Original Article

Salivary Flow rate, pH and buffering capacity in pregnant and non pregnant women - A comparative study

Dr. Naveen S[1], Dr. Asha ML[2], Dr. Shubha G[3], Dr. Atul Anand Bajoria[4], Dr. Anju Anu Jose[5]

[1,3,5] Senior Lecturer, Department of Oral Medicine and Radiology
[2] Professor and Head, Department of Oral Medicine and Radiology
[4] Post- Graduate, Department of Oral Medicine and Radiology
Sri Hasanamba Dental College and Hospital, Hassan

ABSTRACT

Objective: To evaluate salivary flow rate, pH and buffering capacity of saliva in pregnant and non pregnant women.

Sample and design: The present study was a comparison between 30 pregnant women in their third trimester and 30 non pregnant women, in the age group of 19-34 years.

Method: The salivary flow, pH, and buffering capacity was measured using Saliva-check BUFFER kit (GC Corporation). Both unstimulated and paraffin stimulated saliva was measured for 5 min by asking the subjects to spit passively into a measuring jar provided in the kit.

Main outcome measures: The pH and buffering capacity of unstimulated saliva was measured using a pH and buffering strips provided in the kit.

Results: Unpaired Student t test showed a statically significant increase in the salivary flow and a decrease in the pH and buffering capacity in the pregnant group when compare to the non pregnant group.

Conclusion: 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 was due to the decrease in the plasma HCO_3^- ion concentration and an increase in α amylase concentration during pregnancy.

Key Words: Pregnancy, salivary flow rate, pH, buffering capacity.

INTRODUCTION

Saliva is one of the most important, complex and versatile body fluid, performing a large range of physiological needs. [1] Healthy adult subjects normally produce 500–1500 ml of saliva per day, at a rate of approximately 0.5 ml/min.[2] The buffering action of saliva is an important defense mechanism. A buffer is a solution that tends to maintain a constant pH. Whenever the pH starts falling after the ingestion of a substrate, it returns back to the original resting level after a period of time because of the inherent buffers in the saliva. Critical pH is the pH of the saliva below which the inorganic material of tooth starts dissolving and it varies according to the calcium and phosphate ion concentration. The value of Critical pH is usually about 5.5 ranging anywhere between 5.2 and 5.7.[3]

Reduced salivary flow rate and the alteration in the constituents of saliva may result in the reduction of oral defense systems and may cause severe caries and mucosal inflammations. Dental caries is probably the most common consequence of hypo salivation. Subjects with impaired saliva flow rate often show high caries incidence or caries susceptibility.[4]

Female sex hormones (estrogen, progesterone & human gonadotropin) are secreted primarily by the placenta. These hormones are responsible for most of the physiologic changes during pregnancy. The main salivary changes in pregnancy involve its flow, composition, pH and hormone levels. [5]

Pregnancy increases the propensity to gingival inflammation known as pregnancy gingivitis, with an enhanced gingival bleeding tendency without specific plaque association; periodontal pocket formation and dental caries can increase during pregnancy. These changes are reversible after delivery and the exact etiology for this is still unclear.[6]

Salivary analysis has become an important resource for the evaluation of salivary conditions with physiologic and pathologic implications and is a useful tool for disease diagnosis, mainly due to its origin, composition, functions, and interactions with other organ systems. With the addition of modern techniques and chemical instrumentation equipment, there has recently been an observable increase in the use saliva for laboratory investigations. The value of saliva as a diagnostic tool for oral and systemic diseases has been an area of study for many researchers with the aim of increasing its use as a possible complementary exam.[7]

The aim of the study was to assess the stimulated and unstimulated saliva flow rate, pH and buffering capacity in pregnant and non-pregnant women.

MATERIALS AND METHODS

Thirty pregnant women aged between 19-34 years in the third trimester who attended Gynecology Clinic in Hassan constituted the study group and 30 non pregnant women of the same age group who visited the clinics of Oral Medicine and Radiology Department at Sri Hasanamba Dental College and Hospital made up the control group. Exclusion criteria were subjects with salivary gland disorders, oral mucosal diseases and with systemic illness. All subjects signed an inform consent to participate in the study. An ethical approval (no. SHDCH/2010-11/ETH/14) was taken from the institutional ethical committee before the start of the study.

The salivary samples were collected between 9- 11.30 a.m in both the study and control group. The salivary flow, pH, and buffering capacity was measured using Saliva-check BUFFER kit (In Vitro test for pH and Saliva Buffering Capacity) manufactured by GC Corporation. The kit is provided with a ph strips which measures the pH between 5-8, saliva collection cups, paraffin wax for saliva stimulation, saliva dispensing pipette and buffer test strips.

One hour prior to collection of the sample, the subjects were asked not to use any mouthwash, smoke, consume food and beverages. In order to test the flow of resting unstimulated saliva the patient was asked to sit passively for 5 minutes and expectorate into a sterile collection cup with ml marking. The resting salivary flow rate is measured as ml/min. The stimulated salivary flow was assessed by asking the patient to chew a piece of paraffin wax. After 30 second, the patient was asked to expectorate into the spittoon. The patient was instructed to continue chewing the wax for 5 minutes and the saliva was collected in a collection cup with ml marking.

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.

The buffering capacity of the unstimulated saliva was measured by using a buffer strip provided in the kit. Using pipette sufficient saliva from the collection cup was dispensed on to the test pad. At the end of 2 min the test pad would change its color, comparing the change in color with the chart provided in the kit the buffering capacity was scored and recorded.

STATISTICAL ANALYSIS

Data were analyzed by descriptive statistics and comparison between the pregnant and non pregnant group were performed using unpaired Student t test for salivary flow rate, pH and buffering capacity. All statistical tests were two-tailed and a P-value of 0.05 was considered statistically significant, by using SPSS Version 17.

RESULTS
The mean stimulated and unstimulated salivary flow rate in the study group was 8.38, 4.32 and that of the control group was 6.76, 3.47 respectively indicating a significant increase in the salivary flow rate in the study group.
There was a reduction in the pH and buffering capacity in the study group with a mean pH and buffering capacity of 6.36 and 7.50 respectively. The control group had a mean pH of 6.87 and the buffering capacity of 9.93.

Table-1 and Graph-1: shows mean unstimulated salivary flow rate was 3.47± 1.44 and 4.82±1.62 in the non pregnant and pregnant women respectively. An Unpaired Student t test was used which revealed that there was statistically significant difference between the two groups (p<0.001).

Table 1: mean unstimulated salivary flow among pregnant and non pregnant women

FACTOR	Non Pregnant		Pregnant		UNPAIRED - t TEST		
	Mean	SD	Mean	SD	t - VALUE	p value	Significance
Unstimulated Flow	3.47	1.44	4.82	1.62	3.500	0.001	S

P<0.05, S – Significant, NS – Non Significant

Graph 1: mean unstimulated salivary flow among pregnant and non pregnant women in ml/per 5 min

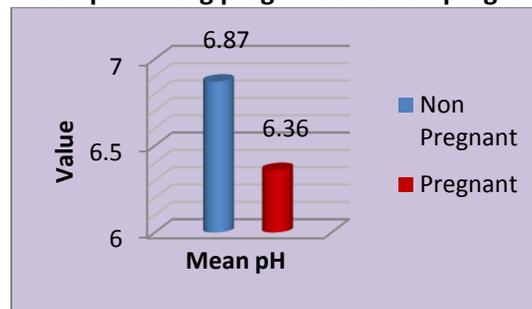**Table-2 and Graph-2**: shows a mean pH of 6.87± 0.37 and 6.36± 0.33 in the non pregnant and pregnant women respectively. Statistically significant difference was found between the two groups when Unpaired Student t test was used (p<0.001)

Table 2: mean pH among pregnant and non pregnant women

FACTOR	Non Pregnant		Pregnant		UNPAIRED - t TEST		
	Mean	SD	Mean	SD	t - VALUE	p value	Significance
pH	6.87	0.37	6.36	0.33	5.585	0.000	HS

P<0.05, S – Significant, NS – Non Significant

Graph 2: mean pH among pregnant and non pregnant women

Table-3 and Graph-3: shows the mean buffer capacities of non pregnant and pregnant groups were 9.93±1.43 and 7.50±1.69 respectively. A statistically significant difference was found between the two groups when a Unpaired Student t test was used (p<0.001)

Table 3: mean buffering capacity among pregnant and non pregnant women

FACTOR	Non Pregnant		Pregnant		UNPAIRED - t TEST		
	Mean	SD	Mean	SD	t - VALUE	p value	Significance
Buffering capacity	9.93	1.43	7.50	1.69	5.994	0.000	HS

P<0.05, S – Significant, NS – Non Significant

**Graph 3: mean buffering capacity among pregnant and non pregnant women**
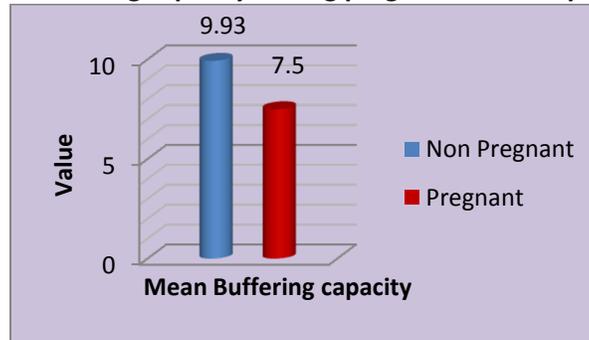
Table-4 and Graph-4: shows stimulated salivary flow rate had a mean of 6.76± 1.87 and 8.38± 2.16 in the non pregnant and pregnant women respectively. An Unpaired Student t test was used and reveled a statically significance (p<0.003)

Table 4: mean stimulated salivary flow among pregnant and non pregnant women

FACTOR	Non Pregnant		Pregnant		UNPAIRED - t TEST		
	Mean	SD	Mean	SD	t - VALUE	p value	Significance
Stimulated flow	6.76	1.87	8.38	2.16	0.328	0.003	S

P<0.05, S – Significant, NS – Non Significant

Graph 4: Mean stimulated salivary flow among pregnant and non pregnant women in ml/5 min
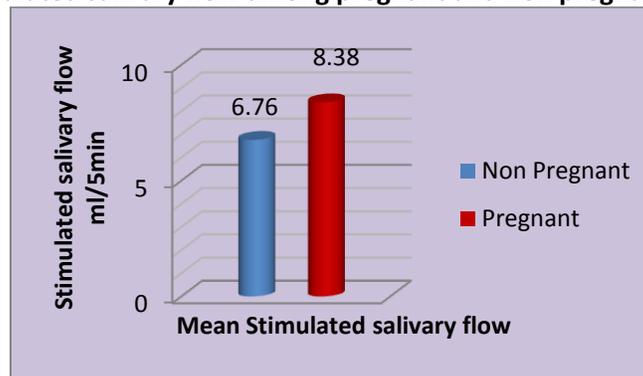
DISCUSSION

Main Findings

The pregnant group showed increased flow rate and decreased buffer and pH when compared to the non pregnant women.

Strength and Limitation: Saliva is regarded as one of the important factors in regulating oral health.[8] Each day, the human salivary gland produce about 600ml of serous and mucous saliva containing minerals, electrolytes, buffers, enzymes, enzyme inhibitors, growth factors and cytokines, immunoglobulin's, mucin and other glycoprotein's. At the same time it possesses antimicrobial components and buffering agents that act to maintain oral tissue.[9] Saliva as a diagnostic medium has advanced exponentially in the last 10years. 3 Most researches have shown the advantage of using saliva in detecting physiologic or pathologic conditions because there is a close relationship between saliva and serum parameters.

Pregnancy is a physiological process associated with many functional and compositional alterations in almost all systems of the body. It is a state of physiological stress which is accompanied by profound hormonal, biochemical and metabolic changes.[10]Studies undertaken previously to estimate the stimulated and unstimulated salivary flow rate between pregnant and non pregnant women have shown mixed results. The studies done by Marja Lane and others shows no significant change in the salivary flow rate between the pregnant and non pregnant women.[11,12] other studies shows significant reduction in the salivary flow rate in the pregnant groups.[10, 13]

Unstimulated whole saliva reflects basal salivary flow rate and it provides protection to oral tissues[3]. The study of unstimulated salivary secretion is an accurate method to analyze salivary gland status, while stimulated saliva is useful for the study of the functional reserve.[14]

The increase of salivary flow in this study may be due to the hormonal changes that take place during pregnancy. Although many hormones are known to regulate saliva composition and secretion, the specific mechanism by which hormones modulate human salivary gland function is poorly understood.[10]

The increased production of hormones during pregnancy is mainly due to the placenta, which takes over the production of progesterone and estrogen in the pregnancy. Estrogen levels rise more than 100- folds from the beginning of pregnancy.[15] Estrogen has a vasodilatory effect on the major arteries and increases blood flow in the target tissue. The possible effects of estrogen on blood flow in the salivary glands is not known but increased blood flow is associated with increased secretion of saliva.[16]

A number of studies have reported an increase in salivary flow rate when estrogen is used for hormonal replacement therapy (HRT), this suggests that estrogen may play an important role in oral mucosal and salivary gland physiology.[13,17]

For direct action steroid hormones require specific receptors in the target tissue.[16] The effects of estrogens are mediated by estrogen receptors (ERs), two different subtypes of ERs have been identified, namely ERα and ERβ. ERβ is mainly expressed in tissues that have only recently been identified in colonic and prostatic epithelia, keratinocytes and salivary gland acinar and ductal cells.[17]

Importantly, the expression of ERβ in oral epithelial cells and salivary gland acinar and ductal cells suggests that estrogens may regulate the physiology of these tissues through the ERβ subtype. These findings may also serve to explain clinical observations of sensitivity of oral tissues to estrogens and the beneficial effects of HRT on oral symptoms in postmenopausal women[17] and also in the present study which showed increase in the un-stimulated and stimulated salivary flow among the pregnant women.

Interpretation: Prenatal patients usually are distressed and uncomfortable by this profuse salivation referred to as sialorrhea or ptyalism.[18,19] The increase in the salivary flow during pregnancy in the present study can be attributed to these factors.

It is believed that nausea and vomiting are necessary components of sialorrhea in pregnancy and certain hormones contribute to this relationship (morning stickiness). In this respect, more than 70% of all pregnant women encounter nausea and vomiting which is accompanied by excessive salivation.[18,19] Human chorionic gonadotropin (HCG) has been implicated in nausea, increased salivation and vomiting because of the high levels produced during pregnancy.

During pregnancy stomach has prolonged gastric emptying times the gastroesophageal sphincter has

decreased tone. Together, these changes lead to reflux and possibly combined with decreased esophageal tone to cause ptyalism. The large bowel also has decreased motility, which leads to increased water absorption and constipation.[19] Hence these factors can be hypothesized to the increase in the saliva flow.

Salivary pH is closely related to the buffer capacity.20 The most important buffering system in saliva is the carbonic acid / bicarbonate system. The dynamics of this system is complicated by the fact that it involves the carbon dioxide dissolved in the saliva. The complete simplified equilibrium is as follows:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+$$

The increased carbonic acid concentration will cause more carbon dioxide to escape from the saliva. Salivary bicarbonate increases the pH and buffer capacity of saliva, especially during stimulation.

The second buffering system is the phosphate system, which contributes to the buffer capacity at low flow rate. The buffering action of inorganic phosphate is due to the secondary phosphate ion, HPO_4^{2-}, to bind a hydrogen ion and form an $H_2PO_4^-$ ion. The third buffering system is the protein system. In the low range of pH the buffering capacity of saliva is due to the macromolecules (proteins) containing H-binding sites.

The inorganic and protein composition of saliva changes during the course of pregnancy.[13] Salivary gland HCO3- originates partly from plasma and partly from the salivary gland carbon dioxide. The reduction in pH value during pregnancy, is related to the effect of progesterone hormone, which is known to decrease plasma bicarbonate level during pregnancy resulting in a decrease in the pH and buffering capacity.[13]

The activity of salivary peroxidase a marker enzyme of estrogen action increases significantly during pregnancy along with specific progesterone receptors in human salivary glands. Progesterone receptors are induced by estrogen receptors but it is still not known which type of cells are the potential targets in the salivary gland.16

The most important protein of saliva is α-amylase which is secreted by parotid gland. Increasing trend of this enzyme activity may lead to increased microorganism substitution and reduced pH of saliva. It was found that α-amylase activity increase during 10 and 21 weeks of gestation.[10]

Hormonal changes may also affect the composition of saliva. During pregnancy, when the serum concentration of estrogens is elevated, IgA increases, whereas sialic acid and the pH and buffer capacity decrease in saliva.[13]These factors have led to the decrease in the pH and the buffering capacity of saliva in the pregnant group.

CONCLUSION

A significant increase in the flow rate of both unstimulated and paraffin stimulated saliva was seen in pregnant women in the third trimester with a reduction in pH and buffering capacity when compared to the non pregnant women in the same age group. The increase in the salivary flow may be attributed to the increase in estrogen and progesterone secretion and the decrease in the pH and buffering capacity may be due to the decreased plasma HCO3- ion concentration and increase in the α amylase concentration. However to obtain a more conclusive conformation of this hypothesis more studies have to be carried out. In conclusion the present study provides further evidences for the modification of saliva during pregnancy.

CONTRIBUTION TO AUTHORSHIP

Dr. Naveen S was the principal investigator for the study. Collection of all the data and samples were done by him.We are grateful to our respected teacher Dr. Asha ML, professor and Head, Department of Oral Medicine and Radiology for her relentless help and suggestions for the study at all times. Dr. Shubha G, Dr. Anju Anu Jose and Dr. Atul Anand Bajoria for their help

in interpreting the study results and analyzing the discussion of the study.

Details of ethics approval:-

An ethical approval (no. SHDCH/2010-11/ETH/14) was taken from the institutional ethical committee before the start of the study.

REFERENCES

1. Amerongen AV, Veerman ECI. Saliva-the defender of the oral cavity. Oral Dis 2002;8:12-22.

2. Chicharro JL, Lucia A, Perez M, Vaquero AF, Urena R. Saliva composition and exercise. Sports Med 1998;26(1):17-27.

3. Newbrun E. Quintessence publishing Co. 3rd ed. Chicago, Illinois: 1989. Cariology.

4. Goran Frostell. A colourimetric-screening test for evaluation of the buffer capacity of saliva. Swedish Dental Journal 1980;4(3):81-6.

5. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000;14:40-7.

6. Loe H, Silness J. Periodontal disease in pregnancy I: Prevalence and severity. Acta Odontol Scand 1963;21:533-51.

7. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 2007;383(1-2):30-40.

8. Salvolini E, Di Giorgio R, Curatola A, Mazzanti L, Fratto G. Biochemical modifications of human whole saliva induced by pregnancy. Br J Obstet Gynaecol 1998;105(6):656-60.

9. Kullaa-Mikkonen A, Mikkonen M, Kotilainen R. The pH of stimulated and resting saliva in different morphological forms of the tongue surface in a young population. Oral Surg Oral Med Oral Pathol 1982;53(5):466-8.

10. Karama MT Al-Nuaimy, Fadhila Sh Al-Doski. Pregnancy related changes in oral health and human unstimulated whole saliva. Al-Rafidain Dent J 2003;3(2):108-15.

11. Laine M and Pienihakkinen K. Salivary buffer effect in relation to late pregnancy & postpartum. Acta Odontol Scand 2000;58(1):8-10.

12. Rockenbach MI, Marinho SA, Veeck EB, Lindemann L, Shinkai RS. Salivary Flow Rate, pH, and concentration of calcium, phosphate, and non-pregnant women. Head Face Med 2006;28(2):44.

13. Eliasson L, Carelen A, Laine M, Birkhed D. Minor gland and whole saliva in postmenopausal women using a low potency oestrogen (oestriol). Arch Oral Biol 2003;48(7):511-7.

14. Fenoll-Palomares C, Munoz Montagud JV, Sanchiz V, Herreros B, Hernandez V, Minguez M et al. Unstimulated salivary flow rate pH and buffering capacity in healthy volunteers. Rev Esp Enferenm Dig 2004;96(11):773-83.

15. Laine MA. Effect of pregnancy on periodontal and dental health. Acta Odontol Scand 2002;60(5):257-64.

16. Laine M, Leimola-Virtanen R. Effects of harmone replacement therapy on salivary flow rate, buffering effect and pH in perimenopausal and postmenopausal women. Arch Oral Biol 1996;41(1):91-6.

17. Valimaa H, Savolainen S, Soukka T, Silvoniemi P, Makela S, Kujari H et al. Estrogen Receptor-beta is the predominant estrogen receptor subtype in human oral epithelium and salivary glands. J Endocrinol 2004;180(1):55-62.

18. Freeman JJ, Altieri RH, Baptiste HJ, Kuo T, Crittenden S, Fogarthy K et al. Evolution and management of sialorrhea of pregnant with concomitant hyperemesis. J Natl Med Assoc 1994;86(9):704-8.

19. Tamara L. Callahan, Aaron B. Caughey. Pregnancy and Prenatal Care. In: Susan Rhyner, editor. Blueprints Obstetrics and Gynaecology, 6th ed. China: Lippincott Williams and Wilkins;2013. p.1-10.

20. Kivela J, Laine M, Parkkila S, Rajaniemi H. Salivary carbonic anhydrase VI and its relation to salivary flow rate and buffering capacity in pregnant and non-pregnant women. Arch Oral Biol 2003;48(8):547-51.