

Clinical Usefulness of pH Papers in the Measurement of Salivary pH

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Purpose: Salivary pH is an easily measurable biochemical marker and related to various intra-oral and systemic conditions. The aim of this study was to evaluate the reliability of the salivary pH measurement using pH paper. In addition, the normal values of salivary pH using pH paper were compared to those of pH meter to investigate the validity.

Methods: Twenty healthy male participants attended this study (mean age, 24.5±1.47 years). Unstimulated saliva and stimulated saliva were collected from each subject two times with the interval of a day and salivary pH was immediately measured by the two experienced examiners using pH paper and pH meter. The salivary pH was compared between the groups and inter- and intra-examiner reliability of pH paper was investigated. The intraclass correlation coefficient (ICC) was used to calculate variations.

Results: All measurements had good to excellent inter-examiner (ICC 0.755 for unstimulated; 0.760 for stimulated saliva), intra-examiner (ICC 0.635 for unstimulated; 0.592 for stimulated saliva) reliability and two measurement methods using pH paper and pH meter also showed high reliability (ICC 0.852 for unstimulated; 0.640 for stimulated saliva). The values measured by pH paper were significantly lower than those measured by pH meter.

Conclusions: pH paper showed adequate inter- and intra-examiner reliability and it presented the validity in terms of comparison with the pH meter as a standard in the salivary pH measurement.

Key Words: pH; pH paper; Reliability; Saliva

INTRODUCTION

Saliva has been known as a very useful diagnostic bio-fluid which contains most of the molecules found in serum and other bodily fluids such as electrolytes, proteins, and hormones.¹⁾ Additionally, collecting saliva has a variety of advantages; it is a readily available, non-invasive, inexpensive method, and easy to apply various diagnostic methods.²⁻⁴⁾ Salivary pH, one of these diagnostic methods, is an easily measurable biochemical marker and related to various intraoral and systemic conditions.¹⁾ Therefore, salivary pH measurement is expected to provide useful diagnostic information.²⁻⁶⁾

In the field of dentistry, salivary pH is helpful in evaluating

risk of dental caries⁵⁾ and it is associated with periodontal diseases and oral infection due to its close correlations with oral microflora.^{2,7)} It is also reported that increased salivary pH with bacterial infection can facilitate the salivary stone formation.⁸⁾ In addition to oral diseases, many studies have found that salivary pH is also related to various systemic conditions such as hypertension,^{5,9)} cardiovascular disease,⁵⁾ insulin-resistance,^{3,5)} renal disease,¹⁰⁾ airway response,¹¹⁾ HIV infection,¹²⁾ acute mental stress,¹³⁾ and depression.⁴⁾ For these reasons, some authors proposed salivary pH as a non-invasive quick chairside diagnostic biomarker not only for obesity, insulin resistance, hypertension and cardiovascular disease⁵⁾ but also for the assessment of psychological symptoms like stress and depression with enough availability

and reliability.^{4,6)}

Salivary pH can be measured by pH paper or pH meter.¹⁴⁾ Despite the current status of pH meter as a gold standard in the pH measurement, some problems limit the clinical access to use it.¹⁵⁾ First, pH meter is relatively expensive, and needs regular calibration, test solution, and user training. Second, it could be challenging to use ordinary pH meter in the patients with xerostomia because most portable pH meters require at least 5 mL liquid sample.¹⁶⁾ Moreover, the tip of its electrode is susceptible to damage by organic solvent. Therefore, disinfection with alcohol or saliva sample itself could shorten the lifespan of pH meter.

Using pH paper is another quick, inexpensive and convenient method for measuring pH.¹⁴⁾ However, studies on the reproducibility of pH paper is few and reliability should be taken into consideration prior to discussing the clinical usefulness of pH paper. Besides, researches to determine whether pH paper is an alternative to pH meter for salivary pH measurement should be performed because there are only a few researches for saliva while there have been a number of studies for other bodily fluids.^{14,15)} Therefore, the aim of this study was to evaluate the reliability of the salivary pH measurement using pH paper. The normal values of salivary pH using pH paper were compared to those of pH meter to investigate the validity.

MATERIALS AND METHODS

1. Participants

The participants consisted of 20 healthy Korean male volunteers including students in the third and fourth grade of College of Dentistry, Dankook University, Cheonan, Korea. Their age ranged 23 to 27 years (mean age, 24.5 ± 1.47 years).

Individuals with history of smoking, taking medicine or both oral and systemic disease were excluded because salivary pH may be influenced by these factors.¹⁶⁾ Prior to examination, enough information was given to all the participants and they consented to this study.

2. Saliva Sample Collection

The participants were instructed to avoid eating and drinking at least 90 minutes before saliva collection, and to keep themselves from oral movement like talking or swallowing for 5 minutes. Collection of saliva samples requires generally to be performed in the morning to minimize the effect of daily variation, which was difficult to be followed due to the schedule of the participants in this study. Therefore, saliva samples for each participant were obtained at the same time in the afternoon on the subsequent two days.

Spitting method was adopted to collect saliva samples. At first, participants were asked to spit out unstimulated saliva into a conical tube for 5 minutes. And then, after chewing a piece of sugar-free xylitol gum for 1 minute, stimulated saliva was collected for 5 minutes into another conical tube.

3. Salivary pH Measurement

Firstly, for the test of inter-observer reliability, each saliva sample was immediately tested by two experienced examiners. The salivary pH was first measured by pH paper. pH indicating paper (Macherey-Nagel GmbH & Co. KG, Düren, Germany) with the resolution within 0.2 pH units (Fig. 1A) was used in the current study. Bromothymol blue (BTB) was chosen as pH indicator and the color of pH paper would change from yellow to blue. Droplets of randomly selected saliva were dropped onto a disinfected stainless-steel plate

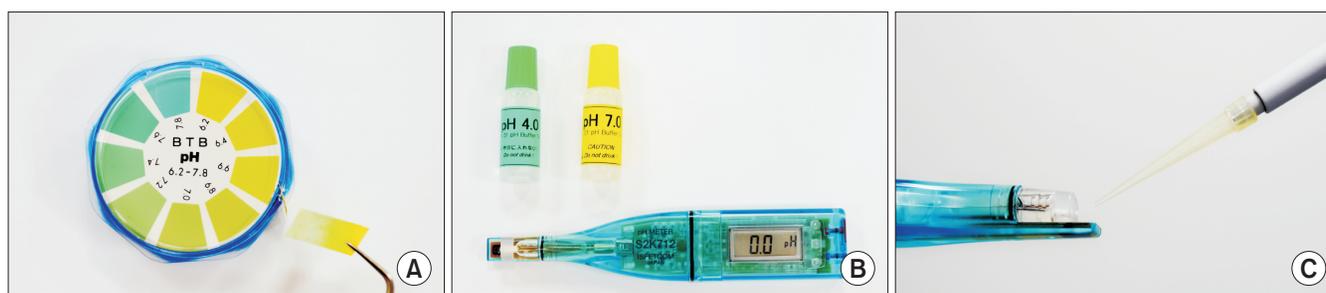


Fig. 1. The pH paper (A; Macherey-Nagel GmbH & Co. KG), pH meter (B; The Lab Depot Inc.), and its tip (C) used in this study.

with a micropipette. An examiner placed a piece of pH paper onto the droplet for one second and its color transition was immediately compared against the color-coded chart by the manufacturer. And then, this procedure was duplicated by the other examiner. No communications between the examiners was allowed during all tests.

Secondly, one examiner subsequently measured the same saliva sample with a micro portable pH meter (S2k712; The Lab Depot Inc., Dawsonville, GA, USA) to compare the pH values between pH paper and pH meter as a gold standard of salivary pH measurement (Fig. 1B). The resolution and accuracy of this pH meter is within 0.1 pH unit. The pH meter used in this study can detect as small as 0.05 mL of saliva and the pH value is displayed digitally. Two-point calibration using pH standard solutions (pH 7.0, pH 4.0) was done prior to each test. A drop of the sample was applied on the tip of the pH meter (Fig. 1C) and the digital value was recorded by a single examiner.

Lastly, for the intra-observer reliability, the procedures mentioned above were duplicated by one examiner on another day with the interval of one day. All the procedures were performed for unstimulated and stimulated saliva samples.

4. Statistical Analysis

Simple descriptive statistics were used for the calculation of means and standard deviations of salivary pH measurements. To estimate the reliability of quantitative variables, the inter-examiner, intra-examiner variations and reproducibility between two measurement methods were analyzed using the intraclass correlation coefficient (ICC), which takes a value from zero (no agreement) to 1 (perfect agreement). The ICC is often judged as providing agreement which is: poor if $ICC < 0.4$; fair if $0.4 \leq ICC < 0.6$; good if $0.6 \leq ICC < 0.75$; excellent if $ICC \geq 0.75$. The differences between the measurements using the pH meter and pH paper were calculated using the paired t-test. IBM SPSS Statistics version 20.0 for Window (IBM Co., Armonk, NY, USA) was used for statistical analysis and significant level was set at $p=0.05$.

RESULTS

A total number of 80 saliva samples were collected from

20 young healthy male participants in this study. Their salivary pH ranged from pH 7.0 in the unstimulated saliva to pH 7.7 in the stimulated saliva. The average pH was pH 7.23 ± 0.13 for the unstimulated saliva, and pH 7.39 ± 0.16 for the stimulated saliva.

1. Inter-examiner Reliability

Table 1 indicates the average salivary pH values measured by the two examiners using pH paper at the first measurement. The ICC showed high agreement between two examiners in the measurement of unstimulated saliva ($ICC=0.755$, $p=0.002$). For the stimulated saliva, high ICC was also obtained ($ICC=0.760$, $p=0.002$).

2. Intra-examiner Reliability

Table 2 shows the result of salivary pH measured by a single examiner using pH paper at first and second measurements. The estimate of the ICCs presented good agreements in the repeatability of the pH measurements ($ICC=0.635$, $p=0.017$ for the unstimulated saliva; $ICC=0.592$, $p=0.029$ for the stimulated saliva).

3. Comparison between pH Meter and pH Paper

Table 3 presented the pH values of unstimulated and stimulated saliva measured by a single examiner using pH paper and pH meter at the first measurement. The pH values measured by pH paper were significantly lower than

Table 1. Inter-examiner reliability of pH paper (n=20)

Saliva	Examiner 1	Examiner 2	ICC	p-value ^a
Unstimulated	7.13±0.11	7.12±0.11	0.755	0.002
Stimulated	7.22±0.11	7.23±0.13	0.760	0.002

ICC, intraclass correlation coefficient.

Salivary pH values are presented as mean ± standard deviation.

Measurement of salivary pH was done at the 1st experiment.

^aStatistical significance from intraclass correlation analysis.

Table 2. Intra-examiner reliability of pH paper (n=20)

Saliva	Test	Retest	ICC	p-value ^a
Unstimulated	7.13±0.11	7.09±0.18	0.635	0.017
Stimulated	7.22±0.11	7.28±0.15	0.592	0.029

ICC, intraclass correlation coefficient.

Salivary pH values are presented as mean ± standard deviation.

The retest was performed on a day after the test.

^aStatistical significance from intraclass correlation analysis.

Table 3. Comparison of salivary pH between the measurements using pH meter and pH paper (n=20)

Saliva	pH meter	pH paper	ICC	p-value ^a	p-value ^b
Unstimulated	7.23±0.13	7.13±0.11	0.852	0.001	p<0.001
Stimulated	7.35±0.13	7.22±0.11	0.640	0.016	p<0.001

ICC, intraclass correlation coefficient.

Salivary pH values are presented as mean±standard deviation.

^aStatistical significance from the intraclass correlation analysis.

^bStatistical significance from the paired t-test.

those by pH meter in both unstimulated saliva (paired t-test, $p<0.001$) and stimulated saliva (paired t-test, $p<0.001$). The measurements between two methods showed an excellent agreement of 85.2% for the unstimulated saliva (ICC=0.852, $p=0.001$). The stimulated saliva exhibited also a good agreement between two tests (ICC=0.640, $p=0.016$).

DISCUSSION

The current study investigated the reliability of pH paper in the measurement of salivary pH, and pH paper demonstrated high levels of inter-examiner (Table 1) and intra-examiner reliability (Table 2). These results indicate that pH paper, as an instrument of salivary pH measurement, has the sufficient reproducibility and could be regarded as valid in the clinics. Consistent with this study, studies as to measurement of urine, vaginal and intragastric pH using pH paper presented high levels of test-retest and intra-observers reliability.¹⁷⁻¹⁹⁾

Portable pH meter in this study was regarded as reference method without assessing reliability. Portable pH meter is widely used due to its accuracy and convenience although benchtop pH meter is generally considered to be gold standard in pH measurement.²⁰⁾ In the studies comparing accuracy and reproducibility between benchtop pH meter and portable pH meters, portable pH meters had nearly perfect agreement with the benchtop pH meter.^{20,21)}

In the current study, the salivary pH measurements using pH paper showed adequate agreement with those using pH meter. However, pH paper exhibited lower pH values compared to those by pH meter (paired t-test, $p<0.001$; Table 3). Previous studies also reported about this disagreement between pH paper and pH meter in the pH measurement of other bodily fluids including gastric acid, urine, vaginal and

pleural fluid.^{14,15,22-24)} There are two reasons for this. First, validity of measurement with pH paper can be influenced by several factors such as color blindness of the reader, environmental lighting, and the presence of blood, chemical factors and proteins reacting with pH paper.^{18,25)} Second, the result of pH paper is susceptible to imprecise readings due to the subjective determination of color change.

The pH difference in this study ranged from 0.10 pH unit (unstimulated saliva) to 0.13 pH unit (stimulated saliva). In some studies, the pH difference of <0.5 was considered as clinically insignificant because the resolution of dipstick they used was 0.5 pH unit.^{14,18)} Therefore, pH difference of the current study is thought to be within clinical acceptance limit because it was almost the same as the resolution of pH meter (0.1 pH unit) or lower than that of pH paper (0.2 pH unit).

There were differences between unstimulated saliva and stimulated saliva. The ICC of stimulated saliva was lesser than that of unstimulated saliva in the intra-examiner reliability and comparison between two measurement methods using pH paper and pH meter. The inter-examiner reliability did not show different ICCs between unstimulated and stimulated saliva. Although it cannot be fully explained yet, several hypotheses could be conjectured. First, the different compositions between unstimulated and stimulated saliva might cause the difference between pH paper and pH meter. Stimulated saliva, unlike unstimulated saliva, is less affected by resorption and secretion of the salivary ductal system; thus, ionic concentration and the composition is different from unstimulated saliva.²⁵⁾ Since inorganic and organic compounds in saliva, not hydrogen ion, can react with pH indicator, the different compositions between unstimulated saliva and stimulated saliva could affect color change of pH paper.²⁴⁾ Second, wide variations of the pH

value of stimulated saliva could influence on the result. The concentrates of saliva have wide variations both between individuals and even within an individual.²⁾ Particularly, pH of stimulated saliva is sensitive to various stimuli such as diet, exercise and innate metabolic differences.²⁶⁾ In this study, time interval of one day between tests might cause pH difference between measurements. In addition, the sampling was not done in the morning, and this daily variation also could contribute to the result.

As a result, unstimulated saliva, rather than stimulated saliva which is relatively unstable in pH, is recommended for salivary pH measurement by using pH paper. This outcome is supported by the study of Tremblay et al.,⁵⁾ i.e., they also chose unstimulated saliva as a sample for salivary pH measurement because it is the greatest contributor to the total salivary output and it covers oral mucosa in much of the time.

The limitations of this study should be mentioned. First, since different indicators of pH paper may have different range of optimal pH and dissimilar results, the result in the current study using only one kind of pH paper cannot be generalized for all pH papers. The BTB as an indicator in the current pH paper can cover the normal range of physiological salivary pH (pH 6.4-7.4) in terms that the optimal pH range of BTB is pH 6.0-7.6.²⁵⁾ This indicates that the pH paper used in this study might not be applicable in the pathologic condition beyond the normal range of salivary pH. Therefore, further study should be tested with various types of pH paper and also in the patients with problems in the salivary condition. Second, we used different saliva samples from each subjects in the time interval of one day to evaluate the intra-examiner reliability. Strictly speaking, the same sample should be used for the assessing the reliability of measuring methods. However, due to the possible pH change of collected saliva as time passes, we tested the different but fresh saliva in the same time with the gap of one day in the evidence that healthy subjects show circadian rhythms in salivary pH.²⁷⁾

Conclusively, pH paper, as a simple and quick method, has proven to be reliable for measuring salivary pH and high reliability of pH paper will make a clinical test more sensitive and useful.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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