Original Article

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Physiology Section

Can Menstrual Cycle Length Predict Cardiovascular Risk in Healthy Indian Females? A Cross-sectional Study

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ABSTRACT

Introduction: Several studies show that variations in the length of the menstrual cycle significantly affect lipid and C-Reactive Protein (CRP) parameters. There is an acute paucity of literature comparing lipid profiles and CRP in women with short and prolonged menstrual cycle length in the absence of Polycystic Ovary Syndrome (PCOS) and other gynaecological conditions.

Aim: To determine the lipid profile and CRP levels in women with short and prolonged menstrual cycle length and compare them with women with a normal menstrual cycle length to identify women at cardiovascular risk.

Materials and Methods: The association of lipid and CRP parameters with menstrual cycle length was evaluated in the present cross-sectional study involving 226 women aged 15-45 years selected from GSMCH, Patiala, Punjab, India, from May 2014 to December 2018. Based on a questionnaire about menstrual bleeding, healthy females were divided into three groups: women with short, normal, and long menstrual cycles. A menstrual cycle length of 24-38 days was considered normal. Lipid profile and CRP were analysed during the menstrual phase of the female monthly cycle in women with short, normal, and prolonged cycles, and the results were statistically analysed using one-way ANOVA. A p-value of <0.05 was considered statistically significant.

Results: A total of 111 (49%) of the 226 females had a normal menstrual cycle duration, whereas the remaining 38 (17%) and 77 (34%) had short and lengthy cycles, respectively. Mean levels of Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein cholesterol (LDL), Very Low-Density Lipoprotein cholesterol (VLDL), lipid ratio, and CRP increased in women with short and long menstrual cycles compared to women with normal menstrual cycles. This increase was significant for TC, TG, VLDL, TC/HDL, and TG/HDL ratios, while HDL was significantly lower ($p<0.05$).

Conclusion: Women with short and long menstrual cycles have a higher risk of developing Cardiovascular Disease (CVD) in the coming years compared to women with a normal menstrual cycle length.

Keywords: Cardiovascular disease, C-reactive proteins, Predictor, Lipid profile

INTRODUCTION

Menstrual cycle length has been correlated with infertility [1], ovarian and breast malignancies [2], type 2 diabetes mellitus [3], and CVD [4]. Fluctuations in sex hormone levels are thought to be related to the length of the menstrual cycle because hormones present during the menstrual cycle affect the proliferation and shedding of the endometrium [5-7]. The prevalence of CVD in women aged 20-39 years is half that of men in the same age group [8]. The difference in cardiovascular risk between men and pre- and post-menopausal women is due to the cardioprotective effect of oestrogen's role in younger, menstruating women [9]. However, pre-menopausal and post-menopausal females have notably dissimilar hormonal profiles of which oestrogen is the only factor. Various markers of CVD risk determinants, including lipid and CRP levels, are considered to be related to female ovarian hormones [10].

Overall, short menstrual cycles are associated with reduced per cycle exposure to estradiol, whereas long cycles have decreased mean concentrations of progesterone and marginally decreased mean estradiol concentrations compared with normal-length cycles [7]. Various studies in PCOS women with irregular menstrual cycles show that lipid and CRP parameters are significantly affected by variations in menstrual cycle length [9,11-15]. However, to our knowledge, there is little in the literature regarding the relationship between lipid profiles, CRP, and menstrual cycle length in the absence of PCOS and other gynaecological diseases. Hence, the study was undertaken to provide information to fill this gap and investigate whether menstrual cycle length could be a simpler vital sign, like blood pressure and heart rate, to better predict cardiovascular status in resource-poor locations. The present study aimed to evaluate lipid profile status and CRP levels in women with short and prolonged menstrual cycle lengths and compare them with women with normal menstrual cycle length.

MATERIALS AND METHODS

The study was conducted in the Department of Physiology in close collaboration with the Department of Gynaecology and Biochemistry of Gian Sagar Medical College and Hospital, Punjab, from May 2014 to December 2018. The research plan was in accordance with the guidelines of the Declaration of Helsinki and was duly approved by the ethical committee of the institute with letter Ref no. Prin/GSMCH-14/PA/94.

Participants were selected from among the family members (relatives) accompanying patients attending the gynaecology OPD of the institute. Out of 710 females of reproductive age 15-45 years selected for the study, only 350 fulfilled the inclusion criteria. A total of 330 females gave consent for present cross-sectional study, and 226 completed the study, while the rest withdrew in between the study and were finally excluded from the study.

A simple random sampling technique was used, and the sample size was calculated using convenient sampling. Before starting the study, written consent was obtained from all participants after a detailed explanation of the study in the vernacular language.

Inclusion criteria: Females in the reproductive age range (15-45 years) with no history of PCOD or any other gynaecological issues were included.

Exclusion criteria: Women who had used contraceptives in the past three months, were currently using nutritional supplements

or prescription medications, pregnant or lactating in the past six months, diagnosed with PCOD, recently infected or diagnosed with a chronic disease, with a Body Mass Index (BMI) of <18 or >35 kg/m^2, or diagnosed with autoimmune disease, thyroid disease, or coronary artery disease before the study by a doctor were excluded along with those who refused to give consent and decided to withdraw in the interim.

Procedure

The length of the menstrual cycle was considered as the time interval between the first day of one bleeding period to the first day of the next bleeding period. These women were divided into three groups based on the average menstrual cycle length from the past six months [16]:

- **Group-1:** Women with a short length of the menstrual cycle of less than 24 days.
- **Group-2:** Women with a normal menstrual cycle length of 24-38 days.
- **Group-3:** Women with a prolonged menstrual cycle length of more than 38 days.

Medical examination: Before performing the study, all participants completed self-administered questionnaires to obtain information about menstrual/medical/family/medical history and lifestyle. To rule out any systemic illness, each subject underwent clinical, biochemical, and ultrasound examinations, and trained personnel performed anthropometric measurements on all participants.

Biochemical analysis: A 5-6 mL fasting (at least 9-12 hours) venous blood samples were collected between 8-10 AM after an overnight fast in a standard vial during the menstrual phase (1-4 days) of the menstrual cycle. Serum total cholesterol (CHOL) and TG concentrations were measured by the Cholesterol-Oxidase-Peroxidase (CHOD-POD) method and glycerokinase peroxidase method, respectively [17]. LDL and VLDL were calculated according to the Friedewald formula. Biochemical analysis of the lipid profile and CRP [18] was performed using a fully automated Mindray calibrated device.

STATISTICAL ANALYSIS

Data related to biochemical parameters and average menstrual cycle length were analysed using Microsoft excel data analyser by one-way Analysis of Variance (ANOVA). Mean standard deviation was used to describe the main variables. The statistical difference between mean lipids and CRP levels during menstruation among women with normal, short, and long menstrual cycles was evaluated using ANOVA.

RESULTS

Compare the demographics and average menstrual length of women with short, normal, and long menstrual cycles [Table/Fig-1]. There were no significant differences in age (p=0.09), while BMI and waist-to-hip ratio were not significantly higher in women with short or long menstrual cycles (p=0.003 and p=0.007, respectively) compared to women with a normal menstrual cycle length. Mean menstrual cycle length was significantly different (p=0.00001) between women with short, normal, and long menstrual cycle length.

Variable	Females with Short menstrual cycle length (n=38)	Females with normal menstrual cycle length (n=111)	Females with prolonged menstrual cycle length (n=77)	F-value	p-value
Mean age (years)	27.42±7.35	26.29±7.97	24.63±4.71	2.38	0.09
BMI (kg/m^2)	25.17±3.35	23.07±3.80	24.66± 4.41	5.81	**0.003***
Waist/Hip (W/H) ratio	0.89±0.05	0.86±0.06	0.88±0.05	4.99	**0.007***
Average length of cycle (in days)	20.89±0.95	29.55±2.7	56.78±17.95	203	**0.00001***

[Table/Fig-1]: Characteristics of study participants.

In women with short, normal, and long menstrual cycle lengths, the mean lipid profile and CRP levels were compared during the menstrual phase [Table/Fig-2]. In comparison to women with normal menstrual cycle length, it was found that women with short and prolonged menstrual cycles had higher mean levels of cholesterol, TG, LDL, VLDL, and CRP. This rise in levels was significant for TC, TG, and VLDL (p=0.04, p=0.00001, and p=0.000001, respectively), while HDL levels were significantly lower (p=0.005).

Variable	Females with short menstrual cycle length (38)	Females with average menstrual cycle length (111)	Females with prolonged menstrual cycle length (77)	F-value	p-value
Total Cholesterol (TC) (mg/dL)	170.94± 31.02	164.34 ±29.38	176.14.80± 34.94	3.20	**0.04***
Triglycerides (TG) (mg/dL)	151.81± 38.33	89.10 ± 14.36	159.11± 32.35	197.59	**0.00001***
HDL (mg/dL)	46.32± 9.45	50.59± 10.71	46.16± 9.19	5.40	**0.005***
LDL (mg/dL)	93.74± 34.37	95.91± 29.22	98.15± 36.93	0.24	0.78
VLDL (mg/dL)	30.89± 7.14	17.84± 2.87	31.82± 6.47	197.59	**0.000001***
CRP(mg/L)	1.91±0.32	1.71±1.15	1.83±1.35	0.50	0.60

[Table/Fig-2]: Comparison of mean lipid profile and CRP levels during menstrual phase in women with short, normal and prolonged menstrual cycle length.

Comparison of mean lipid ratios during the menstrual phase in women with short, normal, and prolonged menstrual cycle lengths [Table/Fig-3]. It was observed that women with short or prolonged menstrual cycle lengths have significantly raised mean levels of TC/HDL and TG/HDL ratios (p=0.0006 and p=0.000001, respectively) compared to women with a normal menstrual cycle length.

Variable	Females with Short menstrual cycle length (38)	Females with average menstrual cycle length (111)	Females with prolonged menstrual cycle length (77)	F-Value	p-value
TC/HDL	3.86±1.18	3.39±0.91	3.97 ± 1.19	7.58	**0.0006***
TG/HDL	3.47 ±1.04	1.86±0.57	3.59±1.07	110.17	**0.000001***
LDL/HDL	2.16 ±1.09	2.02±0.83	2.25 ±1.11	1.35	0.25

[Table/Fig-3]: Comparison of mean lipid ratio during menstrual phase in women with short, normal and prolonged menstrual cycle length.

DISCUSSION

In the current study, the lipid profile and CRP values are compared in females with short, normal, and prolonged menstrual cycle lengths. The fact that the study was conducted on females with irregular menstrual cycle duration for the previous six months, without PCOS or other gynaecological problems, gives the study's findings clinical value. The study's key results were that women with irregular menstrual cycle duration had higher levels of biochemical variables including lipid profile and CRP that are known to contribute to CVD. Lipid profile and CRP are recognised as biochemical indicators of potential CVD in the future.

The majority of earlier research conducted on known cases of PCOD/PCOS discovered that lipid levels were greater in women with irregular menstrual cycle duration compared to controls. The study also showed that women with short or prolonged menstrual cycle lengths had greater levels of lipids than women with a normal menstrual cycle length though LDL did not show a statistically

significant difference between the groups. Similar to this, while the difference did not achieve statistical significance, CRP levels were greater in women with short and long menstrual cycles compared to those with a normal cycle length. The present study results indicate raised lipid levels along with elevated CRP concentrations may be due to lower estradiol levels in irregularly menstruating women [19].

It is possible that lipid parameters may be elevated much earlier, even before inflammation begins in women with irregular menstrual cycle length compared to women with a normal length of the menstrual cycle, as indicated by the significant increase in lipid parameters and the insignificant difference in CRP in these subjects when compared to normal subjects.

The results of the present investigation concur with other worldwide studies of a similar nature [12,14,20-23]. Previous studies have shown that women with longer cycle lengths throughout their lives have higher TG readings. On the other hand, women whose cycle pattern changes to shorter cycles have lower TG readings. Women with longer cycle lengths have higher TC and LDL cholesterol levels, as well as lower HDL cholesterol levels. However, there was no significant relationship between the length of the cycle and other blood lipids (HDL, LDL, TC) [20]. Another study found that, although there was a small correlation between TG concentration and cardiovascular risk indicators (HDL and TG), there was no significant correlation between the menstrual cycle pattern and indicators of cardiovascular risk (HDL, TG, and CRP) [21]. In another study, non-hyperandrogenic women with polycystic ovarian hypertension were found to have higher CRP levels compared to a control group with normal menstrual cycles [22,23].

According to the presently discussed findings, women with short or lengthy menstrual cycles may have elevated lipid and CRP levels as a result of reduced estradiol levels. Oestrogen has a positive impact on lipoprotein metabolism by upregulating the LDL receptors, upregulating ATP-binding cassette transporter-A1 (ABCA1) and apolipoprotein-A1 (APOA1), a key HDL protein, which increases HDL production, and suppressing the activity of the hepatic scavenger receptor class B type 1 (SR-BI), which results in less hepatic cholesterol uptake from HDL [24]. Progesterone, on the other hand, is hypothesised to counteract the stimulatory effects of oestrogen or have no effect on lipoprotein metabolism [25].

Oestrogen appears to largely enhance the light subtype of VLDL, which is weak in atherogenicity, resulting in overall positive benefits [26] and protecting against atherosclerosis, even though these alterations would also tend to raise TG levels. In addition to downregulating adhesion molecules such as intracellular adhesion molecule 1 and E-selectin, oestrogen lowers levels of tumour necrosis factor-alpha [27], interleukin-8, and platelet-activating factor. This results in a reduction in the recruitment of leukocytes [28]. By preventing the release of cytochrome c from the mitochondria, oestrogen can block the apoptotic process in endothelial cells, reducing the subsequent vascular inflammation [29].

By reducing the activity of natural killer cells, macrophage TNF, and inhibiting T-cell growth and activity, progesterone has anti inflammatory effects [30]. Therefore, oestrogen is often responsible for the anti-atherogenic action of reproductive hormones on lipid and CRP levels. Therefore, the authors here may infer that elevated blood lipid levels found in women with short and long menstrual cycles may be caused by an oestrogen-related decline in Lipoprotein Lipase (LPL) function. Lower HDL levels in women with irregular menstrual cycle length are likely caused by a decrease in oestrogen output. Further research is required to corroborate such data.

It's interesting to see that higher BMI was linked to longer menstrual periods. According to the findings of the present study, obesity may cause prolonged cycles and a suppression of reproductive hormones.

Limitation(s)

This study was subjected to bias since it was based on a questionnaire on menstrual bleeding patterns. Another drawback is that the authors here have connected questionnaire data with biochemical data without obtaining estimations of estradiol and progesterone hormone levels due to funding limitations.

CONCLUSION(S)

It can be concluded that variations in the length of the menstrual cycle result in elevated levels of lipids and CRP, which may serve as a predictor of CVD. This is especially useful in our resource-poor countries, where women's health is often put on the back burner. These results highlight the potential negative health effects of menstrual cycle length disorder in women and emphasise the significance of monitoring menstrual cycle features throughout a woman's reproductive life to avoid CVD and atrial fibrillation in females.

REFERENCES

[1] Harris BS, Steiner AZ, Faurot KR, Long A, Jukic AM. Systemic inflammation and menstrual cycle length in a prospective cohort study. Am J Obstet Gynecol. 2023;28(2):215.e1-215.e17.

[2] Wang S, Wang YX, Sandoval-Insausti H, Farland LV, Shifren JL, Zhang D, et al. Menstrual cycle characteristics and incident cancer: A prospective cohort study. Hum Reprod. 2022;37(2):341-51.

[3] Wang YX, Shan Z, Arvizu M, Pan A, Manson JE, Missmer SA, et al. Associations of menstrual cycle characteristics across the reproductive life span and lifestyle factors with risk of Type 2 Diabetes. JAMA Netw Open. 2020;3(12):e2027928.

[4] Huang C, Lin B, Yuan Y, Li K, Xu B, Zhang P, et al. Associations of menstrual cycle regularity and length with cardiovascular diseases: A prospective study from UK Biobank. J Am Heart Assoc. 2023;12(11):e029020.

[5] Chavez-MacGregor M, Elias SG, Onland-Moret NC, van der Schouw YT, Van Gils CH, Monninkhof E, et al. Postmenopausal breast cancer risk and cumulative number of menstrual cycles. Cancer Epidemiol Biomarkers Prev. 2005;14(4):799-804.

[6] Terry KL, Willett WC, Rich-Edwards JW, Hunter DJ, Michels KB. Menstrual cycle characteristics and incidence of premenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2005;14(6):1509-13.

[7] Mumford SL, Steiner AZ, Pollack AZ, Perkins NJ, Filiberto AC, Albert PS, et al. The utility of menstrual cycle length as an indicator of cumulative hormonal exposure. J Clin Endocrinol Metab. 2012;97(10):E1871-E1879.

[8] Mumford SL, Dasharathy S, Pollack AZ, Schisterman EF. Variations in lipid levels according to menstrual cycle phase: Clinical implications. Clinical Lipidology. 2011;6(2):225-34.

[9] Matthews KA, Santoro N, Lasley B, Chang Y, Crawford S, Pasternak RC. Relation of cardiovascular risk factors in women approaching menopause to menstrual cycle characteristics and reproductive hormones in the follicular and luteal phases. J Clin Endocrinol Metab. 2006;91(5):1789-95.

[10] Gozukuçuk M, Gursoy AY, Destegul E, Taşkın S, Şatıroglu H. Homocysteine and C- reactive protein levels in women with polycystic ovary syndrome. Gynaecol Minim Invasive Ther. 2021;10(4):210-14.

[11] Solomon CG, Hu FB, Dunaif A, Rich-Edwards JE, Stampfer MJ, Willett WC, et al. Menstrual cycle irregularity and risk for futurecardiovascular disease. J Clin Endocrinol Metab. 2002;87(5):2013-17.

[12] Alebić MŠ, Stojanović N, Baldani DP, Duvnjak LS. Metabolic implications of menstrual cycle length in non-hyperandrogenic women with polycystic ovarian morphology. Endocrine. 2016;54(3):798-807.

[13] Cobin RH. Cardiovascular and metabolic risks associated with PCOS. Intern Emerg Med. 2013;8(Suppl 1):S61-64.

[14] Boulman N, Levy Y, Leiba R, Shchar S, Linn R, Zinder O, Blumenfeld Z. Increased C- reactive protein levels in the polycystic ovary syndrome: A marker of cardiovascular disease. J Clin Endocrinol Metab. 2004;89(5):2160-65.

[15] Wang ET, Cirillo PM, Vittinghoff E, Bibbins-Domingo K, Cohn BA, Cedars MI. Menstrual irregularity and cardiovascular mortality. J Clin Endocrinol Metab. 2011;96(1):E114-18.

[16] Speroff L, Fritz MA. Clinical gynaecologic endocrinology and infertility. Lippincott Williams & Wilkins; Philadelphia USA; 2011.

[17] Teitz NW ed. Clinical guide to laboratory test. 3rd edition. Philadelphia, Pa: WB Saunders company;1995:130-131

[18] Price CP, Truit AK, Berry D, Gorman EG. Development and validation of a particle enhanced turbidimetric immunoassay for C-reactive protein. J Immunol Methods. 1987;99(2):205-11.

[19] Nafari A, Mohammadifard N, Haghighatdoost F, Nasirian S, Najafian J, Sadeghi M, et al. High-sensitivity C-reactive protein and low-density lipoprotein cholesterol association with incident of cardiovascular events: Isfahan cohort study. BMC Cardiovasc Disord. 2022;22(1):241.

[20] Rubba F, Mattiello A, Chiodini P, Celentano E, Galasso R, Ciardullo AV, et al. Menstrual cycle length, serum lipids and lipoproteins in a cohort of Italian Mediterranean women: Findings from Progetto ATENA. Nutrition, Metabolism & Cardiovascular Diseases. 2008;18(10):659-63.

[21] Hoeger K. Menstrual cycle status and metabolic risk in a cohort of women with and without polycystic ovary syndrome. University of Rochester, NY. MD thesis 2008.
[22] Chensihan H, Bingquan L, Youwen Y, Kangli L, Bingyan X, Peizhen Z, et al. Associations of menstrual cycle regularity and length with cardiovascular diseases: A prospective study from UK Biobank. J Am Heart Assoc. 2023;12(11):e029020.
[23] Rudnicka E, Kunicki M, Suchta K, Machura P, Grymowicz M, Smolarczyk R. Inflammatory markers in women with polycystic ovary syndrome. Biomed Res Int. 2020;2020:4092470. Available from: https://doi.org/10.1186/s43043-023-00158-2.
[24] Knopp RH, Paramsothy P, Retzlaff BM, Fish B, Walden C, Dowdy A, et al. Gender differences in lipoprotein metabolism and dietary response: Basis in hormonal differences and implications for cardiovascular disease. Curr Atheroscler Rep. 2005;7(6):472-79.
[25] Zannis VI, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. J Mol Med (Berl). 2006;84(4):276-94.
[26] Byambaa E, Lars B. Non-genetic influences on lipoprotein(a) concentrations. Atherosclerosis. 2022;349:53-62. Doi: 10.1016/j.atherosclerosis.2022.04.006.
[27] Dama A, Baggio C, Boscaro C, Albiero M, Cignarella A. Estrogen receptor functions and pathways at the vascular immune interface. Int J Mol Sci. 2021;22(8):4254.
[28] Nenad M, Marie R, Ayelet D. Endothelial cell adhesion molecules-(un)Attainable Targets for Nanomedicines Frontiers in Medical Technology. 2022;4:846065. Available from: https://doi.org/10.3389/fmedt.2022.846065.
[29] Razmara A, Sunday L, Stirone C, Wang XB, Krause DN, Duckles SP, et al. Mitochondrial effects of estrogen are mediated by estrogen receptor alpha in brain endothelial cells. J Pharmacol Exp Ther. 2008;325(3):782-90.
[30] Lu A, Frink M, Choudhry MA, Schwacha MG, Hubbard WJ, Rue LW, et al. Mitochondria play an important role in 17beta-estradiol attenuation of H(2) O(2)-induced rat endothelial cell apoptosis. Am J Physiol Endocrinol Metab. 2007;292(2):E585-93.

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