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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, 50 healthy regularly menstruating females aged 15-25 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) ($p<0.001$), Low Density Lipoprotein Cholesterol (LDL-C) ($p<0.001$), Very Low Density Lipoprotein VLDL ($p<0.001$) High Density Lipoprotein Cholesterol (HDL-C) ($p<0.001$), Low Density Lipoprotein Cholesterol (LDL)/ HDL ($p<0.001$), and Triglyceride TG($p<0.001$) was observed in the luteal phase compared to the follicular phase.
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: menstrual cycle phases, plasma lipid, lipoprotein levels

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.

Understanding the role of endogenous estrogen and other cyclic hormones as potential modulators of biomarkers may facilitate a more accurate comparison of biomarker data across sexes. Regarding the mechanisms of variability in cardiovascular markers induced by the menstrual cycle, it has been well documented that steroid hormones, such as estrogen, intimately regulate fundamental cardiovascular functions such as blood pressure, blood flow, vasodilatation/vasoconstriction, and vascular inflammation, playing a critical role in the onset of CVD (08–10). Estrogen has also been shown to influence a variety of the biomarkers for the metabolic risk factors of chronic diseases. In particular, biomarkers of oxidative stress, lipoprotein metabolism, inflammation, and glucose metabolism have been shown to be associated with endogenous estrogen levels, as well as with adverse outcomes. Because literature in premenopausal women has demonstrated that circulating sex hormones are not static and fluctuate during a woman's menstrual cycle (11), it is logical that markers of cardiovascular disease would vary as well in premenopausal women. Measurement of Low-density lipoprotein cholesterol (LDL-C) concentration has been the prime index of cardiovascular disease. However, several lipoprotein ratios or "atherogenic indices" such as TC/HDL-C and LDL-C/HDL-C ratios are considered risk indicators with greater predictive value than isolated parameters used independently, particularly LDL-C [12].

Materials and Methods

The prospective study was conducted on healthy and regularly menstruating female subjects selected from the Moti Lal Nehru Medical College and Hospitals, Allahabad from June

2016 to May 2017. Permission from the IEC and an informed written consent from all the subjects were obtained.

This study includes, healthy north Indian undergraduate medical students population of age group 18-24 years having regular menstrual cycles. Non- compliant subjects, lactating women, having irregular menstrual cycle (heavy Dysmenorrhoea, Oligomenorrhoea, Polymenorrhoea).Those having any significant previous illness.Having any bleeding disorder or hemoglobinopathy. Having history of major surgery, accident or blood transfusion. Taking any regular medication or treatment. Under any kind of hormonal treatment or taking OCP. Suffering from or suffered from any gynecological or hormonal disorder 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 (10th day) and in the luteal phase (22nd day) to find out the plasma levels of lipids and lipoproteins.

Plasma levels of Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein-Cholesterol (HDL-C) were measured by Cholesterol Oxidase-Peroxidase (CHOD-POD) methodology [13], Glycerokinase Peroxidase method [14], and direct method [15] 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 [16]. LDL-C/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 data were summarized as Mean ± SD (standard deviation). Groups were compared by paired t test. A two-tailed (α=2) p value less than 0.05 ($p<0.05$) was considered statistically significant. Analyses were performed on SPSS software (windows version 17.0).

Results

In the present study among the lipids and lipoproteins levels a significant decrease in the mean plasma levels of TC ($p<0.001$) and LDL-C ($p<0.001$) 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 also statistically significant [Table/Fig-1]. Further, a significant decrease was detected in the levels of lipid ratio LDL/HDL-C ($p<0.001$) when measured in the luteal phase compared to the follicular phase of the menstrual cycle.

Table 1: Serum lipid profile levels (Mean ± SD) at two phases of menstrual cycle

Serum lipid profile	Follicular phase (n=50)	Luteal phase (n=50)	t value	p value
TC (mg/dl)	157.84 ± 1.74	153.78 ± 1.71	15.97	<0.001
TG (mg/dl)	57.70 ± 1.99	55.06 ± 2.04	8.03	<0.001
HDL (mg/dl)	50.30 ± 2.49	54.31 ± 1.94	9.14	<0.001
LDL (mg/dl)	94.89 ± 3.18	90.41 ± 3.23	11.83	<0.001
VLDL (mg/dl)	13.00 ± 1.86	11.90 ± 1.72	3.62	<0.001
LDL/HDL	1.89 ± 0.10	1.67 ± 0.10	13.92	<0.001

Numbers in parenthesis indicates the range (min to max)

Discussion

Serum Lipid Profile

Periodical change occurs in anatomical architect and hormonal fluctuation during menstrual cycle in females. It is also very much known to have a fluctuation in their hormone concentration. These hormones influence autonomic & metabolic activities.

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 [16, 17]. Likewise Muesing RA *et al.,* and Mattsson LA *et al.,* noticed in regularly cycling females that LDL-C, LDL-C/HDL-C ratios were significantly lower when estimated in the luteal phase in contrast to the follicular phase sample [18, 19].

The results of present study was also comparable with the previously published articles Shilpi Vashishta *et al.* [20] where 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. 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. Further, a significant decrease was detected in the levels of lipid ratio LDL/HDL-C (p=0.01) when measured in the luteal phase compared to the follicular phase of the menstrual cycle. In contrary to this study, in our results value of HDL shows significant difference in different phases of menstrual cycle.

There was a significant decrease of total serum cholesterol and triglycerides occurred during the luteal phase, as compared

with the follicular phase which was also correlated with our present study done by R.G. DeLeon K.L. [21]

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]. Our study result was similar to this study.

On the other hand in our study there was a significant decrease in all of the lipid parameters as in the present study biochemical analysis was done in the mid follicular and mid luteal phase where the differences in the oestrogen levels between mid follicular phase and mid luteal phase are relatively more leading to a 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 [22-24].

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 significant. These results were contrary with the study results of Kim HJ and Kalkhoff RK *et al.,* Tonolo G *et al.,* and Larsen LF *et al.,* [25-27].

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. Discrepancy between results is likely to have occurred due to differences in study design. A wide range of days have been used to classify follicular and luteal collection dates and many studies have collected samples at just two or three points in the menstrual cycle thus potentially missing the full range of hormonal fluctuations occurring during the cycle and the differences in plasma composition that may occur between these specific phases.

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 [28, 29].

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 [30].

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 (SRBI) activity leading to reduced hepatic cholesterol uptake from HDL-C [31].

It has been observed that the hormone progesterone counters the stimulating effect of oestrogen or has inert effect on lipoprotein metabolism [32].

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.

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