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Biochemical Estimation of Salivary Antioxidants and Electrolytes-A Novel, Non-Invasive Tool to Detect Ovulatory Phases

A Non-Invasive Biomarker Strategy for Ovulation Detection

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Abstract:

- ***Background:***

The menstrual cycle is governed by estrogen and progesterone, which show variation during the menstrual cycle, affecting the physiology of the whole body, including the oral cavity. However, few studies are available to show the normal changes in salivary composition during the menstrual cycle. So this study assessed the changes in the salivary composition of calcium, magnesium, sodium, potassium and inorganic phosphate during the different phases of the menstrual cycle. Additionally, salivary H_2O_2 scavenging activity, total antioxidant properties and changes in pH during preovulatory, ovulatory and post-ovulatory phases were estimated biochemically.

- ***Methodology:***

Unstimulated 5ml of saliva was collected from 50 female patients during pre-ovulatory, ovulatory and post-ovulatory phases using the spitting method. The salivary pH was estimated using a digital pH meter. Salivary electrolyte testing was done using atomic absorption spectrophotometer. Total antioxidant property and hydrogen peroxide scavenging property were determined using a UV spectrophotometer. For continuous variables, ANOVA with Repeated Measures was used and if significant Bonferroni post hoc test was done to determine which menstrual phase significantly differed from the other. For categorical variables, the Friedman test was used. After the existence of variance among the phases was confirmed in the Friedman test, the Wilcoxon signed-rank test was applied to understand how significantly each phase differed from the other. The level of significance was set at 0.05.

- ***Results:***

It was found that during the ovulatory phase of the menstrual cycle, all the salivary tested components increased significantly when compared to pre and post-ovulatory phases.

- ***Conclusion:***

Saliva can be used as a supplementary tool to detect the ovulatory phase.

Keywords: *Menstrual Cycle; Saliva; Estrogen; Progesterone; Antioxidants.*

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I. INTRODUCTION

In recent years, oral fluid, especially saliva, has attracted widespread interest as a diagnostic medium because of its ease of access, noninvasive sample collection, increased patient acceptance, and reduced risks of infectious disease transmission [1]. Saliva testing is used to screen for numerous conditions and disease states, including hereditary disease, autoimmune disease, malignancy, infection, monitoring of levels of hormones and drugs, bone turnover markers, forensic evidence, oral diseases and diagnosis of oral disease with relevance for systemic diseases [2][3].

Hormonal changes govern the menstrual cycle. The pituitary gland produces Luteinizing hormone and Follicle-stimulating hormone which promote ovulation and stimulate the ovaries to produce estrogen and progesterone [4]. This hormone shows variation during the menstrual cycle affecting the physiology of the whole body, including the oral cavity. Studies have shown that whole saliva can be used as an excellent specimen for monitoring estradiol and progesterone levels across the menstrual cycle [5][6]. Variations in the levels of these hormones will lead to variation in the composition and flow rate of saliva, which might affect the hard and soft tissue of the oral cavity.

However, not many studies are available to show the normal changes in salivary composition during the menstrual cycle. So, this study was conducted with the aim to assess the changes in the composition of calcium, magnesium, sodium, potassium and inorganic phosphate, H_2O_2 scavenging activity and total antioxidant property and changes in pH of saliva during the preovulatory, ovulatory and post-ovulatory phases.

II. METHODOLOGY

Fifty females in the age group of 16 to age group before childbirth were selected randomly based on the following inclusion criteria: they should have 1.a normal menstrual cycle of 28 ± 2 days 2. DMFT (Decayed Missed Filled Teeth) score of 0, 3. No history of any systemic or 3endocrine disorders and 4. an interest in participating in the study.

Unstimulated 5ml of saliva was collected from the 50 female subjects during pre-ovulatory ovulatory and post-ovulatory phases using the spitting method. The participants were instructed to abstain from eating/drinking 10 hours prior to testing, and the participants were asked to rinse their mouths with plain water without brushing their teeth before saliva collection. The saliva was collected between 6.00 to 8.00 am in each phase to minimize the effects of diurnal variability in salivary composition [7]. Ferning test was done to categorize the menstrual phases [8][9].

The salivary pH was estimated using digital pH meter [10][11]. The collected saliva sample was then centrifuged at 4000 rpm for 10 mins. The clear supernatant fluid collected was used for electrolyte testing using atomic absorption spectrophotometer [12]. Total antioxidant property and hydrogen peroxide scavenging property were determined using a UV spectrophotometer [13][14].

The collected data was entered in Windows Excel 2007, and the statistics were analysed using SPSS version 16. The level of significance was set as 0.05.

For Continuous variables, ANOVA with Repeated Measures was used to compare the mean of calcium, magnesium, sodium, potassium and inorganic phosphate (composition of saliva) during pre-ovulatory, ovulatory and post-ovulatory phases. Wherever statistical significance was seen among the mean values between the ovulatory phases in ANOVA, the pairwise comparison was performed between the phases using the Bonferroni post hoc test. This test helps to justify which phase has significantly increased/decreased from the other.

For Categorical variables, the Friedman test was used to investigate the presence of variance in the pH during pre-ovulatory, ovulatory and post-ovulatory phases. After the existence of variance among the phases was confirmed in the Friedman test, the Wilcoxon signed-rank test was applied to understand how significantly each phase differed from the other.

III. RESULTS

From table 1, it is evident that ANOVA with repeated measures confirms the significant difference in the composition of saliva during the menstrual cycle. Bonferroni posthoc test was done to identify which phase has a significant change in the composition of saliva during pre-ovulatory, ovulatory and post-ovulatory phases. Pairwise comparison in table 2 shows that each phase significantly differed from the other for all the tested components of saliva. Though the significant shift among the phases is confirmed in the pairwise comparison table, it is important to determine which phase had increased or decreased. Hence, estimated marginal means charts of each composition of saliva (Fig 1(a),1(b),1(c),1(d),1(e),1(f)) were examined. The charts clearly, show that phase 2 (Ovulatory phase) is higher than the other phase in each composition of saliva.

Table 3 shows that in the Friedman test; desired level of significance (0.05) is higher than the P-value (0.002). Hence, there is a statistically significant change in salivary pH during the pre-ovulatory, ovulatory and post-ovulatory phases. This is evidenced by the Fried man test statistic $\chi 2$ (2) = 89.479, p = 0.000.

Since the presence of variance among the phases was confirmed by the Friedman test, we assessed how each phase differed from the other. From the Wilcoxon signed rank test, it was seen that salivary pH had significantly changed in the Ovulatory phase of the menstrual cycle.

IV. DISCUSSION

Salivary diagnostics is a dynamic and emerging field to aid in diagnosing oral and systemic diseases using the salivary biomarker. In recent years, saliva has been used to detect the period of ovulation for treating infertility. A recent report shows that saliva was a very good source of ovarian

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hormones and their levels change in accordance with the menstrual cycle [6].

Various studies have shown significant changes in serum electrolytes with variations in estrogen during the menstrual cycle. Dadlani AG et al (1982) [15], Pandya AK et al (1995) [16], Lanje MA et al (2010) [17], carried out studies and suggested serum electrolytes vary with changes in uterine hormones, especially estrogen, and the higher concentration of serum electrolytes is found to be during the ovulatory phase of the menstrual cycle. Gandara BK, Leresche L, Mancl L (2007) [5], Lu Y et al (1999) [6], Choe JK et al (1983) [18] report the presence of ovarian hormones in whole saliva and their variations during the menstrual cycle. Becerik S et al (2010) [19] reported that changes in salivary ovarian hormones during the menstrual cycle impact oral health.

This study was carried out with the aim of finding out whether there is any change in salivary components, salivary salts, salivary pH and salivary antioxidants during the menstrual cycle. The menstrual cycle was divided into three phases: Pre-ovulatory phase (6-12 days); Ovulatory Phase (13-14 days) and post ovulatory phase (15-26 days). This study showed that there are significant changes in salivary calcium, salivary magnesium, salivary potassium, salivary phosphorous, salivary sodium, pH of saliva and antioxidant capacity of saliva with the mean value of each showing a significant increase during the ovulatory phase. This study is in accordance with many other studies which showed higher concentrations of salivary salts during the ovulatory phase. Alagendran S (2007) [12], Puskulian L (1972) [20] documented that salivary electrolytes increase during the ovulatory phase and suggested that the increase in salivary salts may be due to an increase in ovarian hormones, especially estrogen. However, these electrolytes decrease and return to baseline values during the post-ovulatory phases.

Acidic and alkaline salivary pH greatly influence the oral cavity. Salivary constituents maintain a neutral pH of 6.2 to 7.6 for a healthy oral environment. In this study, it is found that salivary pH increases during the ovulatory phase (pH = 8.26) and then decreases during the post-ovulatory phase. This increase in salivary pH might be due to increased salivary salts during the ovulatory phase. A similar finding has been reported by Wadhwani S and Shetty P (2015) [21] and they suggest that sex hormones affect salivary ph.

Antioxidants play a major role in the body's defense mechanism by neutralising free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS), that cause oxidative stress leading to cell breakdown and tissue damage [22]. The total antioxidant capacity of saliva is the level of total antioxidants in saliva. Our study showed an increase in the total antioxidant capacity of saliva during the ovulatory phase. There are no studies to emphasize this finding in the saliva of healthy women. However, Michos C et al (2006) [23] reported elevated antioxidants during the ovulatory phase in the serum of eumenorrheic women. They suggested that estrogen might function as an antioxidant both directly and indirectly. Estrogen shares a chemical structure with phenolic compounds, allowing it to provide hydrogen from its phenol-hydroxyl ring and neutralize reactive oxygen species. As a result, it acts as an antioxidant directly. According to scientific research, estrogen has indirect antioxidant properties. Estrogen works synergistically with glutathione to provide antioxidant benefits, and it also positively modulates the activity of natural cellular antioxidant enzymes. These properties are general to estrogens and are independent of their hormonal activity.

This study has certain limitations. There were no control groups (e.g., pre-pubertal or post -menopausal women) to strengthen our findings. Although the saliva was collected at a specific time to avoid diurnal variability, it still displays individual variability and is affected by other factors such as diet. Salivary proteins which form a bulk of saliva were not analyzed resulting in a major drawback of the study. Our data offers a non-invasive approach to estimating the ovulation period by analyzing several salivary parameters. These salivary parameters can be used to develop salivary biomarkers to detect ovulation.

V. CONCLUSIONS

To summarize, it has been determined that there is a notable increase in certain components of saliva during the ovulatory phase of the menstrual cycle as compared to both the pre- and post-ovulatory phases. Therefore, it is suggested that this be utilized as an additional aid for detecting the ovulatory phase in women.

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Table 1 Salivary Composition During Menstrual Cycle Tested Using ANOVA with Repeated Measures

Salivary components	Type III sum of squares	df	Mean square	F	Sig	Partial Eta Squared
Calcium	20903.16	1.733	12061.24	1593	0.000	0.97
Magnesium	11606.89	1.655	7011.521	4294	0.000	0.989
Potassium	56981.85	1.446	39416.12	4348	0.000	0.989
Phosphorous	49683.16	1.492	27263.22	4240	0.000	0.989
Antioxidant	141962.7	1.838	77244.82	677.242	0.000	0.933
Sodium	84010.29	1.805	46549.94	1257	0.000	0.962

Table 2 Pairwise Comparison

Salivary measures	Phases (A)	Phase (B)	Mean difference (A-B)	Standard error	Significance	95% confidence interval for difference	
						Lower bound	Upper bound
Calcium	Pre ovulatory	Ovulatory	-21.66	0.565	0.000	-23.062	-20.258
		Post ovulatory	5.76	0.4	0.000	4.77	6.75
	Ovulatory	Pre ovulatory	21.66	0.565	0.000	20.258	23.062
		Post ovulatory	27.42	0.555	0.000	26.045	28.795
Magnesium	Pre-ovulatory	Ovulatory	-14.18	0.258	0.000	-14.82	-13.4
		Post ovulatory	6.96	0.257	0.000	6.323	7.597
	Ovulatory	Pre ovulatory	14.18	0.258	0.000	13.54	14.82
		Post ovulatory	21.14	0.171	0.000	20.715	21.565
Potassium	Pre-ovulatory	Ovulatory	-38.44	0.634	0.000	-40.011	-36.869
		Post ovulatory	5.3	0.514	0.000	4.027	6.573
	Ovulatory	Pre ovulatory	38.44	0.634	0.000	36.869	40.011
		Post ovulatory	43.74	0.347	0.000	42.879	44.601
Phosphorus	Pre-ovulatory	Ovulatory	-31.76	0.436	0.000	-32.84	-30.68
		Post ovulatory	5.66	0.31	0.000	4.892	6.428
	Ovulatory	Pre ovulatory	31.76	0.436	0.000	30.68	32.84
		Post ovulatory	37.42	0.538	0.000	36.085	38.755
Antioxidant	Pre-ovulatory	Ovulatory	-74.72	2.267	0.000	-80.34	-69.1
		Post ovulatory	-45.82	2.102	0.000	-51.032	-40.608
	Ovulatory	Pre ovulatory	74.72	2.267	0.000	69.1	80.34
		Post ovulatory	28.9	1.737	0.000	24.593	33.207
Sodium	Pre-ovulatory	Ovulatory	-38.28	1.29	0.000	-41.477	-35.083
		Post ovulatory	18.56	1.195	0.000	15.598	21.522
	Ovulatory	Pre ovulatory	38.28	1.29	0.000	35.083	41.477
		Post ovulatory	56.84	0.958	0.000	54.464	59.216

Table 3 Mean Rank of Salivary pH During Three Phases of Menstrual Cycle

Phases	Preovulatory	Ovulatory	Post ovulatory
Mean pH	6.56	8.26	6.86
Fried man test statistics	N= 50; Chi square = 89.479; df = 2; Asymp. Sig =0.000		
Wilcoxon Signed Rank Test			
	Ovulatory Phase v/s Pre-Ovulatory Phase	**Post Ovulatory v/s Ovulatory Phase**	
Z	-6.278	-6.312	
Asymp. Sig (2 tailed)	0.000	00.000	

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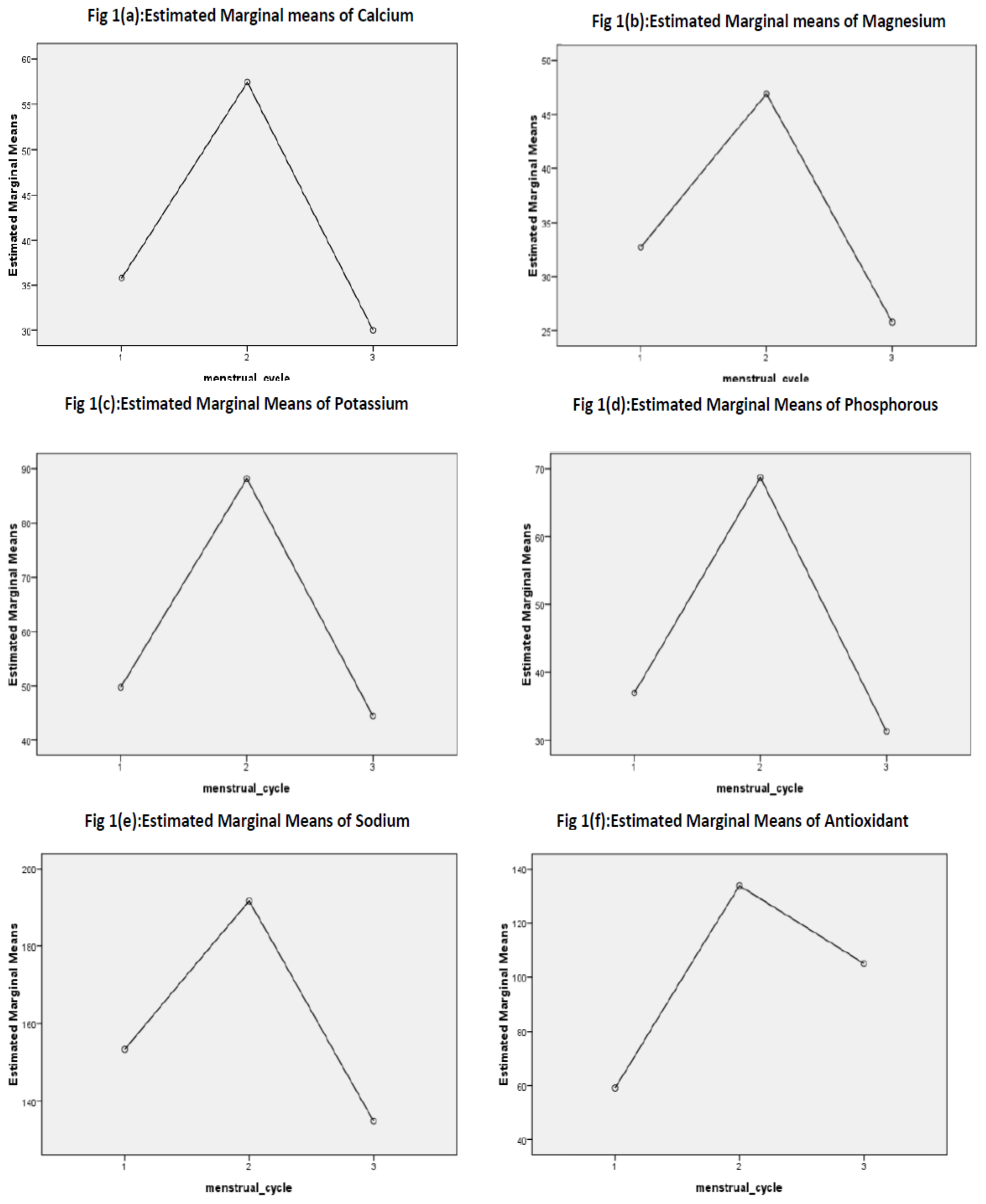

1– Pre-Ovulatory Phase; 2 –Ovulatory Phase ; 3 – Post -Ovulatory Phase

Fig 1 Estimated Marginal Means of 1(A) Calcium, 1(B) Magnesium, 1(C) Potassium, 1(D) Phosphorous, 1(E) Sodium, 1(F) Antioxidant