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## Measuring fluoride in human saliva and water

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### Abstract

**Background and objective:** Fluoride is used to prevent dental caries, and it has been one of the most effective and widespread agents. Fluoride level has been shown in saliva, plaque, and oral soft tissues after use of fluoridated toothpaste that persists at potentially active concentrations for hours. This study aimed to evaluate how fluoride in toothpaste intake can affect the salivary fluoride and test fluoride in tap water in the different places of Erbil city.

**Methods:** Forty five volunteers were examined. In the morning after overnight fasting before brushing teeth, the saliva of the subjects (healthy non-smoker adults) was taken before brushing teeth, which was a baseline. After 10 and 20 minutes of washing mouth with water (after brushing), saliva was taken and then the cotton pad was held under the tongue for 5 min. Saliva samples were frozen at -20 C° for later analysis. Tap water samples in different places in Erbil city were analyzed.

**Results:** Toothpaste significantly ( $P = 0.002$ ) increased salivary fluoride after brushing teeth. The fluoride concentration of tap water in Erbil city was very low at the places that use groundwater.

**Conclusion:** This research indicates that salivary fluoride significantly increases after brushing teeth. The fluoride concentration naturally occurring levels in the tap water in the places where the source is groundwater are not enough.

**Keywords:** Fluoride; Dental caries; Saliva.

### Introduction

Fluorine is a common element because of its high reactivity does not occur in the elemental state in nature. It has been reported that the total fluoride concentration in seawater 1.3 mg/liter.<sup>1</sup> In places rich in fluoride-containing minerals, well water may contain up to about 10 mg/liter of fluoride. The highest natural level reported is 2800 mg/liter. Fluorides may go to a river as a result of industrial discharges.<sup>1</sup> Human saliva is a mouth fluid over 99.5% being made up of water, which has many functions. It is involved in homeostasis and has a vital role in maintaining oral health.<sup>2,3</sup> In humans, salivary glands can be divided into two main types, the major and minor salivary

glands. The major salivary glands are parotid, sublingual, and submandibular. Minor salivary glands are the von Ebner glands and Blandin-Nuhn mucous glands.<sup>4</sup> The composition of saliva varies according to the mucous or serous component of the glands.<sup>5</sup> The relative contribution from each type of gland to the total unstimulated saliva secretion varies as follows, 65% for submandibular, 23% parotid, 8% von Ebner, and 4% sublingual.<sup>6</sup> Healthy adult subjects produce approximately 0.5 ml/min, which is 500- 1500 ml of saliva per day. Several physiological and pathological factors can affect saliva secretion in both qualitative and quantitative term such as taste stimulation, smell, chewing, age, drugs, oral hygiene, physical exercise,

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psychological state, and hormone status. The type of saliva is different according to the type of salivary gland, with differences in the concentration of ions, salts, and total protein.<sup>7</sup> This study aimed to measure salivary fluoride after fluoride intake (after brushing using toothpaste) and measure the concentration of fluoride in tap water (groundwater and public water supplies) in Erbil city.

## Methods

This study was carried out on healthy subjects only.

### Experimental design

This cross-sectional study was carried out during the period of March to July 2016 at the Department of Clinical Biochemistry, College of Health Sciences, Hawler Medical University, Erbil, Iraq. All the laboratory work was done at the Medical Research Center, Hawler Medical University, Erbil, Iraq. Salivary fluoride and fluoride from different sources of tap water in Erbil city were measured by (Fluoride, Palintest) and Total Ionic Strength Adjustment Buffer (TISAB II), with CDTA, for Fluoride Analysis using Ion Selective Electrodes, Ricca Chemical Company. Measurements were carried out according to manufactured instruction. Forty five volunteers (20-25 years) were examined. On three different days, in the morning after overnight fasting before brushing teeth, the saliva of the subjects (healthy non-smoker adult) was taken before brushing teeth that was a baseline. After 10 and 20 minutes of brushing after using 1.5 – 2 gr of the toothpaste and washing the mouth with water, saliva was taken in which the cotton pad was held under the tongue for 5 minutes. After that, samples were put in the freezer -20C° until the day of analysis. Before measuring salivary fluoride, samples were centrifuged (mini spin (plus) eppendorf) for 5 minutes. The control was distilled water free of fluoride. For measuring fluoride in water in Erbil city, samples (groundwater and surface water) were taken in different places for ten

different days. Briefly, a volume of 0.2 ml of sample was added to eppendorf tubes followed by 0.8 ml of reagent that was prepared according to manufactured instruction according (Fluoride, Palintest) kit. Tubes were mixed well and then incubated at 25 C° for 3 minutes. The absorbance at 570 nm was then measured in a spectrophotometer and compared to a calibration curve prepared using (0-5) mM sodium fluoride. Each measurement was carried out in triplicate. A relevant statement on permission and ethical practices was approved by the Research Ethics Committee in the College of Health Sciences, Hawler Medical University.

### Statistical analysis

The data was presented as the mean of concentration  $\pm$  SD. The different variables were compared to each other using paired t test for Table 2 and t test for Table 1. A *P* value of  $\leq 0.05$  was considered statistically significant.

## Results

Data was gathered for 45 adults (mean age  $22.5 \pm 1.11$  years old) who participated in this study. The mean salivary concentrations for the different collection times are shown in Table 1. The present study demonstrated that salivary fluoride concentration reached to peak after 10 minutes of brushing. Salivary fluoride level in adults after using 1.5 – 2 gm of the toothpaste, the fluoride levels increase 0.06 mg/L after 10 minutes of brushing then decreased by 0.03 mg/L after 20 minutes of brushing. Using toothpaste significantly increased salivary fluoride level as compared to a baseline level (*P* = 0.002). Saliva after overnight sleeping before brushing was used as a control (0.01 to 0.12 mg/L). Table 1 shows the mean salivary fluoride concentration  $\pm$  SD values of baseline and after using toothpaste. The mean salivary fluoride significantly (*P* = 0.002) increased after 10 minutes ( $0.125 \pm 0.012$ ) and 20 minutes ( $0.095 \pm 0.009$ ) of brushing teeth.

The fluoride level in the surface water was significantly higher ( $P = 0.001$ ) than the level in ground water for all the days of measurement (Table 2).

**Table 1:** Salivary fluoride (mean  $\pm$ SD) measured before brushing teeth (baseline), after 10 minutes of brushing and after 20 minutes of brushing (n=45 independent experiments).

Salivary fluoride Baseline (mg/L)	Salivary fluoride After 10 min of brushing (mg/L)	Salivary fluoride After 20 min of Brushing (mg/L)	P value
0.065 $\pm$ 0.06	0.125 $\pm$ 0.012	0.095 $\pm$ 0.009	0.002

**Table 2:** Fluoride concentration was measured in drinking water (tap water) in different places in Erbil city (n=10 different days).

Day	Fluoride of groundwater (mg/L) Mean $\pm$ SD	Fluoride of surface water supplies (mg/L) Mean $\pm$ SD	P values
Day 1	0.2 $\pm$ 0.1	0.4 $\pm$ 0.01	0.001
Day 2	0.09 $\pm$ 0.001	0.45 $\pm$ 0.01	
Day 3	0.00	0.5 $\pm$ 0.02	
Day 4	0.09 $\pm$ 0.029	0.43 $\pm$ 0.1	
Day 5	0.06 $\pm$ 0.011	0.4 $\pm$ 0.019	
Day 6	0.1 $\pm$ 0.02	0.41 $\pm$ 0.01	
Day 7	0.3 $\pm$ 0.031	0.39 $\pm$ 0.1	
Day 8	0.078 $\pm$ 0.041	0.45 $\pm$ 0.02	
Day 9	0.3 $\pm$ 0.08	0.5 $\pm$ 0.03	
Day 10	0.00	0.6 $\pm$ 0.04	

## Discussion

Tooth brushing with fluoride toothpaste can raise salivary fluoride.<sup>8</sup> It has been reported that salivary fluoride level varies from 0.01 to 0.1 mg/L depending on the individual diet, water fluoride, and fluoride usage.<sup>9</sup> Also, the concentration of salivary fluoride depends on many factors, such as dental products used for caries prophylaxis and fluoridated drinking water. Also, saliva can get fluoride via facilitated transport over membranes of salivary gland tissue.<sup>10</sup> It has been reported that concentration of salivary fluoride about 0.019 mg/L and confirmed that fluoride level in saliva was not depending on flow rates and high concentration of salivary fluoride can cause the formation of calcium fluoride.<sup>11,12</sup> The concentration of fluoride in the saliva is only about 0.01 ppm.<sup>9</sup> Although it has been shown that oral ingestion of 1 to 10 mg of fluoride can cause an increase in salivary fluoride levels over about the following two hours, there appears to be little data on the relation of salivary fluoride concentration to the level of dietary fluoride intake at more normal levels of intake. The salivary fluoride concentration is determined by the intake fluoride to the mouth via drinking water, beverages, food, toothpaste, and mouth rinses. In addition, physiological factors such as the volume of saliva before and after swallowing and salivary flow rate can affect the elimination rate of fluoride.<sup>13</sup> Also, It has been reported that salivary fluoride increased immediately after brushing teeth then dropped the baseline after 6 hours.<sup>14</sup> The main natural source of fluoride is drinking water. Adding fluoride to public water supplies can prevent dental caries. Testing fluoride level in drinking water (public water supplies and groundwater) of different places in Erbil city was the second purpose of this study. In this research have been found that in places that the tap water is groundwater, that the fluoride level is very low in some days has not been detected ( $P < 0.05$ ). The samples have been collected in 10 different days, and then measuring fluoride

by different kits (Total Ionic Strength Adjustment Buffer (TISAB II), with CDTA, for Fluoride Analysis using Ion Selective Electrodes, Ricca Chemical Company, and Fluoride, Palintest kit). The government should be more concerned about it because it leads to the threat of dental caries that affects human health. It has been published that fluoride concentration in drinking water about 1 mg/L can help prevent dental caries and also has been shown cause dental mottle and adverse effects on bone.<sup>15,16</sup>

## Conclusion

Tap water is usually the main source of drinking water and fluoride intake. The fluoride levels in tap water is really important in determining total fluoride exposure. It has been proven that saliva contains fluoride, and the results of the current study have shown that toothpaste can increase salivary fluoride temporarily. That assumes fluoride might be absorbed in the mouth, so further studies are required to understand the exact mechanism. It would be important to determine the fluoride concentration in natural sources (tap water) that might be affected of fluoride benefit that caused by excess or less fluoride intake in Erbil city.

## Competing interests

The authors declare that they have no competing interests.

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