ORIGINAL RESEARCH

pH of Saliva – An Efficient Diagnostic Tool

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ABSTRACT

Introduction: Saliva, a non-invasive biofluid is still little used compared to plasma although it offers several opportunities in diagnostic and monitoring. Therefore we need to discover new accessible markers which could be readily included in an integrated screening approach. With this objective we tried to use salivary pH as a quick chairside test. The aim of the present study was to analyze the variation of pH in different groups of females. Also an attempt is being made to review the literature to analyze salivary pH as an efficient diagnostic tool.

Material and method: The study included 80 patients comprising 60 subjects and 20 controls. The subjects were divided into three groups including 20 menstruating, 20 pregnant and 20 postmenopausal females. The randomized unstimulated saliva was collected from each patient and pH was tested. Data was analyzed statistically using Post hoc test.

Results: The salivary pH was significantly lower in postmenopausal women compared with the control group (p < 0.05)

Conclusion: These results indicate significant reduction in pH in postmenopausal women that may make them more prone to the occurrence of oral health problems and could be a potential indicator of metabolic dysregulation. Measuring salivary pH is simple and procedure can be carried out with minimal resources. Salivary pH can therefore serve as an important diagnostic tool in future to monitor health of an individual and can open up a new area of research to develop newer simple techniques.

Keywords: Salivary ph, diagnostic tool, biofluid

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INTRODUCTION

This human body though riddled with complex phenomenon is well programmed and is very systematic. It depends on the researchers to pick up the clues in order to predict the disease and health of the population. But in today's world where the technology is growing so fast we have been able to evaluate certain phenomenon that occurs before manifestation of the actual disease. Every health worker is craving to achieve his dream of a perfect diagnosis so that proper service can be rendered to the patient. Despite the scepticism in the scientific community, saliva has emerged as a valuable tool in various diagnostics and especially mass screening of the population.[1]

Saliva is the most available and non-invasive biofluid of the human body and salivary diagnostics is a dynamic and emerging fluid to aid in the diagnosis of oral and systemic diseases. With a multitude of biomarkers and complexities in their determination, the salivary pH may be tried to be used as a quick chairside test as saliva sampling is an inexpensive, non-invasive and easy-to-use method.[2]

As we know that health needs of women are different mainly because of the distinct changes that occur over their lifetime. This is due to the hormonal fluctuations occurring throughout the woman's life affecting the physiology of the entire body including the oral cavity.[3] Saliva is essential for maintaining oral health and any alterations in salivary function may lead to impairment of oral tissues and have a large impact on the patient's quality of life. With the previous studies it has been shown that, salivary pH plays important role in the oral mucosal defence and reduction in salivary pH makes oral mucosa more susceptible to various oral diseases. Also, at the individual level, women seem to have more variation in their salivary pH.[4] It has been proposed that hormonal fluctuations during events like puberty, menstruation, pregnancy and menopause could explain those differences. The salivary pH is also affected by various cardiometabolic risk components including

the menopausal status. Degenerative alterations in the acinar cells, which cause a decrease of the saliva flow rate and a diminution of salivary pH, are frequently observed among diabetic and dyslipidemic patients.[5] However, various studies have focussed on the significance of salivary pH in the development of caries and sex-dependent differences in salivary pH. There is scanty literature focussing on comparison of salivary pH between menstruating, pregnant and postmenopausal women in a single article. Thus the present study aimed at analyzing the pH of saliva and evaluating the effect of menstruation, pregnancy and menopause on it as determining salivary pH being an inexpensive and non-invasive procedure could be a very promising avenue. Also an attempt is made to review the literature to analyze salivary pH as an efficient diagnostic tool.

MATERIAL AND METHODS

20 menstruating women (with regular menstrual cycle of 28-30 days)[6], 20 pregnant women between sixth and ninth month of pregnancy and 20 postmenopausal women with history of menopause more than 1 year constituted the study group. Thus the study group included 60 patients within an age range of 15-55 years. Also 20 regularly menstruating girls between 15-25 years composed the control group. Hence a convenience sample of 80 subjects was selected from those reporting to the Department of Oral and Maxillofacial Pathology and the Department of Obstetrics and Gynaecology SGT Dental and Medical College, Gurgaon. All of the subjects were healthy individuals attending the clinics for regular examination. A questionnaire covering information on age, sex, systemic disease, daily medication and various oral symptoms was filled out for each individual. All subjects signed an inform consent to participate in the study. An ethical approval was taken from the institutional ethical committee before the start of the study.

Patients free from systemic or local diseases, normal chewing ability, with no evidence of dry mouth or salivary gland disorders and with good oral hygiene were included in the study. Patients having any oral diseases, systemic diseases, on medication, habit of smoking, with high risk pregnancy and those not willing to participate in the study were excluded.

Saliva sampling
Saliva collection was done as per World Health Organization protocol."[7] Subjectswere asked to keep an overnight fast before saliva sample was collected the next morning. They were also asked to rinse the mouth properly and then after 5 minutes were asked to spit the whole saliva. The subjects were instructed not to cough up the mucus while saliva collection. The subjects spit into the collection tube about once a minute for up to 10 min. 5 ml of saliva was thus collected in sterile 10 ml beaker. The salivary sample was collected between 9:00 am and 11:00 am in a comfortable room free from noise and distraction. Salivary samples of the menstruating group were collected within the first three days of menstruation.[8]

The pH of the saliva was immediately measured in order to prevent any deterioration of the sample.

Salivary analysis
Salivary pH was measured with the help of a single electrode pH meter (Slope pH Meter Model 152-R).[8] The pH meter was calibrated every day. The electrode was dipped in hydrochloric acid of 0.1 N when not used. The next day pH meter was again calibrated using freshly prepared buffers of pH 7 and pH 4. The latter was used for finer adjustment to the pH. Following this the electrode was kept dipped in double distilled water. After analyzing the pH, the electrode tip was again washed with a gentle stream of distilled water and then dipped in the double distilled water. The liquids and chemicals were freshly prepared every day.[2]

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) was used for the statistical analysis. To evaluate the significance of the parameters used between the four investigated groups Post hoc and LSD tests were used. P-values lower than 0.05 were taken as statistically significant.

RESULTS

Salivary pHwas evaluated in total 80 candidates including 20 controls and 60 cases (20 menstruating, 20 pregnant and 20 post menopausal). Post Hoc Testwas used for statistical analysis and the comparison of the results was made between all the groups. P < 0.05was considered statistically significant. (Table 1,2).
pH values were significantly lower in post menopausal women when compared with the control group (p<0.05) (Table 1,2) Though no significant difference was found when pH was compared between control group and menstruating and pregnant group respectively. (Table-1,2)

DISCUSSION

The physicochemical and biochemical properties of saliva along with its complex composition endowes this fluid with multiple functions, including: anti-bacterial, anti-viral and anti-fungal properties; buffering capacity; digestive activity; mineralizing capacity; lubricant property and viscoelastic properties essential for the maintenance of oral health; and protective and repairing fluid for mucosal surfaces.[9] Therefore without saliva, mealtimes are difficult, uncomfortable and embarrassing.[10] The fact is that the Women may be more susceptible to salivary changes because of the unique hormonal changes they experience. There are five situations in a women's life during which hormone fluctuations might make them more susceptible to oral health problems – during puberty, at certain points in the monthly menstrual cycle, when using birth control pills, during pregnancy, and at menopause. Many studies have shown that oral mucosa is sensitive to the effect of sex hormones (estrogen and progesterone). Thus there could be a direct link between changing hormonal status and oral health among females.[11,12]

The human being is an integrated organism with so many mechanisms working together to maintain a proper pH balance. pH measures the alkalinity or acidity of body and is the fundamental force for driving all the body functions. To survive, our bodies must maintain the pH very close to 7.4, which is just on the alkaline side of neutral. If our body's pH varies too much from this ideal, it becomes difficult for various enzymes to function properly. Maintaining this slightly alkaline state is a constant challenge, primarily because of the acid-forming functions that take place within the body, and the over-abundance of acid-producing foods we consume.[13]

The normal range for pH of saliva varies from 6.2-7.6 with 6.7 being the average pH. Resting pH of mouth should not fall below 6.3. In the oral cavity, saliva maintains the neutrality of pH(6.7-7.3). The saliva contributes to maintenance of the pH by two mechanisms. First, the flow of saliva eliminates carbohydrates that could be metabolized by bacteria and removes acids produced by bacteria. Second, acidity from drinks and foods, as well as from bacterial activity, is neutralized by the buffering activity of saliva.[2]

In relation to pH the outcome of the present study showed that salivary pH of the postmenopausal group is significantly lower than the control group and this result is similar to the results of Dural S et al.[8] It has been well documented that the dissolution of enamel occurs when the pH falls below critical pH i.e. 5.5[14] and the values obtained in the post menopausal group in our study were very near to critical pH value suggesting the probability for more enamel demineralization in this group. Therefore we can say that the importance of preventive dentistry increases with aging in female subjects. Also oral discomforts such as burning sensations have been strongly associated with the menopause which might be due to the hormonal alterations taking place at menopause.[15] Though the results were not statistically significant but the pH was reduced in

		N	Mean	Std. Deviation
PH	Control Group	20	6.6320	.84481
	Menstruating group	20	6.5570	.63363
	Pregnant group	20	6.4170	.78943
	Post menopausal group	20	5.9840	.52311
	Total	80	6.3975	.72724

Table-1: Descriptive Statistics of Salivary pH value in different study groups

			Mean difference	Std. Error	p-value
pH	Menstruating group	Control group	-.07500	.31718	.814
		Pregnant group	.14000	.31718	.662
		Postmenopausal group	.57300	.31718	.079
	Pregnant group	Control group	-.21500	.31718	.502
		Menstruating group	-.14000	.31718	.662
		Postmenopausal group	.43300	.31718	.181
	Postmenopausal group	Control group	-.64800*	.31718	.048
		Menstruating group	-.57300	.31718	.079
		Pregnant group	-.43300	.31718	.181

p-value <0.05 (Significant)

Table-2: Comparative analysis of pH in different groups (Post Hoc Test)

pregnant group as well. This could be explained on the basis that the inorganic and protein composition of saliva changes during the course of pregnancy. HCO_3^- present in salivary gland originates partly from plasma and partly from the salivary gland carbon dioxide. The reduction in pH value during pregnancy, is due to the effect of progesterone hormone, which is known to decrease plasma bicarbonate level during pregnancy resulting in a decreased pH and buffering capacity.[16]

The pH of saliva is an important component to maintain the integrity of oral cavity. The alkaline pH increases the remineralization of tooth surface because of the increased degree of supersaturation. The acidic pH can cause the maximum incidences of dental caries. It has been well documented that the dissolution of enamel occurs when the pH falls below critical pH i.e. 5.5. Thus salivary pH can be a potential indicator of determining dental caries.[17] Also Saliva can be used as an indicator of prognosis during periodontal treatment. It has been observed that there is a correlation between pH of saliva and periodontal diseases. In patients with chronic generalized periodontitis, the salivary pH is more acidic and this could be of diagnostic value.[2]

It has also been suggested that salivary pH is a significant correlate of plasma adiponectin levels in women. Adiponectin is an adipocyte-derived cytokine, or adipokine, that promotes for normal insulin sensitivity and fat clearance from tissues, along with maintaining a reduced inflammatory state. Although it is made by fat cells, adiponectin is actually found to be abnormally low in obese, insulin-resistant patients.[18] Lower levels of adiponectin associate with lower levels of salivary ph. Any systemic inflammation can cause reduction in the salivary flow rate. Reduced Salivary flow rate produces more acid and thus lower salivary ph. Conditions like Diabetes may dysregulate the cytokine profile and can cause secondary inflammatory changes in the body.. Diabetes is also known to cause salivary hypofunction and thus decreased salivary ph. Although the exact cause for this relationship is still to be unveiled. But certain explanations are being proposed relating to the changes in major salivary glands. The changes occurring in major salivary glands are similar to the one taking place in pancreas. These changes may diminish the response to normal salivary stimulus and cause salivary hypofunction. . Thus measuring salivary flow rate and ph in diabetic individuals might help us to reveal the underlying inflammatory state of the body as it is also one of the possible factors for hyposalivation in Type 2 Diabetes Mellitus.[19-21]

Alterations in salivary pH could be a marker of metabolic dysregulation and may be associated with several factors of the cardiometabolic risk profile. Measuring salivary ph is an inexpensive and simple procedure which can be performed in any clinical set up. It can thus open up another area of research to develop newer simple techniques and promote a wider use of salivary assays in future. The evaluation of various biomarker levels of diseases like Type 2 Diabetes Mellitus and Cardio vascular disease could be performed directly from oral fluids, deleting the need for blood sampling.[4,5] Hence the healthcare costs burden could be reduced while increasing the accessibility to screening tools.[4,5]

Psoriasis which is an inflammatory skin disease has been shortly associated with adverse cardiometabolic profile. A recent study has also shown the lower plasma levels of adiponectin and leptin in psoriasis. Salivary pH being a significant correlate of plasma adiponectin, can be used to substantiate the relationship between salivary pH and psoriasis and to assess whether salivary pH can be used as a biomarker of psoriasis.[22]

The oxidative stress has a significant role to play in the genesis of various cancers including brain tumors. As brain tissue has high rate of oxygen consumption it is highly sensitive to oxidative damage compared to the other tissues of the body. It has been observed that salivarypH is significantly less amongst the patients with brain tumor when compared to healthy controls. Thus salivary pH can be used as an appropriate indicator of antioxidant status in brain tumor patients.Markers of oxidative stress have to be repeatedly analysed during the treatment of brain tumors,in order to establish the exact role of oxidative stress and antioxidants in these patients. And as it is easier and inexpensive to collect saliva at rapid intervals, salivary estimation of these parameters can be helpful.[23]

The results of the present study showed significant changes in the ph of saliva in post-menopausal women as compared with the control group of regularly menstruating women. Previous studies have revealed that postmenopausal women often exhibit oral discomfort and have also shown a low salivary pH. These patients when put on hormone replacement therapy exhibit relief of symptoms. So ph could be non-invasive, easily accessible biomarker which can be used as an adjuvant indicator to start hormone replacement therapy.

CONCLUSION

Salivary ph as a biomarker when successfully validated in large population could potentially be helpful for de-

ciding start of hormone replacement therapy. Moreover being a positive correlate of cardiometabolic risk profile, salivary pH can be utilized as an inexpensive screening tool to improve preventive strategies to reduce burden of healthcare costs.

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