



ida

Indian Dental Association
Madras Branch

E - MIDAS JOURNAL

“An Official Journal of IDA - Madras Branch”

Chennai/Volume:5/Issue:2/Pages:1-20/June 2018

www.idamadras.com

eISSN 2454 - 8928



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Prof. Dr. V. Shankar Ram, M.D.S., Ph.D.,
President
IDA Madras Branch

PRESIDENT'S MESSAGE

Dear Members,

Greetings from IDA Madras Branch!

IDA the premier organization of dental professionals secures the dignity and honour of its members, besides enhancing the image of the profession. Out of more than 450 local branches IDA Madras branch bear more members than any other branch.

Research is an integral part of science and dental science is not an exception. I hereby invite you all to send us well researched articles for publications along with case reports, reviews and professional experience to enrich our scientific knowledge.

My warm regards and good luck is always with dynamic editorial team pioneered by Dr. C.K. Dilip Kumar our editor. May the team continue their saga of continuous publication for the years to come.

A handwritten signature in blue ink, written in a cursive style, is shown next to a hand holding a pen. The signature is positioned above the printed name of the president.

Prof. Dr. V. Shankar Ram, M.D.S., Ph.D.,



Dr. H. Thamizhchelvan
Hon. Branch Secretary
IDA - Madras Branch
Hon. Secretary National CDH
IDA (Head Office)

SECRETARY'S MESSAGE

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H. Thamizhchelvan

Dr. H. Thamizhchelvan



Dr. C.K. Dilip Kumar
Editor-in-Chief
IDA - Madras Branch

LETTER FROM THE EDITOR

IDA Madras branch has done a tremendous team work with Rotary International 3232, Sri Ramachandra University and Colgate to achieve & mark a record in Asia Book of records and India book of records by bringing in 23615 people together in a single venue and making them brush together to create dental awareness among public. Our editorial team wholeheartedly wishes the entire team and organisations for their effort in achieving the milestone, which added another feather to the cap for IDA Madras branch.

If you love life, don't waste time, for time is what life is made up of." - Bruce Lee

Yes time is precious, to go higher in life one should utilize time to its fullest. So whatever time you get start documenting the cases what you do in your practise, colleges, etc.

The documentation will not go unproductive, it can become precious as time when you record it in history by publishing it in the journals so that your case can educate many others.

So I request all the clinicians, practitioners, students, etc to send manuscripts to our journal to make you and your time precious.

A handwritten signature in blue ink, appearing to read 'C.K. Dilip Kumar', is written on a white surface. A hand holding a pen is visible on the right side of the signature.

Dr. C.K. Dilip Kumar

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Prevalence of Oral Mucosal Lesions in Relation to Tobacco and Alcohol Usage

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Abstract

Key Words:

Introduction

Premalignant lesions of the oral cavity represent an important target for cancer prevention. They can be detected by visual inspection and their importance derives from the high proportion of cases in which biopsy reveals dysplasia or even frank carcinoma. The strongest risk factors for these oral lesions are use of inhaled tobacco, chewing substitutes that usually include tobacco such as pan masala and betel nut quid and alcohol, Smoking and Chewing Tobacco.

The prevalence of oral precancerous lesions varies in different countries and suggests a variance from as much as 25% to as little as 0.2%.¹ In India it is estimated that 195 million people use tobacco, 62.46 million use alcohol. The incidence of oral mucosal lesions depends on the method, duration, frequency and intensity of use.²

Smokeless tobacco, which is chewed alone or with betel quid / paan, has a significant detrimental impact on the oral cavity. A wide variety of mucosal changes have been noted in habitual users of smoked and smokeless tobacco.³ These changes most likely result from the many irritants, toxins, and carcinogens found naturally in cured or burned tobacco leaves, but may also arise from the mucosal drying effects, the high intraoral temperatures, intraoral pH changes, local alteration of membrane barriers and immune responses, or altered resistance to fungal and viral infections.

Alcohol could contribute to oral lesions, either directly or indirectly. Chronic exposure to ethanol may be associated with carcinogenic cytological changes in the oral mucosa, even in the absence of tobacco smoking.⁴ In this study, subjects who visited dental outpatient clinic with the habit of smoking, chewing tobacco and consuming alcohol were examined for the prevalence of oral mucosal lesions

Materials and Method

The study group constituted 998 patients, examined over a period of 1 year attending Sri Venkateswara Dental College and Hospital, Kanchipuram, South India.

Patients who visited the Dental outpatient department with the history of tobacco habits and alcohol consumption were selected for this study. Patients were explained orally about this study and those who were willing to reveal their personal habits and willing to undergo oral examination were taken as subjects. Patients who came for medical ailments met the physician first for their chief complaint and were later taken for oral examination in the dental clinic.

Inclusion Criteria

1. Both male and females patients in the age of 18 years and above.
2. Patients who consent to reveal the tobacco and alcoholic habits and consent to subject themselves for oral examination.
3. Individual who practiced the habit for a minimum period of 6 months and still actively continuing the habit.

Exclusion Criteria

1. Patients who were not willing to reveal the habits and/or subject themselves for oral examination.
2. Patients admitted for systemic diseases.
3. Individual who gave up the habit during the past 6 months.

A preformed case sheet, which included detailed recording of the patient's habits, was used for each individual. The oral habits section included questions about regular use of tobacco smoking, tobacco chewing, areca quid and alcohol consumption. All the patients were examined with the help of a mouth mirror and probe under adequate illumination. The lesions if present were photographed with the

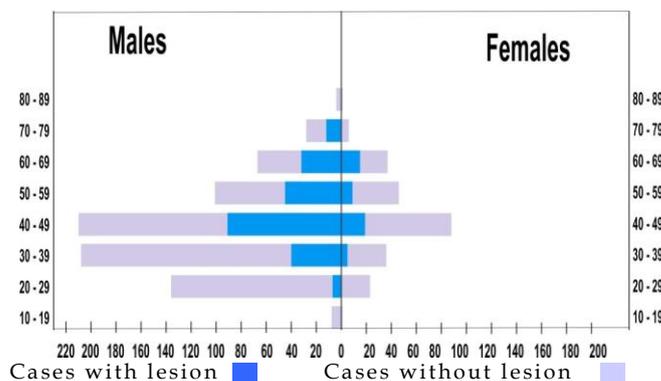
patient's consent. The collected data were compiled and statistical analysis was made.

Results

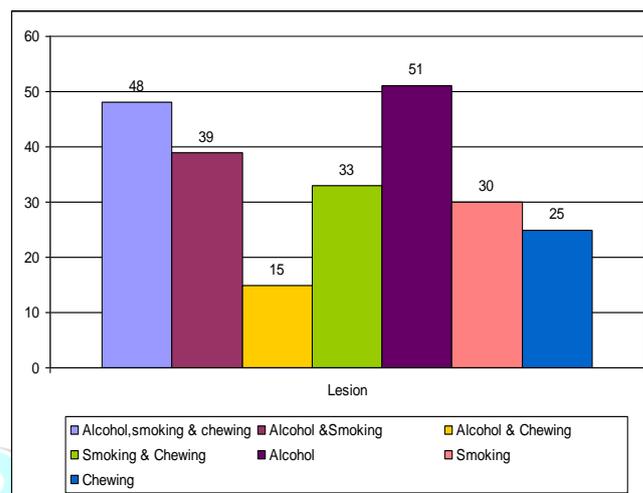
In this study of 998 individuals with habits, 760 (76.2%) were males and 238 (23.8%) were females, with the age range from 18 years to 80 years. The mean age of the study population was 42.7, in which it was 41.6 for males and 45.9 for females. (Graph 1) The different types of habit seen among them was shown in Table 1

Out of the 998 individuals examined 275 of the individuals were found to have 334 lesions. Among them, 227 (82.5%) of the males and 48 (17.5%) of the females had lesions. Based on the age, 2.5% of the individuals had lesions less than 30 years of age, 16.4% of them between 30 - 39, 40% of the between 40 - 49, 19.6% between the age group of 50 -59 and 21.5% of them were above the age of 60.(Graph 2)

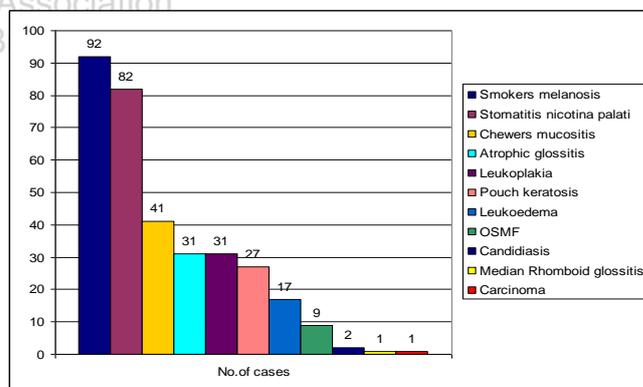
Lesions were present in 30% of the smokers, 25% of tobacco chewers, 51% in alcoholics, 39% of individuals had alcohol and smoking habit, 15% of the individuals consumed alcohol and chewed tobacco, 33% of the individuals smoked and chewed tobacco and 48% of the individuals smoked, chewed tobacco and consumed alcohol. (Graph 3) The mean frequency and duration of various habits among different habit groups are tabulated. The lesions found in 275 individuals and the distribution of different lesions by habits is shown in Graph 4. Assessment of odds ratio and risk estimate for individual habits were done as shown in Table 2.



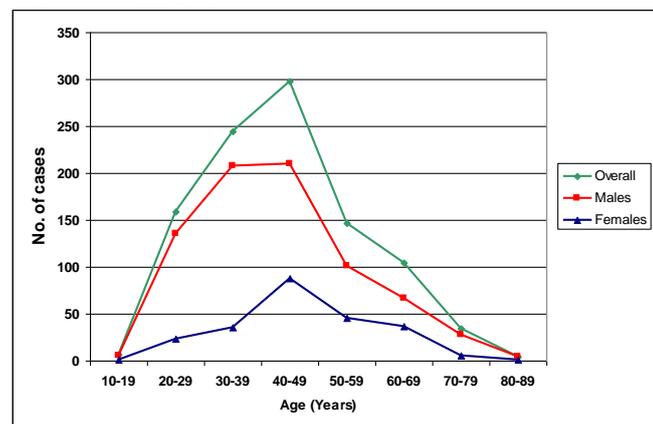
Graph 2. Study Population with and without lesion



Graph 3: Prevalence of lesions based on habits



Graph 5: Distribution of different lesions



Graph: 1 Age and Sex of the study population

Table: 1 Habits in the study population

| Habits | Number |
|----------------------------|--------|
| Smoking | 208 |
| Chewing tobacco | 273 |
| Alcohol | 37 |
| Alcohol & Chewing | 33 |
| Smoking & Chewing | 99 |
| Smoking & Alcohol | 248 |
| Smoking, Alcohol & Chewing | 100 |

Table: 2 Odds ratio and risk estimate for individual habits

| Smoking Duration | Mean | Lesion | | OR | 95% CI | |
|------------------|------|------------|------------|-------|--------|--------|
| | | Present | Absent | | Lower | Upper |
| > 7 | | 40 (19.2%) | 50 (27.9%) | 11.95 | 4.782 | 29.883 |
| < 7 | | 6 (2.9%) | 104 (50%) | | | |

| Alcohol Duration | Mean | Lesion | | OR | 95% CI | |
|------------------|------|------------|------------|-----|--------|--------|
| | | Present | Absent | | Lower | Upper |
| > 15 | | 11 (29.7%) | 5 (13.5%) | 3.5 | 0.903 | 14.153 |
| < 15 | | 8 (21.6%) | 13 (35.1%) | | | |

| Tobacco Duration | Mean | Lesion | | OR | 95% CI | |
|------------------|------|---------------|----------------|-------|--------|--------|
| | | Present | Absent | | Lower | Upper |
| > 10 | | 46 (16.8%) | 60 (22.6%) | 20.57 | 8.356 | 50.646 |
| < 10 | | 6 (2.2%) | 161 (59.0%) | | | |

Discussion

Study on the prevalence of oral mucosal lesions in tobacco habits and alcohol consumption has been done in different parts of the world, differences do exist as the methodologies and sample size vary. The prevalence of oral mucosal lesions in this study (27.6%) is close to the prevalence observed in Thailand (28.4%) and in South London (28.1%).⁵

The various risk habits found in this study are tobacco smoking, chewing, alcohol consumption and a combination of two or three of these. There was correlation between oral mucosal lesions like smokers melanosis, stomatitis nicotina palate, betel chewers mucosa, leukoplakia, leukoedema, oral submucous fibrosis, atrophic glossitis, pouch keratosis, median rhomboid glossitis, candidal infection, cancer to the above habits and found to be consistent with other studies.²

Smokers melanosis (27.5%) had the highest incidence. Hedin CA and Axell T. found smokers had significantly more oral surfaces pigmented than non tobacco users.⁶ Tobacco smoking stimulates oral melanocytes to a higher melanin production along with genetic factors.

The second most prevalent oral mucosal lesion was stomatitis nicotina palate (24.5%). This finding was much higher than in Ljubljana and in Sweden which were population based.^{7,8} The third most prevalent lesion was betel chewers mucositis (12.2%), consistent with that of a Northern hill tribe of Thailand (13.1%).⁹ In our study, this lesion was totally associated with tobacco chewing and was seen more prevalent among females.

The fourth most prevalent lesions were leukoplakia and atrophic glossitis (9.2% respectively). The result of leukoplakia was slightly higher than that of Ching - Hung Chung¹ (7.44%)¹ and Rooban T et al.² (6.6%). In India, oral leukoplakia was reported in people who either smoked and/or had a betel quid chewing habit. Alcohol has been found to increase the risk of oral leukoplakia in the presence of tobacco but the independent association between alcohol and leukoplakia remains unclear. The findings in our study showed that there is a strong association between leukoplakia and toxic habits.

Atrophic glossitis (9.2%) seen in our study group was higher than the finding of Axell T, who showed the

prevalence of 3% in Thai, 1.3% in Malaysian⁶ and 1.1% in Swedish populations.⁸ Chronic consumption of alcohol causes oral mucosal atrophy. It has systemic effects such as malnutrition and immunosuppression also

Regarding pouch keratosis (8%), our finding was consistent with that of Axell T⁶. The development of this lesion is most strongly influenced by habit duration and also by the brand of tobacco used, early onset of spit tobacco use, total hours of daily use, amount of tobacco consumed daily, and number of sites routinely used for placement.

Oral submucous fibrosis was found in nine individuals (2.6% of our study) and is strongly associated with tobacco chewing habits. The predominant use of areca nut results in comparatively an earlier onset of the disease and fibrous bands formation, whereas chewing of areca nut with tobacco, betel leaves and lime results in later onset of the disease.¹⁰

Candidal infection (0.5%) and median rhomboid glossitis (0.2%) were associated with smokers in our study¹¹. Oral cancer was seen in one individual in the buccal mucosa and tongue.

Comparison of the frequency of development of lesion among the various habit groups showed the Atrophic glossitis was the only lesion and has highest prevalence of lesion (51%) among "only alcohol consumption group". But the prevalence of atrophic glossitis reduced when the individuals had multiple habits. The "only chewers" group presented with 25% of chewer's mucositis. Betel quid habit i.e leaf with areca nut, slaked lime and tobacco was more when compared to the commercial forms of areca nut. Females presented with chewer's mucositis and pouch keratosis and males presented with oral submucous fibrosis.

Areca nut is the most important etiologic factor of submucous fibrosis. The nut contains many alkaloids, arecoline being the most abundant, is shown to stimulate collagen synthesis by fibroblasts. According to Reichart PA and Philipsen HP, chewer's mucositis may be a precursor lesion of oral submucous fibrosis.¹²

A high prevalence of lesions of 48% was seen among individuals with all the three habits. The effect of individual habit in causation of various lesions showed either increased frequency or increased duration, supported by a higher mean age of individuals with lesion when compared to individuals without lesions. Assessment of odds ratio showed smoking for more than 7 years had an 11.95 times higher risk when compared to those who smoked for less than 7 years. Individuals who consumed alcohol

for more than 15 years had an 3.56 times higher risk of developing a lesion and chewers who had used tobacco for more than 10 years had an 20.57 times higher risk of developing a lesion.

Conclusion

Oral health professionals should incorporate prevention and cessation services in their routine and daily practice and prevalence study of oral mucosal lesion related to habits will help dentist to provide more effective community based health promotion programs.

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Evaluation of Salivary Ferning for Predicting Ovulation using Smartphone Mounted Microscope

Dr.

1 P

Abstract

Key Words:

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 image¹. 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 infection².

A cost effective optical cell-phone based transmission polarised light microscopy was found effective for imaging the malaria pigment known as hemozoin³. 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 misdiagnosed⁴. A simple, easy to use device that uses a hand-held microscope to visualise ferning pattern in saliva has been recently introduced commercially⁵. 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 gonadotropin 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 hours⁶.

Several researchers have studied ferning pattern in body fluids such as cervical mucus and saliva⁶. Crystallization (with NaCl) of cervical mucus and saliva are characterised by the content of the mucoprotein⁷. 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 content⁸.

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 body⁹. Increased level of estrogen alters vaginal and salivary secretion and forms “fern like pattern” due to crystallisation of sodium chloride on mucus fibre⁸.

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.

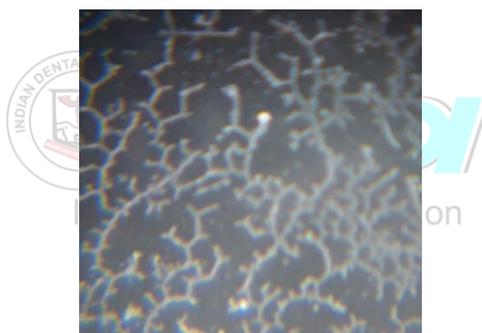
| Sample no | 10 th LMP | | 11 th LMP | | 12 th LMP | | 13 th LMP | | 14 th LMP | | 15 th LMP | | 16 th LMP | | 17 th LMP | | 23 rd LMP | | 24 th LMP | |
|-----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|
| | 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 Positive 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 techniques⁹. 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 day¹⁰.

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 period¹¹.

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% sensitivity⁵. 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 subjects¹³. 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 surveillance¹⁴. 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 concentration¹⁵.

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 hours⁶.

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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