

# Evaluation of pH, buffering capacity, viscosity and flow rate levels of saliva in caries-free, minimal caries and nursing caries children: An *in vivo* study

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## Abstract

**Background and Aim:** The present study was undertaken to evaluate the pH, buffering capacity, viscosity and flow rate of saliva in caries free, minimal caries and nursing caries children and to evaluate the relationship of these on the caries activity of children. **Materials and Methods:** A total of 75 school children of age group between 4 and 12 years were selected and divided into three equal groups: Group I, Group II and Group III, consisting of 25 subjects each. Group I included caries-free subjects, Group II included subjects with minimal caries and Group III included subjects with nursing caries. Saliva samples were collected from all subjects and were estimated for flow rate, pH, buffering capacity and viscosity. **Results:** There was a significant decrease in the mean salivary flow rate, salivary pH and salivary buffer capacity and a significant increase in the salivary viscosity among caries-free subjects, subjects with minimal caries and subjects with nursing caries. **Conclusion:** The physicochemical properties of saliva, such as salivary flow rate, pH, buffering capacity and viscosity, has a relation with caries activity in children and act as markers of caries activity.

**Keywords:** Caries-free, minimal-caries, nursing-caries, salivary buffering capacity, salivary flow rate, salivary pH, salivary viscosity

## Introduction

Among the oral diseases, dental caries is the most common chronic disease of mankind.<sup>[1,2]</sup> It is the main oral health problem in industrialized countries.<sup>[3]</sup> Dental caries development is considered to involve a triad of indispensable factors that can be concluded as bacteria in dental plaque, carbohydrates in the diet and susceptible teeth.<sup>[4]</sup> It affects all people regardless of their sex, socioeconomic strata, race and age. It is also profoundly affected by other factors like oral hygiene and saliva.<sup>[1,2]</sup>

Saliva plays a very important role in oral health. Based on the constituents of saliva, it adopts properties such as lubrication,

clearance of unwanted substances, digestion, neutralization of acids or bases, protection against demineralization and also an antimicrobial role.<sup>[5]</sup> Theoretically, saliva affects the incidence of dental caries in four ways: (1) as a mechanical cleansing agent that results in less accumulation of plaque, (2) by reducing enamel solubility by means of calcium, phosphate and fluoride, (3) by buffering and neutralizing the acids produced by cariogenic organisms or introduced directly through diet and (4) by antibacterial activity.<sup>[6]</sup>

Saliva composition is an important factor in determining the prevalence of caries.<sup>[7]</sup> For relative protection against dental cavities, flow rate, buffer capacity, calcium, phosphate and fluoride concentrations are essentials.<sup>[5]</sup> The saliva circulating in the mouth at any given time is termed as whole saliva, and it comprises of a mixture of secretions from the major and minor salivary glands and traces from the gingival crevicular fluid.<sup>[8]</sup> Saliva definitely promotes oral health and hence lack of its secretion contributes to the disease process. The saliva, by constantly bathing the teeth and oral mucosa, functions as a cleansing solution, a lubricant, a buffer and an ion reservoir of calcium and phosphate, which are essential for the remineralization of initial carious lesions.<sup>[1,2]</sup>

Saliva maintains the integrity of oral hard and soft tissues and protects against immunologic bacterial, fungal and viral infections.<sup>[1]</sup> Saliva controls the equilibrium between demineralization and remineralization in a cariogenic environment. Salivary buffers can reverse the low pH in plaque and allow for oral clearance thus preventing demineralization of enamel. The flow rate and viscosity of saliva may also influence the development of caries.<sup>[4]</sup>

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The purpose of this study was to evaluate the relationship between the physicochemical properties of saliva such as flow rate, pH, buffering capacity and viscosity in caries-free,<sup>[1,2,7]</sup> minimal caries<sup>[9]</sup> and nursing caries children.<sup>[7]</sup>

## Materials and Methods

The proposed study was carried out on patients between the ages of 4 and 12 years. The inclusion criteria were: (1) children should be free from systemic or local diseases that affect salivary gland secretions (such as submandibular duct canaliculi, asthma and diabetes) and (2) children should be permanent residents of Khammam and should be consuming only municipal water (those consuming hard water were not included as hard water consumption predisposes to dental fluorosis). The exclusion criteria were: (1) children who were physically and medically compromised, (2) children who were on medications (antipyretic drugs, bronchodilators, multivitamin syrups; sweet syrups accumulate over teeth and, if not rinsed properly, predispose to bacterial infection and, later, dental caries), (3) children who had arrested carious lesions (children who had taken measures such as use of fluoride toothpaste, improved oral hygiene to combat caries process were excluded) and (4) children who had undergone previous dental treatment, particularly any restorations, fluoride applications, etc.

### Study design

A total of 75 school children of age group between 4 and 12 years were selected. The decayed-missing-filled surface (DMFS) index was calculated for all the subjects. The subjects were divided into three equal groups: Group I, Group II and Group III, consisting of 25 subjects each [Table 1].

**Table 1: Study design**

Group (n=25)	Group name	Group description
I	Caries-free subjects	Caries-free children, having no caries; DMFS=0
II	Subjects with minimal caries	Children having mandibular first permanent molar decayed
III	Subjects with nursing caries	According to the AAPD, the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth in a child of 71 months of age or younger. From ages 3 through 5, one or more cavitated, missing or filled smooth surfaces in the primary maxillary anterior tooth or a decayed, missing or filled score of greater than or equal to 4 in age 3, greater than or equal to 5 in age 4, greater than or equal to 6 in age 5 constitutes S-ECC

DMFS: Decayed-missing-filled surface; AAPD: American academy of pediatric dentistry; ECC: Early childhood caries

### Collection of saliva

Twelve milliliters of unstimulated whole saliva was collected for the study. Sample collection was carried out in the day time between 10 am and 12 pm, 2 h after breakfast. Before collection, the subjects were made to rinse their mouth with distilled water after 15 min. Then, the children were made to sit comfortably in a ventilated and well-illuminated room, and were instructed to expectorate saliva, which was collected for exactly 5 min in a pre-weighed graduated cylinder. A note was made of this value. Saliva collection was then continued till 12 mL of saliva was accumulated in the cylinder.

### Analysis of saliva

All collected samples of saliva were estimated for flow rate, pH, buffering capacity and viscosity. The salivary flow rate was obtained from the volume of saliva collected in the initial 5 min of saliva collection.<sup>[1,2]</sup> The “Saliva-Check Buffer Testing Mat” (GC Dental Products Corp., Kasugai City, Aichi, Japan) was used to estimate the pH [Figure 1] and buffering capacity [Figure 2] of saliva.<sup>[1,2]</sup> The relative viscosity of saliva with respect to water was measured using the “Ostwald’s Viscometer” [Figure 3].<sup>[10-12]</sup>

### Statistical analysis

The results obtained were subjected to statistical analysis. The mean values for flow rate, pH, buffering capacity and viscosity of saliva for Groups I, II and III were analyzed using the “Statistical Package for the Social Sciences” (SPSS) software, version 16.0. One-way ANOVA with *post hoc* Tukey’s test was performed. *Post hoc* analysis was performed to evaluate significant intergroup differences. A  $P < 0.05$  was considered as statistically “significant” and a  $P < 0.001$  was considered as statistically “highly significant.”

## Results

The results obtained are summarized in Table 2.

## Discussion

Dental caries is the most common chronic oral disease of mankind. It affects all people regardless of their sex, socioeconomic strata, race and age. It is also profoundly affected by other factors like oral hygiene and saliva.<sup>[1,2]</sup> Despite advancements in oral disease science, dental caries continues to be a worldwide health concern affecting humans of all ages, especially children, where caries disease is on the rise. Dental caries is an infectious bacterial biofilm disease that is expressed in a predominantly pathologic oral environment.<sup>[13]</sup> Early childhood caries (ECC) is a recent term that describes rampant dental caries in children. Terms describing this affliction have evolved during the last 20 years, and include names like nursing caries, nursing bottle caries and baby bottle caries.<sup>[14]</sup> According to the American Academy of Pediatric Dentistry (AAPD), the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due

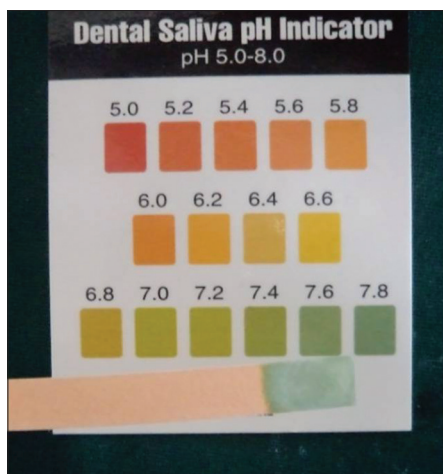


Figure 1: Estimation of salivary pH

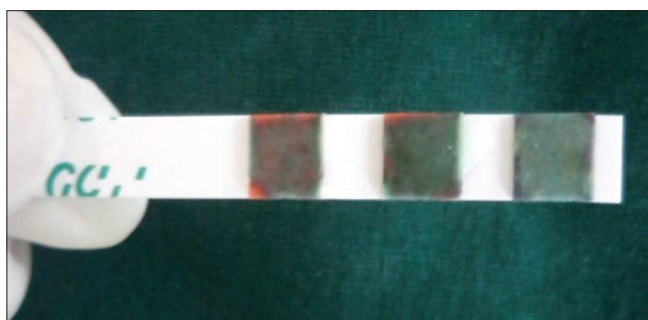


Figure 2: Estimation of salivary buffering capacity

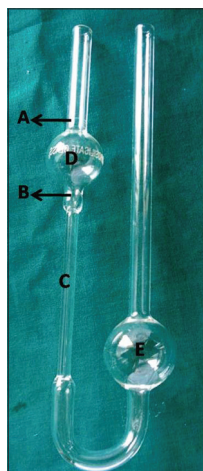


Figure 3: Ostwald's viscometer

to caries) or filled tooth surfaces in any primary tooth in a child of 71 months of age or younger is described as ECC.

Human saliva is a mouth fluid that has several functions involved in oral health and homeostasis, with an active protective role in maintaining oral health.<sup>[15]</sup> Saliva helps bolus formation by moistening food, protects the oral mucosa against mechanical damage and plays a role in the preliminary digestion of food through the presence of  $\alpha$ -amylase and

Table 2: Summary of the results obtained

Parameter	Group	N	Mean $\pm$ SD	P value	Post hoc test
pH	I	25	7.42 $\pm$ 0.23	<0.001	1>2
	II	25	7.29 $\pm$ 0.24		1>3
	III	25	6.40 $\pm$ 0.38		
Buffering capacity	I	25	10.28 $\pm$ 1.28	<0.001	1>2
	II	25	9.04 $\pm$ 1.40		1>3
	III	25	7.04 $\pm$ 0.79		
Viscosity	I	25	1.01 $\pm$ 0.05	<0.001	1<2<3
	II	25	1.18 $\pm$ 0.13		
	III	25	1.21 $\pm$ 0.26		
Flow rate	I	25	0.43 $\pm$ 0.09	<0.001	1>2>3
	II	25	0.29 $\pm$ 0.08		
	III	25	0.19 $\pm$ 0.02		

SD: Standard deviation

other enzymes. It also facilitates taste perception and also has a role in maintaining teeth enamel mineralization.<sup>[15,16]</sup>

Dental caries has been thought of as a multifactorial disease as it is not only influenced by dietary factors but host factors as well.<sup>[15]</sup> In addition, the role of saliva as a defense system against dental caries is well documented. These defense systems include clearance, buffering, antimicrobial agents and calcium and phosphate delivery for remineralization, to name a few.<sup>[17]</sup> The interaction of protective and pathologic factors in saliva and plaque biofilm, as well as the balance between the cariogenic and non-cariogenic microbial populations that reside in saliva, decides the caries process.<sup>[10]</sup> The factors in saliva most frequently related to dental caries are: (a) aciduric/acidogenic bacteria and (b) rate of acid production in the presence of glucose. Other factors that have been suggested as being related to dental caries include (a) amount of saliva secreted in a given time and (b) acid-neutralizing ability (buffering capacity) of saliva.<sup>[18]</sup>

### Salivary pH

There was a significant difference in the mean salivary pH among the study groups ( $P < 0.001$ ). Group I had a significantly higher mean salivary pH value than that of Groups II and III. However, no significant difference was seen between the mean salivary pH values of Group II and Group III. The results obtained are in accordance with the studies performed by Prabhakar *et al.* in 2009<sup>[2]</sup> and Preethi *et al.* in 2010.<sup>[1]</sup> However, the results obtained in their studies were not significant. The salivary pH was only slightly reduced in caries-active children compared with caries-free children. Another study by Zhou *et al.* in 2007<sup>[19]</sup> showed that the pH of saliva from early childhood caries children was statistically higher than that in caries-free children. In contrast, a study carried out by Thaweboon *et al.* in 2008<sup>[7]</sup> revealed that the mean values for salivary pH were similar in caries-free and rampant-caries children. Swerdlove in 1942<sup>[20]</sup> and

Malekipour *et al.* in 2008<sup>[21]</sup> reported no relationship between the incidence of dental caries and the pH of normal resting saliva. Lamberts *et al.* in 1983<sup>[22]</sup> observed no relationship of salivary pH rise activity and caries experience in caries-free and caries-active subjects.

### Salivary buffering capacity

There was a significant difference in the mean salivary buffering capacity among the study groups ( $P < 0.001$ ). Group I had a significantly higher mean salivary buffering capacity than that of Groups II and III. However, no significant difference was seen between the mean salivary buffering capacities of Group II and Group III. The results obtained are in accordance with the studies performed by Prabhakar *et al.* in 2009<sup>[2]</sup> and Preethi *et al.* in 2010.<sup>[1]</sup> However, the results obtained in their studies were not significant. The salivary buffering capacity was only slightly reduced in caries-active children compared with caries-free children. Another study by Zhou *et al.* in 2007<sup>[19]</sup> showed that the buffering capacity of saliva from early childhood caries children was statistically higher than that in caries-free children. A study performed by Malekipour *et al.* in 2008<sup>[21]</sup> showed similar results, although the difference was not statistically significant.

### Salivary viscosity

There was a significant difference in the mean salivary viscosity among the study groups ( $P < 0.001$ ). Group III had a significantly higher mean salivary viscosity than that of Groups II and I. Similarly, Group II had a significantly higher mean salivary viscosity than Group I. Abou El-Yazeed *et al.* in 2009<sup>[4]</sup> reported a mean salivary viscosity of  $1.13 \pm 0.05$  in a group of children aged 8–14 years, which is similar to that obtained in the present study.

### Salivary flow rate

There was a significant difference in the mean salivary flow rate among the study groups ( $P < 0.001$ ). Group I had a significantly higher mean salivary flow rate than that of Groups II and III. Similarly, Group II had a significantly higher mean salivary flow rate than Group III. Lopez *et al.* in 2003<sup>[23]</sup> reported a salivary flow rate of  $0.27 \pm 0.14$  in a group of children aged 5-12 years. The results obtained are in accordance with the studies carried out by Preethi *et al.* in 2010<sup>[1]</sup> and Prabhakar *et al.* in 2009.<sup>[2]</sup> However, the results obtained in their studies were not statistically significant. The salivary flow rate was only slightly reduced in caries-active children compared with caries-free children. In contrast, a study performed by Thaweboon *et al.* in 2008<sup>[7]</sup> revealed that the mean values for salivary flow rate were similar in caries-free and rampant-caries children. The salivary flow rate did not influence the presence of rampant caries.

### Conclusion

It was established in the present study that pH, buffering capacity, viscosity and flow rate of saliva has a definite relation

with caries activity in children. The results indicate that there is a significant decrease in the mean salivary flow rate, salivary pH and salivary buffer capacity and a significant increase in the salivary viscosity among caries-free subjects, subjects with minimal caries and subjects with nursing caries. These results re-emphasize the importance of the various physicochemical properties of saliva, such as salivary flow rate, pH, buffering capacity and viscosity, which act as markers of caries activity. But, in order to extrapolate the findings of this study, studies involving a larger sample size are required.

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