After recording the detailed menstrual history like age at menarche, length and flow of the cycle and variation from cycle to cycle, anthropometric measurements like weight, height, body mass index (BMI) were recorded in Kg/m^2 . Venous blood samples from each participant were collected twice during the menstrual cycle. The first sampling was scheduled at follicular phase (10th day of the menstrual cycle) and the second was scheduled at luteal phase (22nd day of the menstrual cycle) for estimating CRP concentrations. Five ml of fasting blood sample were drawn from the antecubital vein of each subject after 9-12 hrs of fasting during the follicular phase (on 10th day of the cycle) and luteal phase (on 22nd day) of the menstrual cycle under aseptic conditions. Serum concentration of C-reactive protein (CRP) was measured using enzymatic kits by Turbilatex method with high sensitivity & specificity.¹⁶

Statistical analysis

The data collected in the study were entered in Microsoft excel worksheet and was subjected to statistical analysis using the Student paired t test by Microsoft excel software data analysis tool. The significance level was considered at $p < 0.05$.

RESULTS

Table 1 Represents the demographic data for population of 111 study participants who had cycle length between 24 days and 38 days (mean SD, 29.55 2.71), flow between 4 and 8 days (mean SD, 4.88 1.14), variation from cycle to cycle between 0 and 20 days (mean SD, 4.27 3.22). The mean age of the participants was 26 ± 8 years and the mean body mass index (BMI) was $23.07 \pm 3.81 \text{ kg/m}^2$ at the screening visit.

Table 1: Demographic data of regularly menstruating women.

	Mean	SD
Age(yrs)	26	8
Height(m)	1.6	0.065
Weight(kg)	59.3	10.71
BMI(Kg/m^2)	23.07	3.81
Waist/ hip ratio	0.86	0.06
Length of menstrual cycle(days)	29.55	2.71
Flow (days)	4.88	1.142
Variation from cycle to cycle(days)	4.27	3.22

SD: Standard Deviation.

Table 2 presents the concentrations of CRP (Mean \pm SD) during the follicular and luteal phases of the menstrual cycle. The luteal phase measurement of CRP was significantly lower ($P \leq 0.00004$) compared with that during the follicular phase.

Table 2: Serum concentration of CRP during the follicular and luteal phase of the menstrual cycle in regularly menstruating women.

Parameter	Follicular phase	Luteal phase	P value
CRP(mg/L)			<0.00004*
Mean	1.88	1.54	
SD	1.49	1.30	

CRP: C-Reactive Protein, SD: Standard Deviation, *significant (P <0.05)

DISCUSSION

Very scarce literature is available to show the variations in CRP levels during normal menstrual cycle that too with a fewer number of subjects. Also, the present study included regularly menstruating women based on all the three criteria of the normal menstrual cycle i.e. the length and flow days of menstrual cycle and variation in days from cycle to cycle which to the best of our knowledge has not been done in whatever small number of studies done so far.

Additionally the exclusion criteria of the study strengthen the ability to draw conclusion, having reduced the potential for bias from known risk factors for inflammation and hormonal abnormalities. The aim of the present study was to find out the fluctuations in the inflammatory predictor C- reactive protein concentrations during the follicular and luteal phases of menstrual cycle. A significant raised mean CRP levels in follicular (1.88 ± 1.49 mg/L) was observed compared to luteal phase (1.54 ± 1.30 mg/L) in Regularly menstruating women ($p=0.0004$). Similar observations were found by Blum et al, Gursoy AY et al and Wander K et al, Blum et al, reported that CRP concentrations changed significantly during the menstrual cycle.^{14,17,18} The concentrations were highest in the early follicular phase and inversely correlated to estradiol concentrations.

Gursoy AY et al. observed similar results. CRP values were significantly higher in the early follicular phase in contrast to luteal phase (1.8 mg/L vs. 0.7 mg/L respectively).¹⁸ He noticed that Estrogen levels were inversely correlated with CRP levels hence low levels of estrogen during early follicular phase can be underlying physiology related with relatively high levels of CRP during follicular phase. Further Gursoy did not find any correlation between progesterone levels and CRP. Wander K et al observed that progesterone increases CRP, estrogen decreases CRP, and menstruation may increase CRP.¹⁴ He observed a ten-fold increase in estrogen was associated with a 29% decrease in CRP, a ten-fold increase in progesterone was associated with a 23% increase in CRP, and menses was associated with a 17% increase in CRP while no association between ovulation and CRP was found. Further he reported that the negative association between estrogen and CRP is stronger in the follicular phase, while the positive

association between progesterone and CRP is limited to the luteal phase. In contrast to our study findings, increased CRP has been observed by Gaskins AJ et al, Jilma B et al in luteal phase of menstrual cycle in regularly menstruating women, while Wunder K et al, Saxena AR et al did not find any significant differences in CRP concentrations during a menstrual cycle.¹⁹⁻²²

Gaskins AJ et al found in regularly menstruating women that CRP levels varied significantly across the cycle.¹⁹ CRP tends to be highest during menses, decreased during the follicular phase, was lowest on the expected day of ovulation, and increased in the luteal phase. He also reported that CRP was significantly inversely associated with endogenous estradiol and positively associated with luteal progesterone. Moreover, a 10-fold increase in estradiol was associated with a 24.3% decrease in CRP and a 10-fold increase in luteal progesterone was associated with a 19.4% increase in CRP. Jilma B et al in eighteen healthy regularly menstruating premenopausal women found a CRP increase of 44% at midcycle (ovulation) and 31% in the luteal phase in comparison to follicular phase levels.²⁰

This was significant correlated to the relative rise in progesterone levels during midcycle and the luteal phase. Others like Wunder et al found small non- significant differences in CRP concentrations between the follicular, ovulatory and luteal phase of the menstrual cycle in 36 healthy, young, normo-androgenic women, having a normal body mass index.²¹⁻²² Similarly Saxena AR et al also observed a non-significant reduction in CRP levels from follicular to luteal phase of menstrual cycle. The differences between the results of Gaskins AJ et al, Jilma et al, Wunder et al, Saxena AR et al and the present study can most likely be explained by the differences in the assay methodologies used for the determination of CRP.¹⁹⁻²² Further Gaskins AJ et al findings reveal that during the menses (early follicular phase) largest number of women were at moderate-to-elevated risk of cardiovascular disease (hs-CRP, >1 mg/L) while the small number of women were at cardiovascular risk on the ovulation day.¹⁹

He also noticed that except in the midluteal phase, every clinic visit i.e. on mid-follicular, late- follicular, LH/FSH surge day, and early and late luteal phase had significantly fewer number of women with high-to-moderate risk of cardiovascular disease when compared with the menses day visit. The reason for the difference in the present findings by the results of Gaskins AJ et al may possibly that our study time points were limited to two specific times in the menstrual cycle, late- follicular and mid-luteal phase which may not coincide their timepoint in the cycle.¹⁹ These results were interpreted as the effect of estrogen. Estrogen receptors are highly expressed in endothelial and vascular smooth muscle cells throughout the human body.²³ Estrogen has a major anti-inflammatory role as postulated by underlying mechanisms:

- Estrogen reduces levels of tumor necrosis factor-alpha, a major pro-inflammatory cytokine¹⁰ resulting in the reduced formation and liberation of chemokines such as interleukin-8 and platelet activating factor along with the down regulating adhesion molecules like intercellular adhesion molecule 1 and E-selectin, which leads to decreased recruitment of leukocytes.¹¹
- Estrogen can act as an antiapoptotic agent on various cell types including endothelial cells by inhibiting the liberation of cytochrome c from the mitochondria, thus decreasing ensuing vascular inflammation.²⁴
- While there are conflicting results for progesterone:
- Progesterone can act as pro-inflammatory by promoting the chemo-tactic activity of neutrophils and increase production of some of the inflammatory mediators like Interleukin-6 and leukemia inhibiting factor (LIF) through monocytes.²⁵
- Progesterone can act as anti-inflammatory by decreasing natural killer cell activity, macrophage Tumor necrosis factor, and nitric oxide synthase formation and inhibits the T-cell development and activity.²⁶

Hence our study suggests an overall anti-inflammatory effect of estrogen and progesterone.

CONCLUSION

This explains that the women with Regular menstrual cycle have higher CRP levels in the follicular phase where the estrogen levels are relatively low compared to luteal phase. Hence menstrual cycle phase must be taken into consideration by the clinicians and physicians while interpreting a woman's CRP measurement. While the best time to measure CRP during a woman's cycle has yet to be established, measurements should be made at the same time each month for consistent comparisons.

Application of standardized timing of CRP investigation in reproductive-aged women would enhance the proper interpretation in future studies as well as in clinical settings. Hence taking into account the phases of menstrual cycle in the clinical guidelines for reproductive-age women could increase the importance of CRP as a predictor of cardiovascular disease.

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