Original

Evaluation of Salivary Ferning for Predicting Ovulation using Smartphone Mounted Microscope

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Introduction

Providing low cost alternative solutions for medical diagnosis is one of the key constituents of making universal health care practical, affordable and accessible to one and all. In line with this principle, there has been a plethora of innovations with the advent of mobile technology and data networks, making the concept of tele-pathology a reality. The connectivity and portability are very ideal for providing point of care diagnosis or Tele diagnosis or even assist in self detection in remote and resource limited areas. The advanced camera features present in the Smartphone can be used in capturing good resolution images, storing and sharing for further validation

Mobile phone-based microscopy has been designed for diagnosis of malaria in peripheral blood smear. A paper microscope called foldscope uses smart phone to capture the image of blood smear and magnifies the image[1]. Recently, University of Division of Medical Laboratory Technology, Ghana demonstrated that mobile phone-mounted Foldscope and reversed-lens Cell-Scope are more sensitive and specific than conventional light microscopy in diagnosing Schistosoma haematobium-parasitic infection[2].

A cost effective optical cell-phone based transmission polarised light microscopy was found effective for imaging the malaria pigment known as hemozoin[3]. These cost effective technological applications have not yet penetrated to all levels of health care provision.

Our study aims to evaluate efficiency of mobile mounted microscope in visualising salivary ferning (crystallisation or arborisation) pattern that is strongly associated with pre-ovulatory increased estrogen levels, in predicting the fertile days for conception. Since the Leutinising hormone (LH) surge is a consequence of increased estrogen levels, the LH surge is evaluated using commercial LH urine strip in fertile female subjects as a marker for ovulation. Self-detection of ovulation using commercial strip that detect luteinizing hormone in urine sample are commonly used by women to aid in conceiving or avoiding pregnancy. Other natural method that aid in detecting ovulation time includes basal body temperature and cervical mucus characteristics, but they may be misdiagnosed[4]. A simple, easy to use device that uses a hand-held microscope to visualise ferning pattern in saliva has been recently introduced commercially[5]. In this study it is proposed that, evaluating salivary ferning using mobile mounted microscope is far economical than using LH strips for urine analysis for self-evaluation and the images captured can be stored and shared for further validation.

Ovulation is the process of release of secondary oocyte. This occurs on an average, on the 14th day from the last menstrual period (LMP). During last two days of menstrual cycle, the fall in estrogen, progesterone and increase in gonodotropin releasing hormone secondary to it cause rise in level of follicle stimulating hormone (FSH). FSH recruits ovarian follicles that are destined to ovulate in next menstrual cycle. FSH activates the formation of estrogen. When estrogen reaches >200pg/ml for approximately 50hrs duration, surge of luteinising hormone occurs, causing release of ovarian cell. LH surge is a relatively precise predictor for timing ovulation as the peak of LH surge precedes the ovulation by 12 to 24 hours[6].

Several researchers have studied ferning pattern in body fluids such as cervical mucus and saliva[6]. Crystallization (with NaCl) of cervical mucus and saliva are characterised by the content of the muco-protein [7]. During the pre-ovulatory period when estrogen dominates, the mucous secretions are thin and watery called which facilitates migration of spermatozoa through mucus. In mid-luteal stage when the progesterone hormone dominates the mucus is thick and sticky with reduced water content [8].

Thus, increasing levels of estrogen and adreno-cortico tropic hormone before ovulation stimulates the secretion of aldosterone, which regulates the electrolytes and fluid status in human body[9]. Increased level of estrogen alters vaginal and salivary secretion and forms “fern like pattern” due to crystallisation of sodium chloride on mucus fibre[8].

Materials and Method

Study Samples

20 female subjects aged between 23 to 39 years having regular 28 or 30-day menstrual cycle volunteered and participated in this study. The exclusion criteria were non- usage of any hormonal contraceptives, estrogen antagonists, intrauterine devices, pregnancy and breast feeding.

Written consent was obtained from all the subjects. The study was conducted between June 2016 to June 2017 in the SRM Dental College, Ramapuram, Chennai.

Material used

A mini microscope (Universal mobile microscope) which can be clipped on to android Smartphone to view or capture magnified image through phone camera. The microscope has a 200x magnification power and a LED illuminating system attached to it. Ferning pattern of saliva was visualised with it.

Ovulation test strips (Egens biotechnology) that detects luteinising hormone in urine sample was used.

All the subjects were given prior instructions regarding collecting salivary samples and using the ovulation test strips and the test was done at home.

Detection and evaluation of salivary ferning

Samples were collected everyday of fertile window (10th day LMP to 17th day LMP) and on 23rd and 24th day of LMP (to check for absence of salivary ferning). All subjects were instructed to avoid taking food for two hours prior to taking salivary samples. A thick drop of saliva was directly placed on clean glass slide and allowed to dry. The slide with dried salivary sample was studied by the investigator for presence and quality of ferning pattern and the image was captured using the mini microscope and Smartphone camera. The ferning pattern was given a scoring of 0 or 1 (0 if no ferning pattern was detected, 1 when a ferning pattern was seen irrespective of the nature of crystallisation).

Detection of LH surge in urine

The ovulation strip was used every day of fertile window (10th PMD to 17th PMD) and on 23rd and 24th day of PMD (to check for post ovulatory consequential reduction of estrogen levels). As per manufacturer's instruction the subjects performed ovulation test by immersing the strip below the indexed line on the strip and then placed on a flat surface. Any change in colour of the test line and control line are noted. The result is recorded as positive if test and control line changed to pink colour and as negative when there is no change of colour in test line.

Results

The data regarding salivary ferning test and LH surge test as studied throughout the fertile phase (10th to 17th PMD) as well as post ovulatory days (23rd and 24th days) are given in the Table 1.

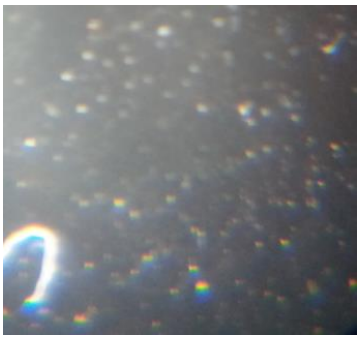
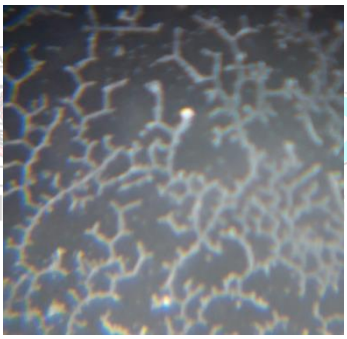
The data was further selected and tabulated (Table 2) to include the details of salivary ferning test in the immediate vicinity of LH surge. In the table 2, the LH surge day is considered as the 0th day. And the immediate preceding three days to LH surge are designated as -1, - 2, -3 days. Following evidence of LH surge, the immediate three days is designated as +1, +2, +3 days.

Sample no	10th LMP		11th LMP		12th LMP		13th LMP		14th LMP		15th LMP		16th LMP		17th LMP		23rd LMP		24th LMP	
	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine	Saliva arborization	LH-urine
1	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0
2	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0
4	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0
6	1	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0
7	0	0	1	0	1	0	1	0	1	0	1	1	1	1	1	0	0	0	0	0
8	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0
9	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0
10	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1	0	1	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0
12	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0
13	0	0	1	0	1	0	1	0	1	1	1	0	0	0	0	0	1	0	1	0
14	1	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0
15	1	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0
16	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0
19	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0

Table 1: Details of salivary ferning and LH surge test results during the fertile phase (10th to 17th PMD) and in the post ovulatory days (23rd and 24th PMD)

samples	-3	-2	-1	LH+Ve D	1+	2+	3+
1	0	0	1	1	1	1	1
2	0	0	1	1	1	1	1
3	0	0	1	1	1	0	0
4	0	0	1	1	1	1	1
5	0	0	0	0	1	1	1
6	1	1	1	1	1	1	1
7	1	1	1	1	1	1	0
8	0	0	0	1	1	1	0
9	1	1	1	1	1	1	0
10	0	1	1	1	0	0	0
11	1	1	1	1	1	0	0
12	1	1	1	1	1	1	1
13	1	1	1	1	1	0	0
14	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1
16	1	1	1	1	0	0	0
17	0	0	1	1	1	1	0
18	0	0	1	1	1	0	0
19	0	0	0	0	1	1	1
20	0	0	0	1	1	1	0

Table 2: Presence or absence of salivary ferning in the immediate pre-ovulatory and post ovulatory phase in relation to LH surge day

SCORE – 0 (no ferning pattern detected)	SCORE – 1 (small ferning pattern detected)	SCORE – 1 Wellformed arborization
		

Statistical Analysis

	Estrogen peak				
Salivary Arborization	**Present**	**n**	**Absent**	**n**	**Total**
Positive	True Positive	a=66	False Pasitive	c=4	a + c = 70
Negative	False Negative	b=14	True Negative	d=36	b + d = 50
Total		a + b = **80**		c + d = **40**	

Table 3: Cross tabulation of details of salivary ferning test correlating to estrogen peak results on the day preceding LH surge (-1 day), the day of LH surge positivity (0th day), and the two days following LH surge (+1 and +2 days)

A total of 80 observations were analysed in the selected 4 days (n= 20, and observations repeated for four consecutive days). Fisher's Exact Test as done to check for level of significance between salivary ferning and its causal factor, estrogen peak that is known to occur one day prior to LH surge. The two-sided p value was found to be <0.001 and was statistically highly significant. Following that accuracy tests including sensitivity, specificity, positive predictive value and negative predictive value were calculated and given in the Table 4.

STATISTIC	VALUE	95%CI
Sensitivity	82.50%	72.38% to 90.09%
Specificity	90.00%	76.34% to 97.21%
Positive Predictive Value	94.29% (*)	86.62% to 97.68%
Negative Predictive Value	72.00 % (*)	61.24% to 80.71%
Accuracy	85.00% (*)	77.33% to 90.86%

Table 4: Statistical evaluation of accuracy tests for salivary ferning in relation to estrogen peak based on LH surge day positive result

Discussion

Ultrasonography is standard reference for ovulation detection since it can be used to observe the maximum growth of dominant follicle (>15mm on ultra sound). It is used extensively as an investigative tool in assisted reproductive techniques[9]. Detection of the luteinizing hormone (LH) surge in serum or in urine is also accurate for determining ovulation and hence for predicting favourable time for conception.

The interval of potential fertility was defined as the fertile window beginning 8 days prior to and ending two days after the identified ovulation day[10].

Since the objective of the study was to narrow down the most fertile day(s) within the 10th to 17th day, it was essential to study the salivary ferning pattern in the immediate vicinity of LH surge day. Also, as the estrogen peak happens just prior to (approximately 24 hours) LH surge day, it is important to study the crystallisation that happens in saliva in the preceding days to the LH surge. It is known that the LH surge lasts for approximately 12 to 24 hours immediately after which ovulation occurs. Hence the analysis was focussed on the -1 day, 0th day of LH surge and +1 and +2 days after LH surge day. The +1 days after LH surge is considered as the day of ovulation. The additional day (+2 day) after LH surge was also considered due to the impracticality of determining the exact time when oocyte will be released, in the absence of ultrasound test.

The results of this study showed that among the 80 observations of test for salivary ferning and presumed estrogen peak days (derived from LH surge positive test result), the true positive observations are 66 meaning salivary ferning exactly predicts and coincides with the estrogen peak. While the 14 false negative observations indicate that despite estrogen peak the salivary ferning is absent and does not appear to coincide and be predictive. Thus, giving a sensitivity of 82.5% (72.38% to 90.09% of 95% CI).

On analysing the post ovulatory phase (23rd and 24th PMD) when estrogen levels are considerably reduced, there is no evidence of salivary ferning in 36 out of 40 observations (true negative). Thus, giving a specificity of 90 %. (76.34% to 97.21% of 95% CI.) These results are similar to several previous studies.

In 1992, a study involving 300 women found a definite correlation between oestrogen activity and crystallization of saliva, claiming that accuracy of detecting ovulation is 98%, hence will be helpful for women as an additional aid in detecting the fertile period[11].

Two studies conducted to evaluate commercially available ovulatory monitoring system (Knowhen and Geratherm) in saliva, found salivary ferning to have good predictive value. In the study to evaluate Knowhen monitoring system, the salivary ferning pattern had 96.5% sensitivity[5]. Salmassi et al evaluated the Geratherm ovu control kit which showed specificity of 78% and sensitivity of 89.4%[12].

Contentious results were found by studies on salivary ferning predictability. Berardona in 1993 showed that salivary ferning was not reliable as their study showed that salivary ferning can be formed during any time in menstrual cycle and more so can occur in pre-pubertal, post-menopausal, pregnant woman and even in male subjects[13]. Maruzio Guida et al evaluated the efficacy of different ovulation detection methods used in natural family planning in comparison with pelvic ultrasonography and concluded that measuring urinary LH levels is an excellent method for determining ovulation, while the salivary ferning test is not an accurate method for detecting ovulation. The results revealed the urinary LH correlated 100% with ultrasound evidence of ovulation, while the sonographic detection of ovulation with salivary ferning was only 36.8%. Interestingly 57.8% of salivary specimens were reported as being "uninterpretable" by the subjects. The authors suggested that the large percentage of interpretable results could have been due to the fact that many of the patients were not taught to use the microscopes or interpret the slides properly. This point is important because adequate patient instruction is the key to the successful use of this method of ovulatory surveillance[14]. In current study analysis of slide was done by the investigator allowing accurate interpretation of result.

Gunther stated that salivary ferning can be affected by numerous factors like fluid intake, dehydration, drugs and systemic disease that modify salivary secretions and solute concentration[15].

The results of the current study show cyclical changes in saliva as well as in urinary LH concentration. Previous studies have shown that the time sequences of hormonal changes in estrogen and LH are responsible for salivary ferning and LH urine test positivity respectively. The mean interval from the estrogen peak to ovulation was 34 hours and the interval from the estrogen peak to the LH peak was 24 hours[6].

The results of current study show saliva can be used to narrow down the fertile day(s) in the menstrual cycle. Women can use the salivary ferning test as a indicator for ovulation when planning a pregnancy to help maximize their chances of conceiving. The difference caused by the time sequences of hormonal changes

could be an advantage of the saliva test that it allows to identify the fertile period that much earlier.

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