



Chromatography paper: A novel approach to estimate the salivary flow

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Abstract

Background: To evaluate a simple and safe method to estimate the salivary flow using chromatography paper.

Materials and Methods: A total of 30 volunteers participated in the study. The salivary flow rate was estimated using chromatography filter paper and compared with the salivary flow rate per minute. The assay consisted of 3 spots containing starch and potassium iodide per spot on filter paper. Based on the color reaction on the chromatography filter paper the salivary flow rate was estimated.

Results: Pearson's correlation test showed highly significant negative correlation when the salivary flow rate and the number of colored dots were compared.

Conclusion: This method can be used as a chair side diagnostic aid in the estimation of salivary flow.

Introduction

Quantitative and/or qualitative alterations in salivary secretion may lead to local adverse effects like caries, oral mucositis, candidiasis, oral infections, chewing disorders, halitosis and functional adverse effects such as dysphagia, hypersalivation (sialorrhoea) and hyposalivation (xerostomia).^[1]

Whole saliva (mixed saliva) is a mixture of oral fluids that includes secretions from both the major and minor salivary glands, in addition to several constituents of non-salivary origin, such as gingival crevicular fluid, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components, and food debris.^[2]

Stimulated saliva is produced on account of some mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around 80-90% of daily salivary production.

In adults, the total stimulated salivary flow ranges from 1 to 3 ml/min^[3] whereas the un-stimulated salivary flow at rest ranges from 0.25 to 0.35 ml/min.^[4]

Un-stimulated salivary flow rate is most affected by the degree of hydration, olfactory stimulation, exposure to light, body positioning, and seasonal and diurnal factors.

There are various methods for the estimation of salivary flow including measurement of glandular salivary flow, resting salivary flow and stimulated salivary flow.

The best two ways to collect whole saliva are the draining method, in which saliva is allowed to drip off the lower lip, and the spitting method, in which the subject expectorates saliva into a test tube.^[2]

The above methods are time consuming and needs special apparatus like collection tubes or volume meter.

Materials and Methods

The study consisted of 30 volunteers comprising of 21 females and 9 males, aged 19-25 years. The subjects were asked to sit in an upright position with their head bent in a downward position and were asked to collect the saliva in their mouth for 1 min and asked to spit into the collecting test tubes at the end of 1 min to determine the salivary flow rate/min.

Method of preparation of filter paper

The filter paper for the estimation of salivary flow was prepared according to the method given by Takashi *et al.* using soluble starch, potassium iodide and chromatography paper (70 mm × 21 mm).

4 µl of the detection reagent (1% starch solution and 0.3 mol/L potassium iodide solution mixed at a ratio of 3:1) was placed at 3 places on the chromatography paper using a micropipette.

The filter paper was left overnight in a cool dark room and then stored in a light-resistant container until use.

The soluble starch and potassium iodide were dissolved in 0.1 mol/L Tris-HCl buffer the adjusted pH of 7.3.^[5]

Method of preparation of coloring reagent

The coloring reagent was prepared from a solution of 31% hydrogen peroxide, ethanol and distilled water at a ratio of 1:7:1.

Method of collection of saliva

Subjects were asked to open their mouths and then to lift the tongue.

The tip of the paper was put on the center of the floor of the mouth in the sublingual region.

After 1 min, the filter paper was taken out and then the coloring reagent (approximately 2 µL) was added dropwise to the spots in the filter paper.

Via the iodine-starch reaction, colorless spots immediately turned blue as represented in Figures 1-3 (salivary flow rate of 1 ml).

The number of blue spots, including partly colored spots (50%), was grossly counted as an integer.

Based on this we examined the relationship between the number of colored spots and salivary flow rates after insertion of filter paper for 1 min in the sublingual regions of healthy subjects.^[5] The results obtained are shown in Tables 1 and 2.

Results

The results are shown in Tables 1 and 2. Table 1 shows the number of coloured spots and salivary flow rates. The correlation between the coloured spots and flow rates were assessed by a Pearsons test and a highly significant correlation between the two parameters was obtained [Table 2].

Table 1: Number of coloured spots and resting salivary flow rate

Number of subjects	Number of colored spots	Resting salivary flow/min
12	1	3 ml
17	2	2 ml
1	3	1 ml

Table 2: Pearson's correlation test

Flow of saliva	Total number of individuals	Number of colored spots	r value	P value	Significance
1 ml	1	3	-1.000	0.000	HS
2 ml	17	2			
3 ml	12	1			

P<0.05, HS: Highly significant. Pearson's correlation test was done to determine the correlation between salivary flow rate and the number of dots. There was a negative correlation seen (r=-1)



Figure 1: Colored dot showing salivary flow rate of 3 ml



Figure 2: Colored dot showing salivary flow rate of 2 ml



Figure 3: Colored dot showing salivary flow rate of 1 ml

Discussion

Common methods for collecting whole saliva include draining, spitting, suction and swab (absorbent) method. In draining method, saliva is allowed to drip off the lower lip into a pre-weighted container or graduated test tube. In a spitting method of collection, the saliva is allowed to collect in the floor of the mouth and subjects are asked to spit in the collecting tubes whereas, in suction method, saliva is continuously aspirated from the floor of the mouth into graduated test tubes. In absorbent method pre-weighted cotton rolls, swabs, or gauze were inserted into the opening of the ductal orifices of the salivary glands and reweighed after the collection is completed. The suction and swab method causes some degree of stimulation and variability and thus are not recommended for un-stimulated salivary collection whereas swab method is said to be least reliable among the above mentioned methods.^[6] Other disadvantages of these methods are that they are time consuming and need special apparatus like collection tubes or volume meter.

Takashi *et al.* proposed the present method the present assay for salivary flow assessment. The assay system consists of 3 spots 1 mm apart containing starch and potassium iodide on the filter

paper. Potassium iodide in the spots easily gets displaced with the flow of saliva, in contrast to this starch does not get displaced and gets retained in the original spot. Thus, the colorless spots on the paper not infiltrated with saliva immediately turned blue with the addition of the coloring reagent that contained hydrogen peroxide, whereas the spots on the paper infiltrated with saliva does not show the color. Colored spot is based on the reaction of saliva between potassium iodide and starch in the chromatography paper and the color reaction of iodine-starch with hydrogen peroxide.^[5]

Un-stimulated saliva reflects the basal salivary flow rate while stimulated saliva represents the functional reserve of the salivary glands. So the study of un-stimulated saliva is useful for the study of the salivary gland status.^[1] The present method can be of help in case of elderly patients suffering from xerostomia, in screening tests, post-radiation therapy for cancer in elderly patients where salivary flow measurement can play a chair side diagnostic test.

In addition, the method can easily be adjusted to variations in cutoff values and accuracy by changing the number of spots and distance between the spots on the filter paper. With the present method, Good Correlation was obtained [Table 2] between the salivary flow rate and the colored spots with an $r = -1$ and $P = 0.0$ in healthy individuals. Thus, the routine use of this method as a chair side assessment assay for salivary flow would be of benefit to all practicing dentists and patients alike.

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