ORIGINAL ARTICLE

Level of Lysozyme on Saliva After Drinking Black Tea *(Camellia sinensis)*

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ABSTRACT

Introduction: Saliva plays a critical role in preventing dental caries through regulation of pH, flow rate and proteins of saliva. Tea leaves from black tea *(Camellia sinensis)* is one of the plants containing catechins that has shown to influence secretion of saliva, thus it can inhibit the development of caries. This study aimed to investigate the effect of consuming black tea *(Camellia sinensis)* on level of salivary pH, flow rate and secreted lysozyme in caries and caries-free subjects. **Materials and methods:** Saliva was obtained from twenty four participants divided into two categories, caries and caries free group. Subjects were asked to collect saliva before and 30 minutes after drinking a cup of tea. Salivary pH was measured using a digital pH meter, while the flow rate was measured by weighing the collected saliva divided by time. The concentration of lysozyme was assessed by using ELISA kit. The data were analyzed using paired t-test and Mann Whitney test. **Results:** The results showed that black tea significantly increased pH and salivary flow rate, but had no significant effect on salivary lysozyme. It also demonstrated that there was no significant difference on salivary pH, flow rate, lysozyme after drinking black tea in the caries subjects compared to the caries free. **Conclusion:** Although drinking black tea could raise salivary pH and flow rate, but it did not promote the increase of lysozyme. This finding suggests that black tea had no unfavourable impact on saliva in oral environment.

Keywords: Saliva, Flow rate, Lysozyme, *Camellia sinensis*, Black tea

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INTRODUCTION

Dental caries or cavities has become a major oral disease problem with a high prevalence that is induced by many factors. The cause of dental caries is related to four main elements, such as oral bacteria in dental plaque, the presence of fermentable carbohydrates, the tooth surface, and period of time [1]. Genetic and environmental factors have also been reported to contribute to an elevated susceptibility to dental caries. Genetic factors influence the introduction of antigens, immune response, and dietary patterns. Several studies on humans and animals have demonstrated that genetic variations can lead to immunomodulatory deviations from antigens, thereby controlling the development of dental caries [2]. Mutation in the HLA-DRB1 gene is a genomic factor associated with dental caries, playing a significant role in the immunogenetic pathway involved in developing this oral disease [3]. In addition, other supporting factor such as body mass index is correlated to caries risk at young age [4]. However, the quantity and quality of saliva in oral cavity has become the most potentially contributing factor in the occurrence of caries. Both flow rate and pH of saliva play an important role in regulating the demineralization and remineralization processes of teeth [5–7]. The increased salivary flow directly impacts on removing food debris and neutralizing acid from tooth surface, thus can inhibit the development of dental caries lesions. Moreover, a higher salivary flow allows an increase in the availability of organic and inorganic components of saliva [6,8]. Organic components including proteins have various functions in oral environment [9]. Lysozyme in saliva belongs to innate immune system serving as first line of body defense against microorganisms in oral cavity [10]. Monocytes, neutrophils, Paneth cells found in the intestinal tract and cells in the salivary glands belong to lysozyme producing cells [11,12]. This enzyme is released from salivary glands, crevicular fluid in the gingiva and leukocytes within the oral cavity. The higher concentration of lysozyme is found in serum as well as saliva but slightly detected in CSF, urine and bile. In certain circumstances, caries may potentially induce the upregulation of this protein, taking the protective role against pathogens [13]. Other factor including bacterial lipopolysaccharide can trigger the stimulation of lysozyme derived from monocytes or other immune

cells [14].

Lysozyme also act as an antimicrobial agent that plays a role in lytic action on bacteria by hydrolyzing bonds between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of bacterial cell walls [10,15]. This enzyme has the ability to cause cell lysis from Gram positive bacteria, but together with IgA and complements enabling it to destroy Gram negative bacteria [16]. The level lysozyme in children under 3 years showed a higher value in caries-free group compared to the caries one, while there was no difference found in the concentration of sIgA between caries-free and severe early childhood caries (SECC) children [17]. A study by Lertsirivorakul et. al demonstrated the enhanced activity of lysozyme in SECC preschool children implying that the involvement of this protein in oral immune system relating to caries development [18]. In contrast, another study found that the level of sIgA between caries-free and active caries children remained unchanged [19].

Saliva can be mechanically or chemically stimulated to elevate flow rate. Chemical stimulation from several types of meals or beverages has shown to significantly increase salivary flow, resulting in the raise of organic and inorganic saliva components [6]. Drinking black tea is a chemical stimulation which may improve the quantity of saliva. Black tea is originated from *Camellia Sinnesis* leaves that undergoes the process of fermentation [20,21].

Black tea that is commercially available in the market generally has a bitter taste. The taste is processed on the dorsum of the tongue by the Taste 2 Receptor Protein (TAS2R) functioning as a bitter taste receptor. Activation of taste receptors eventually stimulates saliva production. Higher temperature of the tea induces vasodilation of the salivary glands causing the increase of saliva flow rate. The greater saliva flow rate result in the raise of pH levels due to buffer capacity of saliva in oral cavity [22].

Black tea contains various active substances, such as catechins. Its antibacterial effect inhibits the growth of *Streptococcus mutans* causing the reduced carbohydrate fermentation and acid production so that it leads to maintaining the pH of oral cavity [23]. The dimished number of *Streptococcus mutans* causes the decrease of the secreted lysozyme, because this enzyme is stimulated when the colonization of bacteria becomes greater [13]. Catechin is able to promote the activation of helper T cells on CD40L to bind CD40 expressed by B lymphocytes. Then cytokines result in B cells differentiation to produce Immunoglobulin. Catechins that have a ring B triphenolic component increase IgA production [24,25].

Drinking black tea has been shown to be good for the oral cavity environtment, particularly saliva. However, study relating to the effect of black tea to protein of saliva such as sIgA and lysozyme remains unclear. Therefore, current study aims to investigate whether consuming black tea *(Camellia sinensis)* can influence the saliva protein like lysozyme and sIgA.

MATERIALS AND METHODS

Subject criteria

This study was approved by the Health Research Ethical Committee of Universitas Sumatera Utara, Medan, Indonesia with the ethic number of 1271/KEP/USU/2021. The study was a quasi-experimental design with a non-randomized pretest and posttest control group design. This experimental design was carried out by measuring or observing before and after treatment. All subjects were students from Faculty of Dentistry, Universitas Sumatera Utara, that were divided into two categories, caries and caries-free, with twelve participants in each group. The criteria include subjects with good health, DMF-T scores 1-5 for the caries subjects, and DMF-T = 0 for the caries-free subjects. While subjects who had smoking and drinking alcohol habit, experienced any systemic disease or allergy, received an orthodontic treatment or dentures, as well as radiotherapy treatment, and performed strenuous sports activities belong to the exclusion criteria.

Preparation of black tea solution and saliva collection

Subjects were requested to sign an informed consent prior to sample collection. The subjects were informed to stop eating, drinking, brushing teeth, and exercising 1 hour before collecting saliva. Oral cavity was rinsed by gargling with distilled water to remove debris. Black Tea solution was prepared by brewing 2 grams of Sidamanik black tea powder in 150 mL of boiling water (100°C). The tea liquid was allowed to cool down until the temperature reached to 50°C. The subjects were instructed to drink the tea for 2 minutes.

Saliva was collected around 9 to 12 a.m which was obtained before and 30 minutes following drinking tea. Accumulated saliva was allowed to drain by dripping it through the lower lip into the saliva tube. The saliva was stored in a refrigerator at -20oC until the saliva was used for examination.

Saliva measurement

The collected saliva was weighed using a digital scale to determine the volume of saliva. Salivary flow rate was assessed by dividing the volume during a period of time measured in minutes (5 minutes). Salivary pH was measured using the Hanna Instrument, a tool with a single-line LCD screen, replaceable electrode, and low power requirement for measuring the alkalinity of a substance. The pH measurements were presented with a precision of 0.1 pH and a margin of error of ±0.1 pH,

ranging from 0.0 to 14.0 pH.

Saliva was centrifuged with a centrifugal machine (Eppendorf centrifuge) 1000 revolutions/minute for 20 minutes at 80C. Saliva was then diluted in dilution buffer with a ratio of 1 : 100. Human lysozyme C (LZM C) ELISA kit (® Fine test) was used to examine salivary lysozyme concentration and then the optical density was determined using a microplate reader with 450 nm wavelength.

Analysis of the data

Data were presented as mean ± SD and analysed using SPSS Software program. To verify the effect of drinking black tea on salivary pH, flow rate, and lysozyme, the paired t-test was selected. In addition, Mann Whitney test was used to examine the difference effect of drinking black tea between caries and free caries group.

RESULTS

Distribution of the subjects is shown in Table I. Most of them predominantly included in the study were females rather than males. The result demonstrated that there was a significant increase of salivary pH following drinking black tea in the caries and caries free groups, from 6,73 ± 0,15 to 6,99 ± 0,20 and from 6,79 ± 0,26 to 7,08 ± 0,27, respectively (Figure 1). Interestingly, as shown in Figure 2. The salivary flow rate was also significantly enhanced in both groups. The increased flow rate might affect components of saliva including organic portion such as salivary proteins. We further assessed the level of lysozyme, an enzyme involved in immune defense against oral bacteria, to examine the effect of black tea to this protein. However, the concentration of salivary lysozyme after consuming black tea decreased in both groups. Although the reduction of lysozyme was not significant but it exhibited the lower content of the enzyme in black tea treated group (Figure 3). Furthermore, we analyzed the difference towards the level of salivary pH, flow rate and lysozyme after dringking black tea between caries and caries free groups. It showed there was no significant difference in salivary pH, flow rate, and concentration of lysozyme after drinking tea between those groups (Figure 1 - 3).

Table I: Data distribution of the subjects

Variable	n (24)	% (100)
Gender		
Male	2	8.3
Female	22	91.7
Age		
19 - 21 years	19	79.2
22 - 24 years	5	20.8
DMFT score		
1 - 3	8	66.7
4 - 6	4	33.3

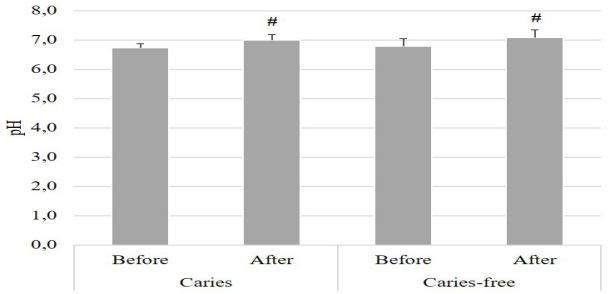

Figure 1: Average Salivary pH Before and After Drinking Black Tea. The pH was increased after the consumption of black tea in both caries and caries free subjects. (#) denotes the significant effect compared to baseline (before).

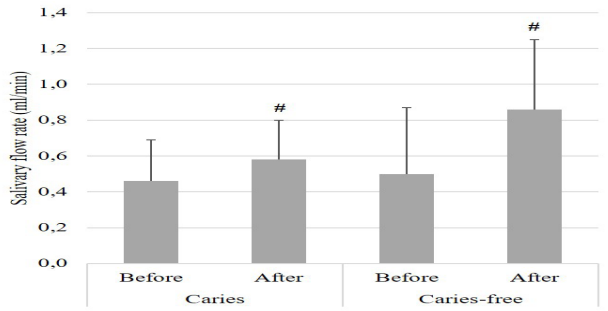

Figure 2: Average Salivary Flow Rate Before and After Drinking Black Tea. The flow rate of saliva was shown to significantly become higher after the consumption of black tea in both groups. (#) denotes the significant effect compared to baseline (before).

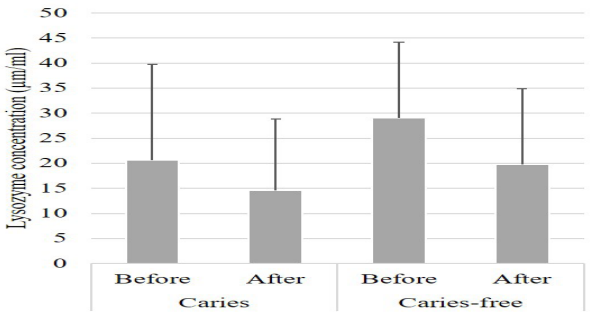

Figure 3: Average Salivary Lysozyme Concentration Before and After Drinking Black Tea. There was no significant change of lysozyme concentration either in caries or caries free subject, yet the value of lysozyme after drinking black tea is lower than baseline (before).

DISCUSSION

The current study demonstrated the increase of salivary pH in both groups. Our result corresponds to previous study showing the raise of salivary pH and flow rate after consumption of black tea [22,26,27]. The presence of polyphenolic compounds which mostly consisting of catechins in black tea makes the salivary pH increased. Catechins from black tea act by inhibiting the activity of the glycotransferase preventing the attachment of bacteria to the pellicle. This leads to the inhibition of plaque formation so that the pH of saliva becomes alkaline. The pH value is also influenced by the concentration of bicarbonate. A high bicarbonate concentration is associated with a high flow rate.

Black tea solution in a higher temperature increases vasodilation of the salivary glands inducing the saliva production. The higher the salivary flow rate occurs, the greater the bicarbonate concentration is obtained, so the value of salivary pH also elevates [22]. Our study demonstrated black tea could stimulate the increase of saliva flow rate. It corresponds to the other study showing the effect of drinking black tea on salivary pH and salivary flow rate where drinking black tea significantly increased salivary flow rate from 0.53 ml to 0 ,56 ml [22]. Interestingly, the results showed that the pH and flow rate of saliva in caries subjects were lower than those in caries-free subjects. Although these values are not significantly different, the lower pH and flow rate values in caries subjects are likely due to caries teeth facilitating the accumulation of plaque, while the lower flow rate inhibits saliva to clean tooth surface. This further increases *S. mutans* colonization leading to the fermentation of substrates by bacteria which can reduce the pH of saliva [28,29]. The acidic oral environment induces tooth demineralization that subsequently result in higher risk for caries [29].

Commercially available black tea generally has a bitter taste. Bitter taste is processed on the dorsum of the tongue by the Taste 2 Receptor Protein (TAS2R) which functions as a bitter taste receptor. The active molecules derived from tea activate taste receptors and stimulate saliva production [22]. Although there was no significant different in the lysozyme concentration following drinking black tea, but the level of this protein was lower after drinking tea in the caries and caries free groups. This finding is similar to other study that compared the lysozyme concentrations between caries resistant people and people with caries risk, where the level of lysozyme reduced in stimulated saliva subjects [30].

Lysozyme is a notable antibacterial protein found in human saliva. The antibacterial characteristics can be attributed to its muramidase activity, which facilitates the destruction of the murein-containing layer of the bacterial cell wall, ultimately leading to bacterial lysis. The action of lysozyme has been associated with oral streptococci [31]. On the other hands, black tea contains catechins which act as an antibacterial agent. The negatively charged catechins bind strongly to the lipid molecule layer of the positively charged gram-positive bacteria. The binding of catechins in lipid membranes results in loss of cell integrity and function and eventually leads to cell death [32]. Black tea had a tendency to block the growth of *Streptococcus mutans* as well as *Lactobacillus* [33].

In this study, the lower lysozyme may occur due to the antibacterial properties of black tea, causing the diminished bacteria number in oral cavity. As lysozyme can be induced by lipopolysaccharide from bacteria, indicating that the activity of this protein is attenuated as a result of bacteria reduction [14]. However, the correlation between tea components, such as catechins and lysozyme remain unclear. Several studies exhibited that tea could interact with some type of proteins, thus interfering the action of those proteins [34–38]. Those probably can describe why the lysozyme level became lower after tea consumption in the current study. Although the study demonstrated the less number of lysozyme concentration following drinking black tea, but there might be other proteins in saliva involved in the action of tea to inhibit bacterial growth for preventing caries development.

CONCLUSION

The current study showed that salivary pH and flow rate were significantly increased after drinking black tea in both caries and caries free groups, while the level of lysozyme was found to be diminished. Our findings indicated that consuming black tea did not result in any unfavourable effect to saliva. Further study is required to investigate the influence of black tea with other possible salivary proteins that may take part in modulating saliva homeostasis.

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