CRP at early follicular phase of menstrual cycle can cause misinterpretation for cardiovascular risk assessment

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(Received: February 20, 2015; Revised manuscript received: June 7, 2015; Accepted: August 3, 2015)

Abstract: *Objective:* C-reactive protein (CRP) is a well-known marker of inflammation and infection in clinical practice. This study is designed to evaluate CRP levels in different phases of menstrual cycle, which might end up with misleading conclusions especially when used for cardiovascular risk assessment. *Methods:* Twenty-seven women were eligible for the cross-sectional study. Venous blood samples from each participant were collected twice during the menstrual cycle. The first sampling was held at 2nd to 5th days of the menstrual cycle for FSH, estradiol, CRP, and sedimentation, and the second was done at 21st to 24th days of the menstrual cycle for measurement of progesterone, CRP, and sedimentation values. *Results:* CRP values were significantly higher in the early follicular phase compared to luteal phase (1.8 mg/L [0.3–7.67] vs. 0.7 mg/L [0.1–8.3], $p < 0.001$, respectively). In both phases of the menstrual cycle, sedimentation rate was similar (12.1 ± 6.7 vs. 12.3 ± 7.7; $p = 0.717$, respectively). *Conclusions:* CRP levels in early follicular phase of the menstrual cycle (menstruation) are significantly higher than CRP levels in luteal phase of the same cycle. In reproductive age women, detection of CRP for cardiovascular risk assessment during menstruation might not be appropriate.

Keywords: CRP, early follicular phase, menstruation

Introduction

C-reactive protein (CRP) is a well-known marker of inflammation and infection in clinical practice. It is mainly produced by hepatocytes, but also coronary-artery smooth-muscle cells, inflamed kidneys, human neurons, alveolar macrophages, and adipose tissue [1]. CRP levels vary between genders mostly higher in women compared to men [2, 3] and increase with age in both genders [2]. However, there is not a consensus about changing reference values among different races [2, 3]. In clinical practice, CRP is used for two main purposes: evaluation of infectious processes and low-grade inflammation. When used for the diagnosis of infectious diseases, the cut off value has a wide range, changing between 10 and 80 mg/L [1]. If used as a marker of chronic low-grade inflammation, then high sensitivity C-reactive protein (hs-CRP) form is prefered to accurately detect CRP levels within the range needed for cardiac risk detection [4, 5].

Low-grade inflammation has been shown to have a pivotal role in the pathophysiology of atherosclerosis and cardiovascular disease. Inflammatory activation is either a trigger in the acute phase or a substrate in the chronic phase of the atherosclerotic process [6]. The injurious processes that promote atherosclerotic plaques result with a contour inflammatory reaction in which cytokines, bioactive molecules, and related cells are included [7]. This is why inflammatory markers like adhesion molecules, cytokines, white blood count, and acute pkase reactants such as fibrinogen and CRP have been considered as predictors of cardiovascular risk [7]. High quality evidence points out that hs-CRP is mostly useful in subjects with a history of cardiovascular events and intermediate risk of events at 10 years, where adding hs-CRP to the classical models for event risk estimation improves risk staging [8]. As a result, CRP has been accepted as one of the criteria for cardiovascular risk assesment by Centers for Disease Control and Prevention and American Heart Association. The criteria defines

an increament in risk, with increasing CRP values, subgrouped as low, average, and high (<1, 1–3 or >3 mg/L, respectively) [7].

CRP levels should be evaluated cautiously since it has been known to be affected by many clinical and external intervening situations. Among these situtions, physical activity [9], stress [10], genetic polymorphisms [11], oral contraceptive use [12], blood pressure, and body mass index (BMI) [8] have been reported. As the baseline levels of CRP in apparently healthy [13] men and women are highly predictive of future risk of heart attack, stroke, and sudden cardiac death, any factor influencing the baseline level of CRP gains significance.

In women of reproductive age, detection of basal CRP levels in different phases of the menstrual cycle has been evaluated previously [14–23]. Nevertheless, the data about menstrual cycle variability of CRP is conflicting. In this study, CRP levels of women during follicular and luteal phases of the menstrual cycle were evaluated, and the results are represented with review of the literature.

Materials and Methods

The study was performed in the Obstetrics and Gynecology outpatient clinic of the University Hospital between March 2013 and June 2014. Twenty-seven apparently healthy hospital staff was included in the study. The participants of this study also contributed to another study performed during the same period in our clinic [24]. The ethical committee of the University Hospital approved the study, and informed consent was taken from all participants. All the participants' routine gynecological examination and pelvic ultrasonography were normal. One cycle of each participant was included in the analyses. Exclusion criteria were presence of any chronic disease, any hormonal contraceptive use, irregular menstruation, CRP values over 10 mg/L, smoking, and pregnancy. Venous blood samples from each participant were collected twice during the menstrual cycle. The first sampling was held at follicular phase (2nd to 5th days of the menstrual cycle) for FSH, estradiol, CRP, and sedimentation, and the second was held at luteal phase (21st to 24th days of the menstrual cycle) for progesterone, CRP, and sedimentation values. Ovulation was defined as progesterone level ≥3 ng/mL in the luteal phase. BMI was calculated as weight (kg)/height $(m)^2$.

CRP levels were measured by immunoturbidimetric assay (Abbott® Architect *c*8000, USA). Sedimentation was measured by Vacuette SRS 20/II, and results were given as mm/h. FSH, estradiol, and progesterone levels were measured by Chemiluminescent Microparticle Immunoassay technology (Abbott® *i*1000, USA). The intra-assay and inter-assay coefficients of variation for all parameters analyzed were <2.7 and 3.2, respectively.

Statistical analysis was performed using SPSS for Windows Version 21.0 (SPSS Inc., Chicago, IL, USA). Data were shown as mean ± standard deviation (SD) or median (minimum–maximum) and numerical or percentile where applicable. The differences between groups were compared by Student's *t*-test. Otherwise, Wilcoxon test was used for comparison of values which did not meet parametric test criteria. Independent variables were compared by Mann–Whitney *U* test. Correlation between numeric variables was reported by Spearman correlation coefficient. A p value less than 0.05 was considered statistically significant.

Results

The mean age and BMI of the participants were 25.9 ± 5.1 years and 23.2 ± 3.6 kg/m^2. The characteristics of the menstrual cycle and the results of the hormone analyses of the cases are given in *Table I*. CRP values were significantly higher in the early follicular phase compared to luteal phase (1.8 mg/L [0.3–7.67] vs. 0.7 mg/L [0.1–8.3], $p < 0.001$, respectively) *(Table II)*. Among the participants, 18 had ovulatory and the remaining 9 had anovulatory cycles. In ovulatory cycles, the levels of CRP were significantly higher in follicular phase than in luteal phase (1.7 mg/L [0.3–7.7] vs. 0.7 mg/L [0.1–8.3], $p < 0.002$, respectively). However, in anovulatory cycles, no significant difference was found in follicular and luteal phase CRP values (2.6 mg/L [0.3–7.2] vs. 0.6 mg/L [0.1–6.3], $p = 0.068$, respectively). In both phases of the menstrual cycle, sedimentation rate was similar (12.1 ± 6.7 mm/h vs. 12.3 ± 7.7 mm/h, $p = 0.717$, respectively).

According to the Spearman's rank correlation analyses, neither the baseline characteristics (age, BMI) nor the hormone results (FSH, estrogen, progesterone) were correlated with CRP values. However, a positive correlation between CRP values and sedimentation rate

Table I Menstrual cycle characteristics and hormone analysis of the study population

Variable	Mean ± SD
Menstrual cycle (day)	29.7 ± 6.6
Duration of menstruation (day)	4.8 ± 1.1
Menstrual bleeding (pad/day)	3.2 ± 1.4
Follicular phase day for sampling	2.6 ± 0.6
Luteal phase day for sampling	21.0 ± 0.7
FSH (mIU/mL)	4.9 ± 1.8
E2 (pg/mL)	42.0 ± 22.7
Progesterone (ng/mL)	6.6 ± 5.1

FSH: follicle stimulating hormone; E2: estradiol; BMI: body mass index

Table II CRP values in different phases of menstrual cycle

	Follicular phase	Luteal phase	*P*
CRP (median) mg/L	1.8 [0.3–7.67]	0.7 [0.1–8.3]	<0.001*
Sedimentation (mean) mm/h	12.1 ± 6.7	12.3 ± 7.7	0.717

*$p < 0.05$

was observed both for follicular and luteal phases of the cycle ($r = 0.422$, $p = 0.035$ and $r = 0.628$, $p = 0.001$, respectively).

Discussion

According to the current study, CRP levels in early follicular phase of the menstrual cycle (menstruation) are significantly higher than CRP levels in luteal phase of the same cycle. Therefore, in reproductive age women, detection of CRP for cardiovascular risk assessment during menstruation is not appropriate and can be misleading since might interfere with the categorization of the risk group.

As in our study, significantly higher levels of CRP during early follicular phase have been reported previously [14, 17]. Among the studies evaluating menstrual cycle variability of CRP, the study of Gaskins [14], with the largest study population, also supports our results. Menstruation is a low-grade inflammatory process within the endometrium, which might be the explanation for high CRP levels during menstruation. As estrogen levels were inversely correlated with CRP [17], low levels of estrogen during menstruation can be another underlying physiology related with relatively high levels of CRP during early follicular phase. The hypothesis that anti-inflammatory effects of estrogen emerging by various pathways such as increased nitric oxide synthesis or reduced proinflammatory cytokines [14] supports this mechanism. On the contrary, other studies either reporting no difference in CRP levels in different phases of the menstrual cycle [16, 22] or higher levels in the luteal phase are with very limited number of cases [15, 19].

Ovulation is another condition of low-grade inflammation in which increased values of CRP might be expected. Supporting this data, 44% increase in CRP levels in midcycle was documented previously [19]. On the contrary, others [14] reported that CRP values were lowest on the expected day of ovulation. In our study, the levels of CRP were not obtained in midcycle. Therefore, the current study cannot conclude about midcycle variability of this marker. However, intragroup analysis in this study revealed lack of difference between follicular and luteal phases of CRP in anovulatory cycles. The lower levels of CRP in the luteal phase of ovulatory cycles favor the hormonal dynamics following ovulation. The progesterone rise after ovulation might have a subtle effect on CRP levels. The change in hormonal milieu and their effects on inflammation might be related with this issue. Moreover, progesterone has been known to have a plenty of effects on immunological system. Previous literature about the subject, concludes that luteal phase of the menstrual cycle is accompanied by a shift in Th1/Th2 ratio favoring Th2 cells, mostly involved in humoral immunity [25] which might result with a shift in abundance of synthesized cytokines by these cell populations, which are known to be one of the triggering factors for CRP synthesis. Although, larger number of patients was required for optimum interpretation of statistical analysis, our results did not find any correlation between progesterone levels and CRP.

In conclusion, many physiological, metabolic, and psychological [20] factors can easily effect baseline CRP values. In reproductive age women, other than the well-established factors, menstruation is another physiological condition that might lead to erroneous assessment. Therefore, future studies concluding more accurate and undoubtfull sampling is urgently needed.

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Funding sources: Nothing declared.

Authors' contribution: AYG performed the project development and article writing; GSC, data analysis and article writing; MK, data analysis; EP, data collection; TC, data collection; and SD, project development. All authors had full access to all data in the study and take the responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgement: The authors thank to the participants for their significant contribution.

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