DOI: 10.7860/JCDR/2017/26031.9799

Original Article

Physiology Section

Effect of Menstrual Cycle Phases on Plasma Lipid and Lipoprotein Levels in Regularly Menstruating Women

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ABSTRACT

Introduction: Physiological cyclic variability in levels of sex hormones during a menstrual cycle may possibly have an impact on lipids, lipoprotein levels and therefore on cardiovascular health status of females.

Aim: To ascertain the effect of menstrual cycle phases on the levels of cardiovascular risk predictors i.e., plasma lipids and lipoproteins in healthy menstruating women.

Materials and Methods: In the present study, 111 healthy regularly menstruating females aged 15-45 years were examined at two points of a menstrual cycle i.e., in the follicular phase (10th day) and in the luteal phase (22nd day) to find out the plasma levels of lipids and lipoproteins. The data were statistically analysed using paired t-test.

Results: A significant decrease in the mean levels of Total Cholesterol (TC) (p=0.006), Low Density Lipoprotein Cholesterol (LDL-C) (p=0.004), TC/High Density Lipoprotein Cholesterol (HDL-C) (p=0.006), Low Density Lipoprotein Cholesterol (LDL)/ HDL (p=0.01), and Triglyceride (TG)/HDL (p=0.02) was observed in the luteal phase compared to the follicular phase while TG (p=0.18), HDL-C (p=0.16). VLDL-C (p=0.17) did not show a significant difference although lower levels were observed in the luteal phase of TG and VLDL-C and higher levels for HDL-C.

Conclusion: The present findings reveal high levels of oestrogen in the luteal phase compared to follicular phase of menstrual cycle. Clinicians should take menstrual cycle phase in account when inferring a women biomarker measurement in the analysis of cardiovascular diseases, particularly females possessing marginal levels. Moreover research studies should take the menstrual cycle phase in consideration which may be a confounding factor in framing and concluding studies on reproductive age group women.

Keywords: Biomarkers, Cardiovascular diseases, Gonadal steroid hormones, Women's health

INTRODUCTION

In the research studies young healthy women are assumed as a reference group for women affected with cardiovascular diseases and also functions for determining of normal limits for clinical concern. Since young healthy women are subjected to hormonal variations in various phases of the menstrual cycle, the relationship between the menstrual cycle phase and possible impact on the levels of plasma lipid and lipoproteins and hence Coronary Heart Disease (CHD) risk is of considerable interest [1].

Oestrogen, the principal sex hormone among the female species, besides being important for the reproductive system, is assumed to assist in maintaining the healthy activity of the heart by improving the levels of HDL-C (good cholesterol) and decreasing the levels of LDL-C (bad cholesterol) [2].

Various researches have reliably demonstrated the impact of exogenous sex hormones on lipid and lipoprotein levels [3-5], however the researches related to phases of menstrual cycle and its effect on plasma lipid and lipoprotein levels have been scarce and inconsistent [6,7]. Hence this study was proposed to identify the effect of menstrual cycle phases on the levels of cardiovascular risk predictors i.e., plasma lipids and lipoproteins in healthy regularly menstruating women.

MATERIALS AND METHODS

The prospective study was conducted on 111 healthy and regularly menstruating female subjects selected from the Gian Sagar Medical College and Hospitals, Rajpura from May 2014 to April 2015. Permission from the IEC and an informed written consent from all the subjects were obtained.

Formula for calculating sample size: we used the formula of prevalence for calculating sample size i.e.,

$n=4pq/d^2$

where;

n= required sample size

p=prevalence in the study

q=1-p

d=allowable error.

Assuming a 0.05 level of significance with 20% difference in group and power of study being 80%. With 20% dropouts the sample size was 95 and we took 111 participants.

Females aged 15-45 years having menstrual cycle length of 24-38 days with flow of four to eight days and variation of two to 20 days from cycle to cycle were considered and included in the study as regularly menstruating women as per the International Consensus Conference proposed terms for the most important features of menstrual bleeding [8].

Women using oral contraceptives during the last three months, currently using of supplements or prescription medications, pregnancy or breastfeeding in the past six months, diagnosis of polycystic ovary syndrome, recent history of infections or diagnosis of chronic medical conditions, women having Body Mass Index (BMI) at screening of <18.5 or >24.9 kg/m^2, autoimmune disease or thyroid disorder, and a history of coronary vascular disease were excluded.

Measures

After recording the detailed menstrual history like age at menarche, length and flow of the cycle and variation in number of days from cycle to cycle, anthropometric measurements like weight, height, BMI were recorded. The subjects were then subjected to sample collection. A 5 ml of fasting blood sample were drawn from the antecubital vein of each subject after 9-12 hours 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.

Plasma levels of Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein-Cholesterol (HDL-C) were measured by Cholesterol Oxidase-Peroxidase (CHOD-POD) methodology [9], Glycerokinase Peroxidase method [10], and direct method [11] respectively using enzymatic kits. Plasma levels of Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) were calculated by Friedewald's Formula [12]. TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios were also calculated.

STATISTICAL ANALYSIS

The data collected in the study was entered in Microsoft excel worksheet and was subjected to statistical analysis using the Student's paired t-test by Microsoft excel software data analysis tool. The significance level was considered at $p<0.05$.

RESULTS

In the present study among the lipids and lipoproteins levels a significant decrease in the mean plasma levels of TC (p=0.006) and LDL-C (p=0.004) and a non significant decrease in the mean plasma levels of TG and VLDL-C was observed in luteal phase compared to follicular phase of menstrual cycle of regularly menstruating women. While HDL-C showed an increase in the mean plasma levels in luteal phase compared to follicular phase of the menstrual cycle though it was not statistically significant [Table/Fig-1].

Further, a significant decrease was detected in the levels of lipid ratio TC/HDL-C (p=0.006), LDL/HDL-C (p=0.01) and TG/HDL-C (p=0.02) when measured in the luteal phase compared to the follicular phase of the menstrual cycle [Table/Fig-2].

Parameters	Follicular Phase (Mean±SD)	Luteal Phase (Mean±SD)	p-value
TC (mg/dl)	164.34±29.38	160.43±28.18	0.006*
TG (mg/dl)	98.98±40.62	95.86±40.56	0.18(NS)
HDL-C (mg/dl)	51.04±11.45	53.04±13.09	0.16(NS)
LDL- C (mg/dl)	93.54±28	88.23±24.21	0.004*
VLDL-C (mg/dl)	19.79±8.12	19.16±8.11	0.17(NS)

[Table/Fig-1]: Plasma levels (Mean±SD) of lipids and lipoproteins during the follicular and luteal phase of the menstrual cycle in regularly menstruating women.
TC: Total Cholesterol, TG: Triglycerides, HDL-C: High Density Lipoprotein Cholesterol, LDC-C: Low Density Lipoprotein Cholesterol, VLDL-C: Very Low Density Lipoprotein Cholesterol, SD: Standard Deviation, NS: Non Significant.
* Significant (p<0.05).

Parameters	Follicular Phase (Mean±SD)	Luteal Phase (Mean±SD)	p-value
TC/HDL-C Ratio	3.37±0.93	3.13±0.72	0.006*
LDL/HDL-C Ratio	1.96±0.79	1.76±0.62	0.01*
TG/HDL-C Ratio	2.09±1.14	1.89±0.95	0.02*

[Table/Fig-2]: Plasma levels (Mean±SD) of lipid ratios during the follicular and luteal phase of the menstrual cycle in regularly menstruating women.
TC: Total Cholesterol, TG: Triglycerides, HDL-C: High Density Lipoprotein Cholesterol, LDC-C: Low Density Lipoprotein Cholesterol, VLDL-C: Very Low Density Lipoprotein Cholesterol, SD: Standard Deviation, NS: Non Significant.
* Significant (p<0.05).

DISCUSSION

The novelty of this study is that it included regularly menstruating women based on all the three criteria of the normal menstrual cycle i.e., the length and the flow days of menstrual cycle and variations from cycle to cycle. To the best of our knowledge no such study has been done yet. All the previous studies were performed taking in account only the length of menstrual cycle.

Further only one study so far has evaluated the TG/HDL ratio in various phases of menstrual cycle in regularly menstruating women [6].

Present study findings reveals a significant raised levels of TC (p=0.006), LDL-C (p=0.004), TC/HDL (p=0.006) and LDL/HDL (p=0.01) in the follicular phase compared to the luteal phase of menstrual cycle. Mumford SL et al., and Barnett JB et al., also observed peak levels of TC and LDL-C during the follicular phase compared to the luteal phase of the menstrual cycle [13,14]. Likewise Muesing RA et al., and Mattsson LA et al., noticed in regularly cycling females that LDL-C, TC/HDL-C and LDL-C/HDL-C ratios were significantly lower when estimated in the luteal phase in contrast to the follicular phase sample [15,16].

There was a decrease in the levels of TG, VLDL-C and an increase in the levels of HDL-C from follicular to luteal phase of menstrual cycle though it was non significant. These results were consistent with the study results of Kim HJ and Kalkhoff RK et al., Tonolo G et al., and Larsen LF et al., [17-19].

In contrast a recent study by Gupta K et al., found a significant reduction in all the lipid parameters in the luteal phase of menstrual cycle in comparison to the follicular phase of menstrual cycle which may be due to the biochemical analysis performed in mid follicular and mid luteal phase where the difference in oestrogen levels between both the phases are relatively high [6].

On the other hand in our study there was a significant decrease in some of the lipid parameters as in the present study biochemical analysis was done in the late follicular and mid luteal phase where the differences in the oestrogen levels between late follicular phase and mid luteal phase are relatively less leading to a less significant results.

Other studies by Elhadd TA et al., Haines CJ et al., Alves RJ et al., showed no differences in lipid levels in the various phases of the menstrual cycle [20-22]. Differences from above mentioned studies may possibly be due to the differences in the framing of the study as these previous studies were carried on a very small sample of population.

In the present study, the difference in the findings in both the phases of lipid and lipoprotein levels may be due to high levels of oestrogen in the luteal phase compared to follicular phase of menstrual cycle though both the oestrogen and progesterone levels are at their peak levels in luteal phase. But the impact of oestrogen was negated to a large extent by equally high levels of progesterone and other endogenous hormones, like androstenedione, having opposite effect to that of estrogen [23,24].

Oestrogen exerts a favourable effect on lipoprotein metabolism by various mechanisms:

- Increasing VLDL-C synthesis leading to subsequent decrease in LDL-C and increase in HDL-C [25].
- Upregulate the LDL receptors.
- Upregulate ATP Binding Cassette Transporter-A1 (ABCA1) and Apolipoprotein-A1 (APOA1, a most important HDL protein, which enhance HDL production).
- Suppress hepatic Scavenger Receptor Class B Type 1 (SR-BI) activity leading to reduced hepatic cholesterol uptake from HDL-C [26].

It has been observed that the hormone progesterone counters the stimulating effect of oestrogen or has inert effect on lipoprotein metabolism [27].

TG/HDL-C ratio

In the current study TG/HDL-C ratio declined significantly (p=0.02) in the luteal phase compared to the follicular phase of menstrual cycle.

The TG/HDL ratio originally proposed by Gaziano JM et al., as an atherogenic index is a proven highly significant independent predictor for CHD, even stronger than TC/HDL and LDL/HDL [28].

TG/HDL ratio determines the type of LDL particle elevated i.e. if TG/HDL ratio is less than 2, than there are predominantly large, fluffy LDL particles also called as good "bad" cholesterol that are not harmful. But if the ratio is greater than 4, then there are lot of small, dense LDL particles also called as bad "bad" cholesterol that are more prone to oxidative damage, readily taken by arterial tissue causing oxidative damage accelerating atherosclerosis hence CHD. As the estimation of sdLDL concentrations is technically demanding, expensive, labour intensive and slow to produce results, hence TG/HDL might be a surrogate marker for sdLDL with better clinical and economical significance [29].

LIMITATION

The present study did not do time blood sampling to ovulation, collected only a single follicular and luteal phase sample and did not verify cycle phase with measured oestrogen and progesterone concentrations. Further LDL-C was indirectly measured by Friedwald's formula. No specific diet was instructed to the subjects to be followed before taking the sample.

CONCLUSION

The knowledge of cyclic fluctuations in plasma lipid and lipoprotein cholesterol levels during the menstrual cycle is essential for determining the suitable phase of measurement which may be a confounding factor in the framing and concluding studies on reproductive age of women. Notably, changes in these biochemical parameters are evident long before the evidence of clinical symptoms or a cardiovascular event. Thus, screening of such parameters allows the possibility for timely clinical intervention. Although in the present study the changes observed in mean levels by cycle phase were modest, but women possessing marginal levels are prone to cross clinical boundaries of acceptable lipoprotein cholesterol levels when tested at different phases of the menstrual cycle.

ACKNOWLEDGEMENTS

We would like to thank all the volunteers who participated in the study. We acknowledge all the Biochemistry staff members of Gian Sagar Medical College and Hospital for helping in the biochemical measurements.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 16, 2016**
Date of Peer Review: **Feb 14, 2017**
Date of Acceptance: **Mar 13, 2017**
Date of Publishing: **May 01, 2017**