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Research Article

Salivary Flow Rate, pH and Buffering Capacity in Pregnant and Non Pregnant Women - A Comparative Study

Authors

Naveen, S., Asha, M. L., Shubha, G., Atul Anand Bajoria and Anju Anu Jose

Department of Oral Medicine and Radiology, Sri Hasanamba Dental College and Hospital, Hassan, India

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Abstract

The objective of this article is to evaluate salivary flow rate, pH and buffering capacity of saliva in pregnant and non pregnant women.

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.

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.

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

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.

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 HCO_3^- ion concentration and an increase in a amylase concentration during pregnancy.

Keywords: Pregnancy, salivary flow rate, pH, buffering capacity.

Introduction

Saliva is versatile and complex fluid and is necessary for various physiological functions in the oral cavity. A healthy adult individual produces about 500-1500ml of saliva per day with an average rate of about 0.5ml/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

Hyposalivation causes alterations in the oral defense system leading to increased caries susceptibility and mucosities .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 unstimulated saliva flow rate, pH, buffering capacity and stimulated saliva flow rate 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, 4.82 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

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

Table-3 and Graph-3: shows the mean buffering 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 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

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 revealed a statistically 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

Discussion

The pregnant group showed increased flow rate and decreased buffer and pH when compared to the non pregnant women. Saliva is regarded as one of the important factors in regulating oral health.⁸ About 600ml of serous and mucous saliva containing minerals, electrolytes, buffers, enzymes, growth factors enzyme inhibitors and immunoglobulin's, cytokines, mucin and other glycoproteins is produced by the human salivary gland produce every day. At the same time it possesses antimicrobial components and buffering agents that act to maintain oral tissue.⁹ Many studies have shown that saliva has a close relationship between the serum parameters, hence it can be used in detecting physiological and pathological changes in the body.⁵⁻⁹

Pregnancy is a process which brings about alterations in the composition and functions of all systems of the body. It is also accompanied with profound metabolic biochemical and hormonal 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 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 tissues³. Unstimulated salivary provides a precise parameter to analyze the salivary gland status

while the stimulated saliva provides information about the functional reserves .¹⁴

The increase of salivary flow in this study may be due to the hormonal changes that take place during pregnancy. Saliva composition and secretion is modulated by many hormones but the exact mechanism how these hormones bring about these changes 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 Estrogen receptors (ERs) are responsible for the effects of estrogen. There are basically two types of receptors, ER α and ER β . ER β is identified recently in salivary gland acinar and ductal cells.¹⁷

Importantly, the expression of ER β in oral epithelial cells and salivary gland acinar and ductal cells suggests that estrogens may regulate the physiology of these tissues through the ER β subtype. Thus suggestive of the sensitivity of oral tissue to estrogen and its application in HRT¹⁷ and also in the present study which showed increase in the un-stimulated and stimulated salivary flow among the pregnant women.

Pregnant patients are uncomfortable and distressed due to the profuse salivation which is termed 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.

Pregnancy induces decreased gastroesophageal sphincter tone and prolonged gastric emptying times. These changes along with decreased esophageal tone lead to ptyalism. Further decreased large bowel motility which leads to increased water and constipation absorption.¹⁹ Hence these factors can be hypothesized to the increase in the saliva flow.

Salivary pH is closely related to the buffer capacity (Figure 1).²⁰

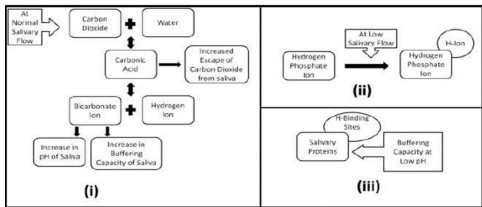


Figure 1: Salivary Buffer Systems (i) Bi-Carbonate Buffer System: Activates at Normal Salivary Flow were in the Bicarbonate Ion Modulates the Salivary pH (ii) Phosphate Buffer System: Activates at Low Salivary Flow were in the Hydrogen Phosphate Ion Modulates the Salivary pH (iii) Protein Buffer System: Role of Various Salivary Proteins in Maintaining Salivary pH.

The inorganic and protein composition of saliva changes during the course of pregnancy.¹³ Salivary gland HCO_3^- 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 HCO_3^- 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.

Conflict of Interest:

None.

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Disclosure of Interests: None

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

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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.

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