

The Role of Mastication in Improving TGF- β Levels on the Inhibition of *Streptococcus sanguinis* and *Streptococcus mutans* in Gingivitis

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Abstract

Dental plaque in gingivitis containing 56% gram-positive bacteria and 44% gram-negative bacteria. The most dominant gram-positive species are *Streptococcus sanguinis* and *Streptococcus mutans*. Moreover, Transforming Growth Factor Beta (TGF- β) is a peptide for regulating proliferation, differentiation, and mortality of various cells, that can be increasingly secreted by parasympathetic stimulation through a mastication.

To evaluate the effect of mastication intervention to TGF- β levels, the number of *Streptococcus sanguinis* and *Streptococcus mutans*, and the correlation between TGF- β levels and the number of *Streptococcus sanguinis* and *Streptococcus mutans*.

42 men of 17-22 years old were divided into 3 groups: group of healthy with mastication (G1); group of gingivitis with mastication (G2); and group of gingivitis without mastication (G3). The subjects who got a treatment had to chew normal gums (equal size, without sweetener and tastener) and did the mastication for a minute as much as 32 times, every morning after waking up in a week. After a week, the subject's saliva sample was collected and the TGF- β levels, the number of *S. sanguinis* and *S. mutans* colony were counted with ELISA and RT PCR. The data was statistically analyzed with Mann Whitney U-test and Spearman's correlation test.

There was a significant difference of TGF- β levels on all groups ($p < 0.05$) and the number of *S. sanguinis* between group G1:G3 and group G2:G3 ($p < 0.05$). The numbers of *S. mutans* did not show any difference among all groups ($p > 0.05$). Besides that, there was a correlation between the increased of TGF- β levels to the decreased number of *S. sanguinis* ($p < 0.05$).

Mastication can increase TGF- β levels and related with a decreasing quantity of *S. sanguinis*.

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Introduction

Gingivitis is a gingival inflammation with signs and symptoms are red, normal contour, swelling, bleeding accompanied by exudate and occurs after tooth eruption. Gingival inflammation can occur in one or two teeth and also in all teeth. Gingiva bleeds easily due to small stimuli such as brushing teeth or without any stimulation, and it occurs at any time^{1,2}. Dental plaque bacteria in chronic gingivitis consists of 56% gram-positive

and 44% gram-negative species, 59% facultative species and 41% anaerobic species. The dominant gram-positive species in gingivitis are *Streptococcus sanguinis* and *Streptococcus mutans*¹.

Many studies on the development of new classifications of antibiotics that relate to bacterial resistance to the structure and function of *Antimicrobial Polypeptides* (AMPs), are an important part of the innate immune system in living things to deal with pathogenic attacks, act as direct antimicrobials and function as mediators of the initial defense reaction mechanism. Defensin is the first polypeptide antimicrobial found in humans³. The peptides secretion can be increasingly stimulated by parasympathetic stimulation through a mastication.

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Because there is no clear explanation about *S. sanguinis* and *S. mutans* growth inhibition in gingivitis patients due mastication, so that the authors aim to observe the mechanism of bacteria inhibition in gingivitis due to mastication treatment that can increase the peptides' levels.

Materials and methods

Ethics

The ethical approval number of this study was 193/KKEPK.FKG/XII/2014.

Study design

This was an experimental study with observations on post-test design in patients with gingivitis. The population of this research was patients with gingivitis in the Dental Clinic of Dental Health Polytechnic Makassar, South Sulawesi, Indonesia in October 2014. The research subjects were 42 men of 17-22 years old who dont smoke, dont take any antibiotic medication for a month, dont take any corticosteroid, dont have saliva abnormalities, dont have blood abnormalities, and dont have diabetes mellitus condition.

There were 42 participants who were divided into 3 different group and asked to fill out the questionnaire sheets and to sign an informed consent. During the treatment there were six participants dropped out, four people dropped out due to nausea after mastication in the morning, one person took antibiotics on the second day of the treatment and one person left without explanation.

The subjects were grouped into 3 groups consisting 14 men. Group 1 is a group with healthy periodontium condition and will get a mastication treatment. Group 2 is a group with gingivitis and will get a mastication treatment. Group 3 is a group with gingivitis and will not get any mastication treatment. The mastication treatment used normal gums with equal size, without sweetener and tastener. The treatment was given for a week and must be conducted for a minute (32 times) in the morning right after the subjects wake up. The subject's saliva sample was collected after a week of treatment. After one week of treatment, all saliva samples was stored in an ependorf tube that was given Phenylmethylsulfonyl Fluoride (PMSF) as a preservative of saliva. Saliva was examined for TGF-β levels, the number of *Streptococcus*

mutans and *Streptococcus sanguinis*.

The results were statistically analyzed with SPSS application, using normality analysis, comparison test of Mann Whitney, and correlation test with Spearman.

Results

The effect of mastication treatment were increasing TGF-β levels, decreasing the amount of *S.sanguinis* and the amount of *S.mutans* that shows in Table 1.

Variable	Group	Mean
TGF-β	Healthy periodontium with mastication	35.18
	Gingivitis with mastication	19.43
	Gingivitis without mastication	9.89
<i>S. sanguinis</i>	Healthy periodontium with mastication	12.86
	Gingivitis with mastication	19.50
	Gingivitis without mastication	22.14
<i>S. mutans</i>	Healthy periodontium with mastication	14.75
	Gingivitis with mastication	19.50
	Gingivitis without mastication	22.14

Table 1. Description of research data TGF-β levels, the number of *S.sanguinis*, *S.mutans*

The mean value of TGF-β from the highest to lowest are group of healthy periodontium with mastication treatment; group of gingivitis with mastication treatment; and group of gingivitis without mastication treatment. The mean value of *S. sanguinis* from the lowest to highest are group of healthy periodontium with mastication treatment; group of gingivitis with mastication treatment; and group of gingivitis without mastication treatment. Meanwhile the mean value of *S. mutans* from the lowest to highest are group of healthy periodontium with mastication treatment; group of gingivitis with mastication treatment; and group of gingivitis without mastication treatment.

Normality test on Transforming Growth Factor Beta (TGF-β), *Streptococcus sanguinis* and *Streptococcus mutans* variables used One Sample Kolmogorov-Smirnov test (sample less than 50) and the results showed all of them was not normally distributed (Table 2).

Variable	TGF-β	<i>S. sanguinis</i>	<i>S. mutans</i>
p-value	0.004	0.000	0.026

Table 2. Normality test using *One Sample Kolmogorov-Smirnov test*.

*Description: the variable have a normal distribution if p-value > 0.05.

To analyze the differences in TGF-β, *Streptococcus sanguinis* and *Streptococcus mutans* from the 3 groups, the Mann – Whitney Test. Based on Table, 3 TGF-β levels in all three groups shows significant differences ($p < 0.05$). Table 3 also shows there was a significant difference the number of *Streptococcus sanguinis* in group of healthy periodontium with mastication treatment and group of gingivitis without mastication treatment ($p = 0.000$) and in group of gingivitis with mastication treatment compared to group of gingivitis without mastication treatment ($p = 0.003$).

Comparison of TGF-β levels between two groups		p-value
Healthy periodontium with mastication	Gingivitis with mastication	0.000*
Healthy periodontium with mastication	Gingivitis without mastication	0.000*
Gingivitis with mastication	Gingivitis without mastication	0.002*
Comparison of <i>S. sanguinis</i> number between two groups		p-value
Healthy periodontium with mastication	Gingivitis with mastication	0.603
Healthy periodontium with mastication	Gingivitis without mastication	0.000*
Gingivitis with mastication	Gingivitis without mastication	0.003*
Comparison of <i>S. mutans</i> number between two groups		p-value
Healthy periodontium with mastication	Gingivitis with mastication	0.427
Healthy periodontium with mastication	Gingivitis without mastication	0.946
Gingivitis with mastication	Gingivitis without mastication	0.635

Table 3. Mann-Whitney test results on TGF-β levels.

Description: *there is a significant difference, if p-value < 0.05.

Table 3 shows there was no significant difference the number of *Streptococcus mutans* in all three groups ($p > 0.05$). The correlation analysis using Spearman correlation test which performed in Table 4.

Variable	<i>Streptococcus sanguinis</i>	<i>Streptococcus mutans</i>
TGF β	$p = 0.022^*$	$p = 0.945$

Table 4. Spearman's Correlation test results of TGF β level against *Streptococcus sanguinis* and *Streptococcus mutans* colony.

Description: *There is a significance difference, if p-value < 0.05.

There was a correlation between the increased levels of TGF-β with a the decreased number of *Streptococcus sanguinis* ($p = 0.022$). Meanwhile there is no correlation between the increased levels of TGF-β with the decreased number of *Streptococcus mutans* ($p = 0.945$).

Discussion

Normal mastication of the teeth is

differentiated by the presence of food bolus and only comes into contact at the end of the mastication cycle during food ingestion. The total duration of teeth contact over a 24-hour period is 17.5 minutes, consisting of 9 minutes of masticatory contact and 8.5 minutes of contact when swallowing. Therefore teeth contact with functional is rarely obtained and is generally temporary. Mastication of unsweetened gum as much as 32 times (1 minute) in this study was carried out when the subjects woke up around 4-6 o'clock in the morning, due to the teeth mobility was greatest when woke up. The systemic process goes through a deep sleep process around 2-4 o'clock in the morning, then after that around 4-6 o'clock in the morning the body temperature is in the lowest state after a long enough rest⁴.

Mastication does not only affect an oral cavity health, it also affects a systemic health. Mastication can increase a heart rate and blood pressure, cortisol, and brain blood vessel secretions. Mastication increases salivary secretion through stimulated salivary reflexes that occur when chemoreceptors or pressure receptors are in the oral cavity. These receptors initiate impulses in afferent nerve fibers that carry information to the salivary center in the medulla of brainstem. Salivary center sends impulses from electric autonomic nerve to salivary glands, it could increase salivary secretion. Mastication movements stimulate salivary secretion even though there is no food due to manipulation of pressure receptors found in the mouth⁵.

This research focused on the function of mastication that can cause an increase in the body's immune system, specifically the antimicrobial TGF-β polypeptide and also on the inhibition of dominant bacteria in plaque namely *Streptococcus sanguinis* and *Streptococcus mutans*.

The lower number of *Streptococcus sanguinis* compared to *Streptococcus mutans* in this study is in line with epidemiological studies that show that the presence of *Streptococcus sanguinis* that produces peroxide, in the early stages of colonization and with a large number significantly associated with low *Streptococcus mutans* bacteria, whereas the number of S mutants that produce mutacins causes a decrease in the number of S sanguis bacteria^{6,7}.

Streptococcus sanguinis is able to synthesize extracellular dextran from 1-6 chain-

shaped sucrose and is water soluble. *Streptococcus mutans*, synthesizing more dextran insoluble in water with chains 1-3 and this bacterium is stronger in colonization of plaque formation than *Streptococcus sanguinis*.^{4,8} This finding is in line with research on mastication that increases TGF- β levels which shows that only *Streptococcus sanguinis* bacteria which can be significantly reduced by increasing levels of TGF- β , in contrast to *Streptococcus mutans* which does not show a significant decrease by increasing TGF- β .⁴

Metabolism of extracellular sucrose by mutant *Streptococcus*, with 1-3 chain dextran products that are not soluble in water, is very important in the mechanism of dental plaque formation and increased colonization in plaque. This increase in colonization because of bacterial aggregation through heterotypic attachment between cells and heterotypic attachment between different cells. Dextran with chain bonds 1-3 also acts as a mediator of aggregation between *Streptococcus mutans* and *Streptococcus sanguinis*.^{6,9}

Streptococcus mutans in plaque metabolizes sucrose to become acidic faster than other bacteria. The *Streptococcus mutans* colony is covered by glucans or dextran which can reduce the anti-bacterial activity of saliva against dental plaque¹⁰. Oral bacteria can survive from oxygen exposure, host immunity and antimicrobial agents through the formation of biofilms as a barrier unit. The oral cavity bacteria contained in biofilms are more resistant to antimicrobials, because the biofilm matrix is less permeable to antimicrobials^{2,10}. The defense from bacterial biofilms is related to the presence of protective barriers provided by extracellular polymer matrix (EPM). Biofilm bacteria showed higher resistance to antimicrobials compared to free planktonic form¹¹. Factors influencing include presence of extra cellular matrix physically limits diffuse antimicrobial agent. Second, slow growth in biofilms contributes to antimicrobial resistance due to lack of sensitivity for growth-dependent antimicrobials³.

TGF- β levels in this study showed that there were significant differences between the group of healthy periodontium with mastication treatment and also, compared to the group of gingivitis without mastication treatment. TGF- β levels in the group of gingivitis with mastication treatment compared to the group of gingivitis

without mastication treatment showed there was a significant difference. This study showed TGF- β levels did not correlate to the decreased number of *Streptococcus mutans*, while for *Streptococcus sanguinis* variable showed there was a significant difference.

Transforming Growth Factor Beta (TGF β) is produced in the parotid gland, which produces 25% of total saliva per day so that by mastication greatly increases salivary secretion including levels of hBD-2. In vitro and in vivo studies revealed the role of rat saliva on wound healing¹². The study stated that saliva is a source of growth factors, including epidermal growth factor (EGF), basic fibroblast growth factor (BFGF), transforming growth factor (TGF) and insulin-like growth factor (IGF). These growth factors will stimulate inflammatory cells to the wound area, induce keratinocyte and fibroblast proliferation, angiogenesis and form granulation tissue^{1,12,13}.

Wound healing is a dynamic process, including hemostasis, inflammation, proliferation, and remodeling. Growth factor is a polypeptide that initiates growth, differentiation and metabolism, and regulates tissue repair processes¹⁴. Research before using human saliva that was tested on a wound model in the rabbit group resulted in a faster healing process than control rabbit group. TGF- β is a growth factor that plays a role in triggering IgA secretion, by transforming TGF- β which activates B cells to isotope IgA. TGF- β induces B cells to become IgA of 10% to 20%¹³. According to study before there was a strong correlation between TGF- β and sIgA. Another research showed that TGF β directly affects the increase in IgA levels. TGF β production is associated with inhibition of IL-4 production by Th2 cells to inhibit IgE production. IgA that has been actively produced is captured by the Fc receptor in the epithelium to the lumen¹¹. IgA activation requires TGF- β to activate B cells and produce IgA Isotype triggers occur in the inductive mucosa location, whereas IgA production by plasma cells occurs in the effector mucosa, separating the IgA and IgA triggers secreted by B cells into different immune components¹². Each stage requires specific signals, such as costimulatory molecules, cytokines and helper T cells, which give rise to specific sIgA antigens in the effector mucosa⁶.

The mucosal immune system is dominated by one immunoglobulin isotype,

namely secretory IgA (SIgA). IgA is the greatest amount of immunoglobulin in mammals. About 70% -75% of all immunoglobulins produced consist of IgA. SIgA plays a major role in adaptive and natural immunity. The ratio of IgA: Ig G in the secretion of parotid glands to oral cavity is 500 times greater than secretion in serum¹⁵. Density of cell plasma IgA in the parotid gland is 2-3 times higher than the IgA density in the labial and submandibular glands¹¹. IgA has the ability to induce the enzyme lactoperoxidase, ability to hydrophobic bacterial and heterolipycucin which cause bacterial agglutination. IgA is an antibody produced in mucosal lymphoid tissue that is actively channeled through the epithelium, and binds to microbes to neutralize the microbes that attack the organism. Antibodies are secreted bind to microbes to prevent the formation of colonization^{15,16}.

Salivary IgA antibody levels are suppressed with increasing caries. Research on increasing serum IgA levels during caries development shows that immune complexes consisting of serum antibodies and mutant *Streptococcus* antigens are able to suppress the stimulation of the mucosal immune system. This is consistent with research which states that caries treatment can cause elevated levels of IgA antibodies against *Streptococcus mutans*¹⁷.

Saliva contains a variety of proteins that have antimicrobial activity, the most dominant being lysozyme (Lz), lactoferrin (Lf), salivary peroxidase (Spx) and secretory immunoglobulin A (SIgA)¹¹. Antimicrobial effect of these proteins has been tested on oral bacteria, specifically *Streptococcus mutans* and *Streptococcus sanguinis*. Lysozyme, a hydolytic enzyme that breaks the glycoprotein bonds in bacterial cell walls that are synergistic with lactoferrin. Lysozyme in saliva helps control the occurrence of caries and *Candida* infections, an anti-bacterial enzyme found in almost all body tissues and secretions^{11,18}. Lactoferrin as an important component of body's defense system has anti-bacterial, anti-viral, anti-parasitic properties. Lactoferrin is a multifunctional protein that is mainly found in the mucosa, is bacteriostasis by inhibiting the ability of bacteria and viruses to stick to cell membranes so as to inhibit the formation of biofilms. Salivary peroxidase is an enzyme that reacts with hypothiocyanite saliva substrate, through oxidation of thiocyanate ions,

reducing the occurrence of gum disease and halitosis. Salivary peroxidase is bactericidal against lactobacillus and streptococcus bacteria^{11,18}.

SIgA levels are inversely proportional to salivary peroxidase levels, whereas the relationship of SIgA to lysozyme and lactoferrin is positive. Some studies suggest low salivary peroxidase levels, but high levels of SIgA and lysozyme and moderate lactoferrin levels. The results of other studies stated increased salivary peroxidase levels, but SIgA, lysozyme and lactoferrin levels decreased. Other studies state that the comparison of salivary peroxidase, lysozyme and lactoferrin levels is different from SIgA. Several studies on the relationship of salivary peroxidase, lysozyme and lactoferrin levels have concluded that no significant effect on SIgA levels^{13,19,20}.

Conclusions

In the groups of gingivitis patients, mastication treatment increased the TGF- β levels than the group who did not get the mastication treatment ($p = 0.002$). The increased levels of TGF- β ($p = 0.022$) mastication was correlated with the decrease of *Streptococcus sanguinis* colony. The increased levels of TGF- β ($p = 0.945$) mastication did not correlate with the decrease of *Streptococcus mutans* colony.

Declaration of Interest

The authors report no conflict of interest.

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