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Evaluation of Salivary pH of Women under different Reproductive Physiological conditions.

[1]Shweta* and [2]Abhilasha Singh
[1]*Department of Zoology, M. V. College, Buxar-802101, Bihar, India*
[2]*Department of Zoology, V K S University, Ara-802301, Bihar, India*

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ABSTRACT: In human female distinct changes occur due to the hormonal fluctuations throughout life time. There is no denying that blood and urine are the most crucial diagnostic fluids but saliva is rapidly becoming another main diagnostic fluid due to the ease and non-invasiveness of collection procedure. Saliva undergoes changes in terms of various physical and biochemical properties in accordance with ovulatory functions. In human female during ovulation period and in diabetic variations salivary pH was observed. 45 healthy volunteers were selected for the assessment of physico-chemical changes in salivary pH. They are categorized as different age group of different reproductive physiological conditions i.e.Prepubertal (6-9 yrs),Parous(pre-ovulatory (6-12 days), ovulatory(13-14 days) and post-ovulatory (15-26 days), Non-parous (pre-ovulatory, ovulatory and post-ovulatory), Menopausal (Above 45 years) and Diabetogenic where we observed a significant decrease ($p<0.05$) level in parous post ovulatory in comparison to prepubertal and highly significant decreased level ($p<0.01$) in diabetogenic than prepubertal condition. A significant increased ($p<0.05$) level was observed in menopausal condition in comparison to parouspreovulatory and post ovulatory.Diabetogenic female saliva showed highly significant ($p<0.01$) level of pH in comparison to non-parous postovulatory and significant decrease ($p<0.02$) level pH than parous ovulatory and highly significant ($p<0.001$) decreasedlevel of pH was observed in comparison to menopausal women's saliva.

Keywords: Saliva, salivary pH, pre-pubertal, parous, non-parous, Menopause and diabetic.

I. INTRODUCTION:

The human body is built to naturally maintain a healthy balance of acidity and alkalinity. Any changes in body might be seen in saliva thus now a daysaliva is considered as diagnostic biomarker with periodontal disease and during ovulatory period also.Saliva is essential for the maintenance of oral health and it is an important diagnostic biofluid(**Malamud,2011).** Saliva has protective properties and contains a variety of antimicrobial constituents and growth factors **(Zelleset al., 1995; Shugars and Wahl, 1998)**. In addition, saliva has lubricating functions and aids in the digestion of food **(Mandel, 1987)**. The normal pH range of saliva is 6.2 to 7.6. Body fluids like blood, saliva, tears, sweat, urine and cerebrospinal fluid are well known biochemical markers that reflect various pathophysiological disorders. Now a day, based on variation in physico-chemical properties of salivary pH level lead to develop a detection kit for detecting the ovulation phase and metabolic disorders in human.The saliva flow rate is also a modulator of salivary pH. At low flow rate, less bicarbonate is released, and pH decreases. (**Humphrey SP and Williamson RT, 2001).Ahmadi-Motamayel et al., 2013** reported that salivary pH was significantly lower in boys as compared to girls. According to the International Journal of Drug Testing, food and drinks, soft drinks (pH 3), white wine (pH 4), American cheese (pH 5), cherries (pH 4)change the pH of saliva.**Sevon, 2008** indicated that relation between saliva flow rate and saliva composition and reported that the factor which affect the flow rate will affect the composition and one of these factors is age. **Edgar and Dawes, 2004** reported that old age cause diminished salivary flow rate. Age may be a factor as children have an average saliva pH 7.5 while adults tend to be more acidic with saliva of pH 6.5 or lower. Earlier findings of **Tolunoglu et al., 2006** reported no correlation between carries and pH in spite of age and gender. **Leome and Oppenheium, 2001** pointed out that pH reduction of saliva is independent due to buffering capacity of saliva. **Tremblay et al., 2012** reported salivary pH as a marker of plasma adipopectin concentration in women. This adipopectin has been identified as a new biochemical marker of visceral fat accumulation in women. It is an adipocyte derived proteins highly abundant in plasma. Earlier reports of **Cardaet al., 2006** indicated that degenerative

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alternations of accinar cells,which cause a decrease of the saliva flow rate and diminutionof salivary pH were observed in diabetic and dyslipidemic human subjects. Earlier findings of **Sreebny, 2000**, **Dodds and Dodds, 1997 and Streckfuset al., 2002** also indicated that hyposalivation was linked with obesity, ageing and hypertensions. Diabetes is the most frequent metabolic disorder associated with salivary hypofunction**(Tremblay et al., 2012)**.This hypofunction of salivary gland in diabetogenic female subjects might be responsible for the decreased salivary pH. Earlier report of **Choeet al., 1983** indicated that the composition of human saliva may alter during the menstrual cycle and pregnancy, presumably response to change in the levels of circulating ovarian hormones estrogen and progesterone.pH also affect Fertility.Sperm must travel through the vagina and the cervical canal before reaching the fallopian tubes to fertilize an egg. An imbalanced pH level in the cervix or vagina candamage sperm enough to prevent it from fertilizing an egg. The most ideal vaginal pH level for a woman to have who wants to get pregnant is between 3.8 to 4.5 outside thedays she is ovulating. During ovulating days, surges in luteinizing hormone are meant to maintain a pH level optimal to increase the chance that sperm reaches the egg. In fact, when the vaginal pH level is between 7 to 12, sperm can survive as long as 48hours within the female reproductive system.The salivary pH was lower in pregnant women than in non-pregnant women **(Rosenthal, 1959).** Therefore it might possible thathormonal imbalance during various phases of menstrual cycle responsible for changes in pH of saliva. **Lukas andLargaespada, 2006 and Temple, 2011**reported that estrogen have significant role in thesaliva flow.Diabetes causes the body pH levels to become more acidic. The salivary pH was significantly lowered in diabetic patients when compared to normal individuals (**K M Pet al., 2013**)

II. MATERIAL AND METHODS:

The studies were performed in 45 different human female volunteers of age group 19 to 40 years categorized as prepubertal, parous, non-parous, menopausal and diabetogenic.The best two ways to collect whole saliva are the draining method in which saliva is allowed to drip off the lower lip and the spitting method in which the subject expectorates saliva into a test tube **(Navazesh, 1993).**

To test the pH of saliva, pH strips which are available at drug store or online was used following the steps mentioned below.

1. Not allowed to eat or drink for a minimum of two hours before testing.
2. Mouth was filled with saliva and then swallowed or spit it out.
3. Mouth was filled with saliva again and then placed a small amount of it on a pH strip.
4. Changes in colors based on the acidity/alkalinity of saliva was examined and compared with the color chart outside the box of pH strips to determine the saliva's pH level.

Table 1: Level of pH in saliva samples of different conditions in human female subjects:

SL No.	Name of different conditions with symbols	Level of pH Mean ± SD of 5 samples	P-Value
1	**Pre pubertal –(a)**	**6.55±0.14**	
2	**Parous** I. **Pre ovulatory –(b)** II. **Ovulatory – (c)** III. **Post ovulatory–(d)**	 **6.19± 0.16** **6.49±0.17** **6.10±0.18**	**a to d** – (p<0.05)**S**
3	**Non- parous** I. **Pre ovulatory-(e)** II. **Ovulatory-(f)** III. **Post ovulatory-(g)**	 **6.19± 0.25** **6.28 ± 0.16** **6.50±0.12**	
4	**Menopausal –(h)**	**6.68 ±0.14**	**b to h** – (p<0.05)**S** **d to h** –(p<0.05) **S**
5	**Diabetogenic –(i)**	**5.98±0.04**	ato i –(p<0.01)HS **c to i**–(p<0.02)**S**, , **g to i** –(p<0.01)**HS** h to i – (p<0.001)HS

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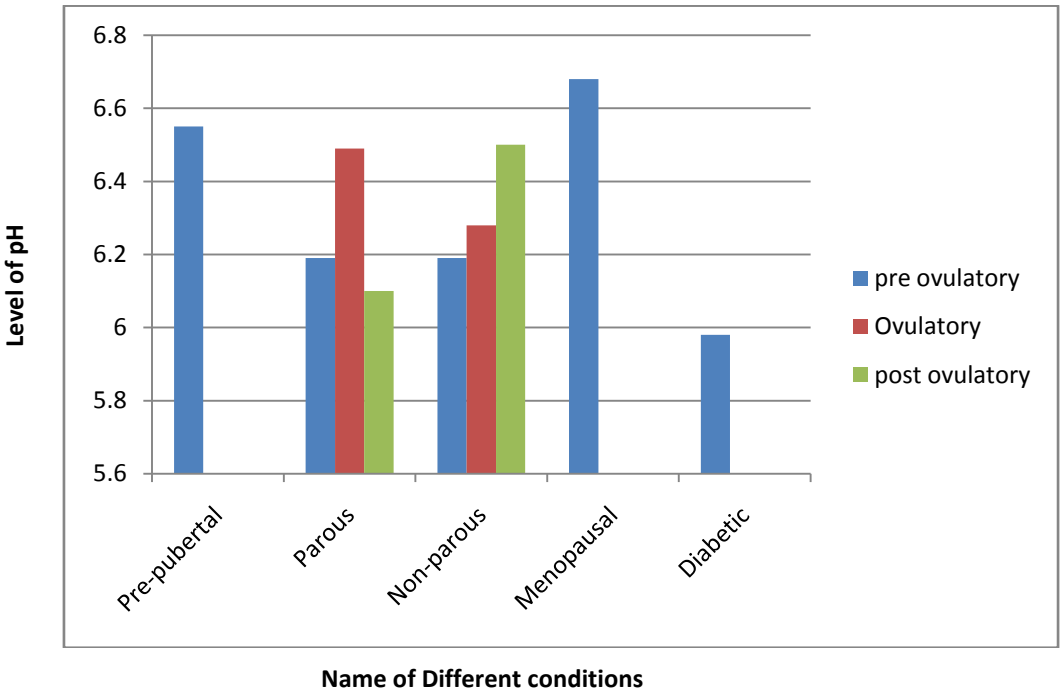

Fig 1: Level of pH in saliva samples of different conditions

III. RESULTS AND DISCUSSION:

The results revealed a fluctuating pattern of increased and decreased level of salivary pH.Diabetogenic female saliva showed highly significant ($p<0.01$) level of pH in comparison to non-parous postovulatory and significant decreased ($p<0.02$) level pH than parous ovulatory and highly significant ($p<0.001$) decreased level of pH was observed in comparison to menopausal women's saliva as shown in Table 1 and Graph 1.Highest level of pH was observed in menopausal conditions (pH 6.68±0.14) followed by prepubertal(6.55±0.14) and non parous postovulatory (6.50± 0.12) as shown in Table 1. Report of Tulunogluet al., 2006 indicated that dental caries affect the pH of saliva in human subjects. Dawes, 2004 reported that the relative concentrations of the organic and inorganic salivary constituents are known to depend of salivary flow rate. Salivary buffering capacity helps in keeping salivary pH at a hormonal level.A significant increased pH in Parous postovulatory than prepubertal and in menopausal women's saliva than parous ovulatory and decreased in diabetogenic than prepubartal, parous ovulatory, nonporous ovulatory and menopausal women's saliva. All these might be possible due to changed salivary flow rate and hormonal fluctuation in different reproductive status of women. The increase in the salivary flow rate in pregnant women could be attributed to the increase in the estrogen and progesterone concentration during pregnancy. The decrease in the pH and buffer capacity is due to the decrease in the plasma $HCO3^-$ ion concentration and an increase in α amylase concentration during pregnancy.

The pH of unstimulated saliva was determined by using a pH strip provided in the kit and placing it in the collected sample of resting saliva for 10 seconds. The color change of the strip was compared with the testing chart available with the kit and recorded.An Unpaired Student t test was used which revealed that there was statistically significant difference between the different groups ($p<0.001$).No differences were found between pregnant and non-pregnant women regarding stimulated saliva pH, and no significant differences were observed in non-stimulated and stimulated saliva pH.

Conclusion: The purpose of this article is to diagnose saliva and monitoring level of salivary pH during human female ovulatory functions and metabolic disorders.This study concluded that salivary pH make the possibility to develop a biomarker for detection of ovulation by non-invasive methods.Significant variations were

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observed in salivary physical and biochemical parameters between diabetics and non-diabetics. Evaluation of salivary parameters can be a cost effective and a non-invasive alternative for screening, diagnosis and monitoring of diabetes and different reproductive physiological conditions.

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