LIPID FLUCTUATIONS IN WOMEN SALIVA DURING MENSTRUAL CYCLE

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Abstract: The present study was designed to identify the lipid and its metabolites in the menstrual cycle of women in order to detect the exact time of ovulation. The human saliva was extracted with 2 ml of chloroform and methanol mixture (2:1) ratio, v/v) and the lipid was isolated using Folch et al. (1957) method. Total cholesterol, phospholipids, triglycerides, HDL-C, LDL-C, and VLDL-C were measured in 50 women during menstrual cycle periods of human saliva using UV-Spectrophotometer. Among these total cholesterol, phospholipids and HDL-C were highly significant during ovulatory phase, which was due to hormone metabolic changes in the period of menstrual cycle. The hormone assay such as Luteinising hormone spiked formation was exposed in total cholesterol and phospholipids were implicated in throughout the cycle shows uppermost during ovulatory phase which is the precursor of the steroid hormone. The result revealed that the total cholesterol and phospholipids were considered as testing the saliva instead of blood is a noninvasive loom and it can be used as the biomarker for ovulation detection.

Key words: Women saliva, Lipids, Luteinising hormone, Menstrual cycle

INTRODUCTION

During the past decade many biophysical and biochemical markers have been proposed to predict a woman's ovulation period. These markers generally are preferred on the basis of specific pathophysiological abnormalities that have been reported in association with ovarian development [1]. Identification of the period of ovulation in woman is critical in the treatment of infertility. Success in in vitro fertilization and embryo transfer has been associated with the exact time of ovulation. Presumptive evidence of ovulation may be obtained by steroid or gonadotropic hormone assays in the blood or urine by observation of peripheral changes in the reproductive tract and other sites associated with ovulation. An understanding of the hormonal events which control the ovulatory process is essential to appreciate the physiologic basis of many tests which have been devised for documentation and timing of ovulation.

Recent years, attention has been paid to the biochemical importance of saliva. Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity of mammals. Most of the saliva is produced by the major salivary glands (parotid, submandibular, and sublingual), but a small contribution is made by the numerous small labial, buccal, and palatal glands which line the mouth [2-3]. The easy noninvasive, stress-free nature of saliva collection makes it one of the most accessible body fluids to obtain. During menstrual cycle the level of hormones, such as, estrogen, and progesterone, varies during different phases. These hormones are the metabolic products of cholesterol. Cholesterol
breakdown leads to higher concentration of fatty acids in the bloodstream which are released in the body fluids (i.e. saliva is a dilute aqueous fluid originating from salivary glands) which serves various functions such as, digestion, lubrication and protection of the oral mucosa [4] during menstrual cycle. The identification of these lipids will certainly help to detect the ovulation. To date, no chemical investigations have been made on saliva at different stages of the human menstrual cycle and its biological functions. The objective of this study was to examine the profiles of lipids across the menstrual cycle so as to ascertain whether women saliva contains information potentially useful in ovulation detection.

**MATERIALS AND METHODS**

**Collection of samples:** Fifty healthy women, ages 19–40 years, who were recruited for a study of lipid profile and steroid hormones determinations by self-collected unstimulated whole saliva specimens each morning for two consecutive menstrual cycles (preovulatory (6-12 days), ovulatory (13-14 days), postovulatory phases (15-26 days) and also from prepubertal (7-9 years) and menopause stages (above 45 years). Subjects were asked to spit the saliva into a 10 ml tube until a 3 ml sample was collected. The specimen was dated and stored in the subjects’ home freezer until the end of the second cycle, when it was picked up by research personnel and stored at 20°C in the laboratory for up to 6 months until analysis [5-6]. The samples were collected in a sterile vial during morning hours and brought to the laboratory for microscopic examination.

**Hormone assay:** On the day of analysis, each specimen was thawed and heated at 57 °C for 2 h and centrifuged at 9000 g for 4 min at 10 °C. Saliva LH levels were measured by radioimmunoassay using the classical double-antibody method. The antiserum to ovine LH employed for the assay was examined for any cross-reactivity it might have with other protein hormones, but was found to be specific for LH. Highly purified ovine LH was iodinated using 125I according to procedure [7-8]. To avoid interassay variations, all the samples obtained during the cycle were analyzed in a single assay. Follicular stimulating hormone concentrations were determined by enzyme immunoassay by commercial kits (Salivary diagnostics, Pantex, Santa Monica, CA, USA). Laboratory personnel were blinded to the day of the cycle. All the determinations were carried out in duplicate.

**Lipid profile:** Lipids were examined using an aliquot of the saliva supernatant with EDTA solution and filtered through millipore filter. The filtrate was dialyzed with distilled water, followed by lyophilization. The resultant dried sample was estimated for total lipid [9]. Total cholesterol was estimated by chod – pod method [10], triglycerides by GPO - PAP method [11] and phospholipids by Rouser method [12]. HDL and LDL cholesterol determination were done by Friedewald kit [13]. All lipid profiles were estimated in Synchron CX – 4 systems, Beckman UV Spectrophotometer (USA).

**RESULTS**

Salivary lipids and their metabolites fluctuate during the menstrual cycle due to variation in LH surge. The levels of total cholesterol, LDL cholesterol and HDL- cholesterol between the preovulatory and postovulatory phases of menstrual cycle were statistically different at P<0.01. Figure 1 represents the level of phospholipids, triglycerides, which are significantly higher during the preovulatory phase, VLDL-C (mean value during ovulatory phase: 24.29 ± 3.57 mmol/L; F value: 16.059; Pd" 0.01). During prepubertal and menopause, the level of triglycerides, LDL-C and VLDL-C apparently declined due to metabolic disparity. Total cholesterol increased during midluteal (ovulatory) phase of the menstrual cycle whose mean range value showed 136.94 ± 13.13 mmol/L; F value: 6.492; P<0.001 while compared to preovulatory and postovulatory phases. Next to this LDL-C mean value during ovulatory phase (16.74 ± 1.59 mmol/L; F value: 14.934; Pd" 0.01) elevated throughout the cycle. During menopause and prepubertal the lipid and lipoprotein variations were consistent prototype during all periods. HDL-C was the lowest at prepubertal and menopause. During the period of preovulatory and postovulatory, phospholipids (mean range: 86.7 ± 10.76 mmol/L; F value: 14.423; Pd'0.01), triglycerides (27.31 ± 3.76 mmol/L; F value: 8.976; Pd'0.01) increased gradually corresponding with peak estradiol levels.

**DISCUSSION**

The present study reveals that the lipid and its metabolites undergo consistent variations during the menstrual cycle, with significant elevations of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), phospholipids and triglycerides corresponding with peak estradiol levels.
Fig. 1: Salivary lipids profile analysis during menstrual cycle (mmol/L)

Absorbance (nm)

T-Cholesterol, Triglycerides, Phospholipids, HDL-C, LDL-C, VLDL-C, LH, FSH

Reproductive periods:
- Prepubertal
- Preovp
- Ovp
- Postovp
- Menopause

Periods:
- (6-9 yrs)
- (6-12 days)
- (13-14 days)
- (15-26 days)
- (Above 45 yrs)
at ovulation. In rat models, estrogen levels inhibit the hepatic lipase and elevate the LDL receptor activity, thereby influencing the levels of circulating lipoproteins. The findings showed that within the subject variation of salivary lipids, the lipoprotein was higher in women, but its timing was more predictable in women than in rats. Furthermore, the lipid variations throughout the menstrual cycle, prepubertal and menopause, showed significant variations from women to women. It was observed that the changes in plasma cholesterol could be mediated through a reduced energy intake (by 17%) at ovulation [14]. Several investigators [15,16] observed high total and LDL cholesterol concentration during the ovulatory phase and preovulatory phase of the menstrual cycle. The mechanisms responsible for variations within a month in saliva lipids were not well understood. Such variations have been partly attributed to estrogen induced alterations in cholesterol metabolism. In contrast to previous findings [16], the increased saliva HDL cholesterol levels in preovulatory phase may be attributed to decreased salivary triglycerides and reduced hepatic lipase activity.

The gonadal hormones not only take part in the excretion of biomolecules, but they also play a significant role in the behavior of women. Higher level of estradiol concentration was reported during the preovulatory and ovulatory phase in the menstrual cycle. This study is unique in that it provides important data of lipid and lipoprotein as well as sex hormones measurement during the preovulatory and postovulatory phases of the menstrual cycle obtained from well characterized subjects under very controlled metabolic conditions.

In their study on the effects of exogenous hormones in premenopausal women, Lyons et al. [15] found significantly higher magnitude of change in lipid and lipoproteins levels. Endogenous levels of estradiol, progesterone and other hormones are periodic throughout the menstrual cycle. In addition to variations in the underlying hormone patterns between and within women, there are considerable variations in the menstrual cycle length [18]. The latter vary from woman to woman and may even fluctuate from month to month for the same woman. It is thus important to control these variations, because these factors make it difficult to study changes in lipid and lipoprotein levels within the menstrual cycle and are likely to partially account for difference in previous findings [19-20]. The levels of total cholesterol, triglycerides, phospholipids and lipoprotein like HDL-C have shown a significant increase between the preovulatory and ovulatory phases of the menstrual cycle. The fact that the timing of ovulation is difficult to ascertain [21]. Fluctuations in levels of lipids and its derivatives between menstrual periods, although short term and small, need to be considered in the screening and medical monitoring of premenopausal women, especially those with borderline levels. Although small, such fluctuations may prove to be clinically significant in the long run.

The current study exhibited that in lipid profile determination which was anticipated with estrogen and progesterone have important effects on fat cell metabolism [22], little is known about their role in modulating lipolysis in humans. Estrogen and progesterone clearly have major effects on the distribution of fat deposition in humans. This influence could be via either change in storage (lipoprotein lipase) [23] or release lipolysis [24]. Insulin is a major regulator of lipolysis in resting humans [25] and the failure to observe differences in insulin-stimulated glucose disposal in different phases of menstrual cycle [26]. Finally, the data obtained in the present study enhances the role of the sex steroid hormone changes during the menstrual cycle in substantially affecting overnight post absorptive free fatty acid flux or the response to hypoinsulinemia [26]. The cyclic changes in estrogen and progesterone production which occur in the normal menstrual cycle appear to be minor, if any, it affects a human adipose tissue lipolysis. It should be noted that no effect of estrogen and progesterone on lipolysis has been detected in some studies [23]. It should also be noted that no effect of estrogen and progesterone on lipolysis has been detected in some studies [22] and that most studies find a positive effect on the rodent model [22].

The present investigations indicate that there is a gradual increase in phospholipid mass occurs in human endometrium during the menstrual cycle. This was consistent with the net enhancement of lipid synthesis which reportedly follows oestrogen stimulation [24]. Our data indicates that such stimulation does not involve all phospholipids to the same extent, and suggests the concerted activity of various enzymes of lipid metabolism throughout the cycle. The reported changes in lipid mass during the menstrual cycle may be related to the differentiation of endometrial tissue. It is noteworthy to what extent
endogenous gonadal hormones determine the systemic fluctuations of lipids and lipoproteins on the menstrual cycle [27]. However, there seems to be an estrogenic influence of lipids and lipoprotein patterns in the postovulatory phase. The systemic fluctuations of lipids found in the menstrual cycle are in some variations of the same magnitude as those induced by certain pharmacological preparations. In certain cases LH may also help screen for polycystic ovarian disease and other hormone related conditions. To conclude, the total cholesterol, phospholipids and HDL-C were used as a biomarker. These results showed that whole salivary samples collected at home by the subject on a daily basis provide a noninvasive, feasible method for determining ovulation.

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