WHITE SPOT LESION RISK ASSESSMENTS IN ORTHODONTIC PATIENTS – WITH AN EMPHASIS ON SALIVARY CARIOGENIC BACTERIAL ACTIVITY LEVELS

A Thesis

by

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ABSTRACT

Purpose

The purpose of this study was to evaluate whether risk factors that have been shown to increase caries susceptibility, including cariogenic bacterial levels and salivary factors, can be used to identify orthodontic patients who have developed white spot lesions.

Materials and Methods

This prospective case-control study included 50 orthodontic patients, ages 11-17, recruited at the Texas A&M University Graduate Orthodontic Clinic. The controls consisted of 25 patients who did not develop new WSLs or increase the severity of existing WSLs during orthodontic treatment. The cases included 25 patients who developed new WSLs or increased the severity of existing WSLs during orthodontic treatment. WSLs, pre-treatment and post-treatment oral hygiene, change in oral hygiene, and fluorosis were evaluated from initial and final intraoral photographs. Risk factors, including snacking frequency, oral hygiene, and fluoride utilization were evaluated using surveys. Salivary buffer, flow rate, bacterial levels, and bacteria activity levels were also evaluated using salivary samples.

Results

There were no between-group pretreatment differences in WSLs (p=.252). The cases reported eating sugary foods significantly (p=.001) more often than the controls, while only 4% of the cases reported eating sugary foods only with meals, compared to 44% of the controls. Most patients had good pretreatment oral hygiene, but only 12% had

good posttreatment oral hygiene, representing a significant ($p<.001$) decline during treatment that was not significantly different between groups ($p=.631$). There were no significant between-group differences in the amount of saliva, buffer, ATP bioluminescence, and bacterial levels. However, both groups showed lower than normal buffer capacity and high bacterial levels. There also was no statistically significant difference in the number of maxillary or mandibular teeth affected by WSLs ($p=0.115$). The most commonly affected tooth was the maxillary canine at 38%, followed by maxillary laterals at 28%, and the maxillary and mandibular molars at 26% and 24%, respectively.

Conclusions

Oral hygiene declined during treatment, bacterial levels were high and salivary buffer was low. Cases had greater sugar intake between meals than controls. ATP bioluminescence with Cariscreen, S. Mutans levels with Saliva Check Mutans, and salivary factors do not accurately identify which patients develop WSLs.

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NOMENCLATURE

WSL(s)	White spot lesion(s)
ATP	Adenosine triphosphate
APF	Acidulated phosphate fluoride
S. Mutans	Streptococcus mutans
RFUs	Relative light units

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1. INTRODUCTION AND LITERATURE REVIEW

Introduction

White spot lesions (WSLs) are the initial manifestations of the carious process. They persist as one of the potential negative side effects of orthodontic treatment. Between 2 to 98 percent of orthodontic patients have been reported to develop WSLs, depending on the method of detection. [1-5] Clinically visible WSLs have been reported to occur in approximately 23% and 28% of orthodontic patients treated in university and private practice settings respectively.[2, 5]

Certain risk factors increase the risk of developing carious lesions, including oral hygiene routine, dietary habits, fluoride exposure, and cariogenic bacterial levels.[6-8] Caries risk assessments have been developed to identify patients with increased caries risk factors via forms relating risk factors and preventative factors. Common factors that increase a patient's risk of developing caries include carious lesions and/or restorations within the last 36 months, poor oral hygiene, frequent intake of sugary drinks or snacks, low socioeconomic status, and the presence of dental/orthodontic appliances. [6, 8, 9]

Caries research has also extensively evaluated how salivary function increases or decreases a patient's risk of developing caries. Salivary buffer capacity has been shown to be one of the best indicators of caries susceptibility.[10] Salivary buffer capacity, defined as the quantitative measure of resistance of pH changes, is indicative of the patient's response to acid challenges.[10, 11] Every time carbohydrates are ingested, the patient's salivary buffer system is activated to neutralize the acid that bacteria produce byproducts. The faster a patient's buffer system can return an acidic environment to a normal environment, the less time the patient is in the demineralization state. The typical amount of time required to

return the oral environment to neutral pH is approximately 20 minutes, but it can take up to one hour. Studies show that low salivary flow may lead to a lengthened amount of time spent in the demineralization state, with increased the risk of dental caries. [12] Normal salivary flow rate is 0.3 ml/min for unstimulated whole saliva, and 1.5 ml/min for stimulated saliva. [13] Generally, less than 0.7 ml of stimulated saliva per minute is considered inadequate. [11] If a patient's salivary flow remains low over an extended period of time, then the risk for caries increases. [11]

In addition to salivary function, the oral flora environment of orthodontic patients has been related to caries development. Differences exist in the microbial composition of patients with dental caries and those without dental caries. Higher levels of S. mutans and lactobacilli increase the risk of dental caries.[14, 15,16] Streptococcus mutans specifically have been found to be associated with white spot lesions due to their highly cariogenic properties.[17,18] Saliva samples from orthodontic patients show increases in overall oral bacteria counts after the placement of orthodontic appliances.[19,20, 21] Following orthodontic treatment and bracket removal, streptococcus mutans levels appear to return to normal levels, indicating that the appliances increase the bacteria counts due to their plaque trapping qualities.[20]

Salivary bacterial levels can be determined using culture methods, chairside tests, or adenosine triphosphate (ATP) bioluminescence. Bacterial culture methods quantify the number of bacteria present in a patient's saliva. Chairside tests classify individuals as having high S. Mutans levels (> 1500 CFU/mL) or low S. Mutans levels (< 1500 CFU/mL). Tests that measure the ATP bioluminescence of saliva measure the salivary bacterial activity levels. ATP bioluminescence tests are based on the fact that active

bacteria in the oral cavity survive in the acidic environment due to their ability to pump hydrogen ions out of cells and maintain a more neutral intracellular pH.[10] However, this requires a large expenditure of ATP. [10] By measuring ATP levels, a determination of overall bacterial load and bacterial activity can be made. [10, 22] The higher the bacterial activity, the higher the caries risk categorization of the patient.

Discovering methods of reducing the incidence of these lesions is vital. Orthodontic research has largely focused on the prevention of WSLs, instead of determining which patients are actually more likely to develop WSLs. Fluoride mouth rinses and fluoride gels have been shown to decrease demineralization, but they depend on patient compliance.[23-25] Professionally applied fluoride varnishes also reduce demineralization and provide a non-compliant method of fluoride delivery.[26-28] Resin-filled sealants create a barrier between the tooth surface and demineralization-causing acid produced by cariogenic bacteria in plaque build-up. [28-31] Due to time, financial constraints, and patient compliance methods, most orthodontists find these methods impractical for everyday use on patients in their practices. If orthodontists were able to identify those patients at increased risk of developing WSLs using risk factors and salivary cariogenic bacterial levels, then treatment could be modified specifically for high-risk patients, reducing the increased burden of time and financial constraints placed on orthodontic practices.

The purpose of this case control study was to determine if caries risk factors, including salivary cariogenic bacterial levels, can assist orthodontists in identifying patients with increased likelihood of developing white spot lesions (WSLs) during treatment. This study will utilize established disease indicators, risk factors, and protective

factors for caries, including poor oral hygiene, high frequency of food intake, WSLs prior to orthodontics, and fluoride exposure. In addition, this study will utilize two assessment systems to help identify and quantify the presence and activity of cariogenic bacteria in the saliva of orthodontic patients.

Problem and Significance

Esthetics, form, function, and stability are the main goals of successful orthodontic treatment. White spot lesions (WSLs) significantly compromise all of these goals, and WSLs remain a substantial problem in orthodontics today despite their preventable nature. Currently, most methods of reducing the incidence of white spot lesions focus on prevention protocols. Studies have shown that patient-compliance based fluoride rinses and fluoride gels, as well as professionally applied fluoride varnishes, decrease the demineralization that typically occurs. [23-28, 32] In addition, resin-filled sealants have been proven to protect tooth enamel from demineralization by providing a barrier against acid insult. [29-31] To date, few studies have attempted to preemptively identify which patients are more likely to encounter this negative side effect during treatment. Current proven caries risk assessment protocols could provide a basis for evaluation of patient risk factors that contribute to WSLs. Treatment time longer than 36 months, poor and declining oral hygiene, younger treatment age, previous carious lesions, and pre-existing WSLs have all been identified as risk factors for WSL development. [2, 33, 34] However, no studies have determined if salivary bacterial testing prior to orthodontics can identify patients with increased cariogenic bacterial counts or activity levels.

The present study will evaluate whether additional risk factors that have been proven to increase caries susceptibility can be used to identify high risk orthodontic patients. The primary focus will be on cariogenic bacterial levels. High ($>10^5$) or low ($<10^5$) bacterial levels will be calculated using the Saliva Check Mutans bacteria protocol. In addition, the CariScreen protocol will be used to determine cariogenic bacterial activity level. The two measures will be compared to determine if the CariScreen protocol provides

reliable and accurate results. Saliva will be collected from orthodontic patients currently in treatment and scheduled for appliance removal. Two groups of patients will be evaluated. Group 1 will consist of patients who have developed WSL during treatment and Group 2 will consist of patients who have not developed white spot lesions over comparable time periods. If the CariScreen protocol can provide accurate results of bacterial activity and if high bacterial activity positively correlates with white spot lesions in orthodontic patients, then orthodontists could easily test their patients' saliva to determine risk prior to treatment. This development would allow orthodontists to determine which patients would benefit from additional preventative measures.

Specific Objectives/Aims

The primary question this study hopes to answer is:

1. Are there risk factors that positively correlate with WSL development?

The specific questions this project intends to answer are:

1. Does the CariScreen system's salivary cariogenic bacteria activity levels adequately predict salivary bacterial levels?
2. Do patient demographics (age, gender, and race) increase or decrease the risk of developing white spot lesions?
3. Can caries risk factors identify patients at higher risk for developing WSLs?

Hypotheses

Null Hypothesis:

1. There is no difference between cariogenic bacterial activity levels in patients who develop WSLs and those who do not develop WSLs.

2. This is no difference in risk categorization of patients by CariScreen or Saliva Check Mutans.
3. There are no significant differences in patient demographics between patients who develop WSLs and patients who do not develop WSLs, including age, gender and race.
4. There are no significant differences in caries risk factors in patients who develop WSLs and who do not develop WSLs, including,
    a. Frequent between meal food intake
    b. Fluoridated toothpaste/rinse/gel use
    c. Professionally provided fluoride varnish
    d. Fluoridated drinking water
    e. Recent caries activity/restorations (last 3 years)
    f. Previous and current oral hygiene

Literature Review

Definition of White Spot Lesions

White spot lesions (WSLs) are a preventable negative side effect that can occur during orthodontic treatment. Even though this side effect is preventable, it persists as a problem for both orthodontic patients and their treating orthodontists. Reports of WSL prevalence vary from 2% to 96%, depending on method of detection. [1-4] More recent studies show that 28% of orthodontic patients develop visible white spot lesions throughout the course of treatment. [5] While WSLs can occur on any tooth surface, they have been reported to develop more frequently in the maxillary arch than in the mandibular arch. [2] In addition, WSLs most commonly develop on the maxillary laterals, followed by

the maxillary canines, mandibular canines, and mandibular premolars. [2, 3, 35, 36] These teeth are more susceptible to demineralization due to their increased exposure to carbohydrates and decreased exposure to salivary flow.[1] White spot lesions formed during orthodontic treatment typically develop along the gingival margin of the facial/buccal surface of the tooth and are symmetrical from left to right. [4] Therefore, not only are patients developing WSLs during treatment that jeopardizes the health of their teeth, but they are developing WSLs in the esthetic zones that will be visible during every day activities such as talking, eating, and smiling. Orthodontic patients seek treatment to improve the form, function, and esthetics of their dentition. However, WSLs negatively affect all these key treatment goal areas. Therefore, preventing this undesirable outcome is vital to providing satisfactory treatment including a healthy, esthetically-pleasing smile for patients seeking orthodontic care.

The development of WSLs is a multifactorial process. Orthodontic wires, brackets, and bands create make it more difficult to remove plaque that accumulates and disrupt the areas of stagnation in the mouth. [20, 21, 37-45] Studies have shown that S. Mutans levels rise in patients who begin orthodontic treatment. [21] In addition, orthodontic appliances create increased adhesion of bacteria, especially where excess resin remains at the bracket/tooth interface. [45-48] Many orthodontic patients are adolescents with less developed oral hygiene capabilities and poor compliance. [3] Removal of daily plaque limits the amount of reaccumulated plaque, therefore, allowing saliva greater access to bacterial colonies to aid in resisting caries formation. [49] Together, these factors lead to greater plaque accumulation and, subsequently, an increased risk of WSL. [5] Since oral hygiene status is known to influence WSL development, studies have evaluated this relationship using several

methods, including the Decayed-Missing-Filled Score method, plaque index, oral hygiene compliance, and the use of fluoride. However, other factors must play a role in WSL development, as shown by those patients with poor oral hygiene who do not develop WSLs. There are also patients with moderately poor oral hygiene who develop WSLs. Therefore, more research into patient characteristics that contribute to the development of WSLs is needed to better identify patients who are at a higher risk of developing these treatment complications.

Etiology of White Spot Lesions

WSLs are the initial sign of a developing dental carious lesion. Hence, the caries disease process can be applied to understand the development and prevention of WSL. Fortunately, the process of developing caries has been extensively researched, analyzed, and documented. [50] First, a pellicle forms on the tooth's surface. Bacterial cells attach to the pellicle and begin growing microcolonies within 24 hours. A mature biofilm develops after approximately 1 week, which includes high numbers of S. mutans bacteria that produce acid as a byproduct of their metabolic processes. [51, 52] This acid byproduct causes demineralization of the tooth's enamel by dissolving the calcium phosphate in the mineral matrix. [50] In addition, S. mutans create extracellular glucans from dietary sucrose that help increase the colonization of bacteria and increase plaque mass. These in turn increase the carcinogenicity of plaque. [53] In the early stages of demineralization, remineralization can redeposit minerals from the saliva into the tooth's enamel. [54] This natural process of demineralization and remineralization occurs continuously in all individuals throughout life. As long as the amount of remineralization equals the amount of demineralization, no caries form. However, when the balance is skewed towards demineralization, caries can

develop. The change towards increased demineralization can be facilitated by a variety of mechanisms, including declining oral hygiene, xerostomia, increased frequency of carbohydrate intake, or appliances that allow greater plaque accumulation. The first evidence of demineralization appears as a milky-white opacity, which is due to subsurface mineral loss as great as 50%. [50] Generally, an intact enamel covering remains over the top of the lesion due to remineralization of the surface layer from saliva. [50] However, the underlying demineralized section can be seen though the surface enamel as a milky white spot on the tooth. This visual effect is now appropriately termed a WSL.

Prevalence of White Spot Lesions in Orthodontic Patients

The prevalence of WSLs following orthodontic appliance placement varies widely, depending on detection method. The prevalence of reported WSLs ranges from 2-98% of patients. Due to the various methods of detection including clinical observation, photographs, light fluorescence, transverse microradiography, hardness tests, polarizing light microscopy, and DIAGNOdent. In the studies reporting prevalence of WSLs greater than 50% range, other factors must have influenced the readings. Mizrahi reported the prevalence of white spot lesions, based on clinical observations, of 796 total patients, 527 pretreatment and 269 posttreatment. The post-orthodontic patients were evaluated for WSLs and the incidence was found to be 84%. [4] However, pre-orthodontic patients also had a high prevalence, reported to be 74%, indicating that other factors or the detection method may have influenced the higher than average WSL reports. Another study that reported a very high (98%) WSL prevalence had a control group with an 85% WSL prevalence, again indicating that the detection method or confounding factors affect the deceptively high percentages. [55] Gorelick et al. reported that 50% of orthodontic patients

undergoing treatment had WSLs, involving 10% of the teeth evaluated. In their control group, 12% had at least one white spot lesion. Methods of detection in the study were clinical evaluations and visual photograph examination. [3] Based on photographs, Lucchese and Gherlone found that 40% of patients had or developed at least one WSL 6 months into treatment and 43% had WSLs after 12 months. [56] Chapman et al. utilized pre and post treatment photographs to determine the prevalence of white spot lesions on the 8 anterior maxillary teeth of 332 patients.[34] In their study, 36% of patients developed at least one new WSL during treatment. [34] Julien et al. compared pre- and post-treatment pictures as well to evaluate WSL on the anterior 6 teeth.[2] Pre-existing lesions were found among 9% of the patients, and 23% of the patients developed at least one WSL during treatment. [2, 5] Based on these studies using clinical or photographic assessments, approximately 11-38% of orthodontic patients develop WSLs.

Studies have also evaluated which teeth are most likely to develop WSLs. Maxillary laterals and mandibular molars, canines, and premolars have been identified as high risk teeth. [35] Lucchese and Gherlone evaluated three groups of patients, one group that had been in treatment for 6 months, one group that had been in treatment 12 months, and the third group that served as the untreated controls. Using clinical visual evaluations on the three groups, the most common site for WSL development was the mandibular first molars (30% of patients) followed closely by the maxillary lateral (29% of patients). [56] Julien et al., who only evaluated the maxillary and mandibular anterior 6 teeth, found the maxillary laterals followed by the maxillary canines and mandibular canines to be the most susceptible to WSL development. [2]

White spot lesions can develop rapidly. White spot lesions have been shown to develop as early as 4 weeks after orthodontic fixed appliances are placed. [57, 58] Current orthodontic treatment modalities have allowed the initial wire to actively straighten teeth for longer periods of time. Therefore, initial appointment intervals have been lengthened to 6, 8, 10 or sometimes even 12 weeks between office visits. This suggests that patients can develop WSL prior to their first adjustment appointment after appliance placement. Consequently, preventing WSLs from the initial bonding appointment is a primary concern for orthodontists and patients.

White Spot Lesion Preventative Methods

Methods for prevention of WSLs have largely centered on educating patients' knowledge about their oral hygiene and diet. However, other methods requiring less patient compliance have also been used, including sealant application and fluoride administration. [59, 60]

Patient Oral Hygiene and Diet Education

Mechanical removal of plaque build-up on oral surfaces by tooth-brushing is an extremely important, irreplaceable, step for preventing white spot lesions.[61] Specialized modifications to the standard toothbrush and floss for improvement plaque removal around orthodontic appliances, disclosure tablets to visualize plaque, and use of daily water irrigation can all assist patients in improving their oral hygiene. [61, 62] Due to the increased caries risk of orthodontic patients, some orthodontists recommend more frequent dental visits and professional prophylaxis. Oral hygiene instruction and regular dental cleanings have proven to be as effective methods of reducing enamel decalcification. [23] Studies have found that a more frequent professional cleaning schedule and chlorhexidine rinses

produce statistically significant reductions in decalcification amount in patients with a high caries risk.[63] However, increased frequency of cleanings increases the cost associated with orthodontic treatment for patients.

Ideally, patient oral hygiene and diet education should be adequate to prevent WSLs. However, at-home oral hygiene programs rely on patient compliance and dedication. Non-compliant patients undergoing fixed orthodontic appliance therapy are at a greater risk for enamel decalcification.[60] Studies have shown that frequent re-education of the effects of poor oral hygiene on dental health can improve patient cooperation.[63] Lovrov et al evaluated patient compliance with oral hygiene at monthly appointments using surveys. They showed that a dedicated oral hygiene regimen and weekly use of a prescribed fluoride gel were effective in decreasing WSLs. [24] Feil showed that the Hawthorne effect can be induced by intentional deception to improve patient oral hygiene in those with poor oral hygiene.[64] Therefore, simply by telling patients they are participating in an oral hygiene study can produce improvements. While the effects have been shown to last up to 6 months, the average length of orthodontic treatment has been reported between 23.5 to 28.6 months.[65, 66] Additionally, studies have indicated that text messaging reminders about oral hygiene increase patient compliance during orthodontic treatment.[67, 68] The plaque index was significantly reduced in patients who received text reminders as compared to their counterparts. [67, 68] In addition, improved bleeding on probing and inflammation scores were seen in patients who received text messaging reminders.[67]

Sealants

Due to the reliance of patient compliance on oral hygiene, orthodontists have attempted to develop methods that do not require cooperation. One method orthodontists utilize to reduce WSLs are sealants. Benham et al. evaluated sealant applications along the gingival margin of the anterior teeth using a split mouth design. [29] The study found a significant reduction in WSLs during orthodontic treatment. Only 6 of the 60 patients showed signs of WSL development. Teeth without sealants had 3.8 times the number of WSLs than teeth that were sealed.[29] Heinig and Hartmann also reported a significant decrease in WSLs in patients who received full-coverage sealants prior to bonding.[69] The study included 78 patients, 38 without sealants and 40 with sealants. The two groups were similar in terms of treatment duration, age, oral hygiene, gender, and fluoride application. In the non-sealant group, 10% presented with white spot lesions compared to 5% in the sealant group. In addition, the WSLs on the non-sealed teeth were deemed to be more severe than the WSLs on the sealed teeth.[69] However, sealants also require maintenance. Over time, sealants erode due to mechanical wear such as tooth brushing and food abrasion. Another potential problem is that WSLs tend to develop along the gingival margins of the teeth where isolation is difficult. Therefore, inability to gain adequate isolation during sealant placement creates a loss of the sealant in the most critical areas of WSL formation. In addition, a tooth can extrude or continue to erupt during treatment, creating areas of exposed enamel that were previously inaccessible for sealant placement. Due to sealant loss and continued eruption, sealants must be reapplied to maintain coverage in the critical gingival margin areas.

Fluoride

The use of fluoride to prevent caries and WSLs has been extensively evaluated. Prevention is based on the enamel's ability to take up ionic fluoride to form fluorhydroxyapatite or calcium fluoride. When the fluoride concentration is low and the oral environment is acidic, fluorhydroxyapatite is formed and integrated into the outer layer of enamel. Below the critical pH level of 5.5, hydroxyapatite is broken down, but, fluorhydroxyapatite can form on the surface layers of enamel if the pH remains above 4.5 and fluoride is available. The remineralization with fluorhydroxyapatite on the surface layers while hydroxyapatite dissolves on the subsurface enamel reduces the total amount of demineralization that occurs. If the pH drops below 4.5, then under-saturation of fluorhydroxyapatite or hydroxyapatite occurs and no remineralization transpires. [70] When the oral environment has higher fluoride levels (greater than 100 ppm), calcium fluoride is formed. The higher the fluoride levels, the greater amount of calcium fluoride that is formed. Furthermore, the solubility of enamel increases at low pH and provides more calcium for binding to create calcium fluoride. By this method, acidulated fluoride gels provide more calcium fluoride to the enamel over a shorter period of time than NaF gels. [71] Decreasing the pH of the fluoride solution, increasing the fluoride concentration, prolonging exposure times, and etching the enamel surface have all been shown to increase the amount of calcium fluoride formation.[72] In vitro studies have shown that calcium fluoride is only formed at much higher fluoride concentrations (300 ppm) when the pH is neutral. Whereas, calcium fluoride is formed at much lower concentrations (100 ppm) when the pH is decreased. [73] Fluoride application causes calcium fluoride to build up in plaque, on the teeth surface, or in incipient lesions. Calcium fluoride then attracts

phosphate ions and protein molecules, providing a cariostatic effect that can serve as a pH controlled reservoir of fluoride for remineralization during a carious attack. [70, 74]

Studies have shown that incorporating fluoride into the enamel's mineral components only slightly reduces solubility. [74-76] Comparisons of caries resistance between different enamel compositions in different species have been performed. For example, Ogaard compared shark enamel, which consists mainly of pure fluorapatite, to human enamel.[77] Microradiography was used to determine mineral loss. The human enamel had more mineral loss than shark enamel unless the human tooth was rinsed daily with .2% sodium fluoride. It was concluded that free fluoride ions in the oral environment are more important than the fluorides that are incorporated into the enamel structure itself. [77] Therefore, daily rinses with fluoride, fluoridated water, or fluoride varnishes may be more effective at reducing WSLs than other methods.

Several methods of delivering free fluoride ions to reduce caries include water fluoridation, fluoride toothpastes, mouth rinses and gels, fluoride varnishes, and fluoride in orthodontic bonding agents.[60] Caries levels are decreased by approximately 50% in fluoridated water communities as compared to non-fluoridated water communities.[78] While many communities in the United States have fluoridated water, some patients may still live in communities with non-fluoridated water or drink from alternative water sources.

Other methods besides fluoridated water exist that can be used by patients. Fluoride toothpastes alone have been shown to be ineffective at reducing enamel decalcification around orthodontic appliances.[26, 79] Topical fluorides used with fluoride toothpastes have proven to decrease the incidence of decalcification in orthodontic

patients.[80] Multiple forms of topical fluoride have been evaluated. Fluoride rinses, either daily with sodium fluoride (0.05% or 0.2%) or weekly with acidulated phosphate fluoride (1.2%), have been shown to reduce enamel demineralization during orthodontic treatment.[32, 61, 81-83] Sodium fluoride rinse (0.5%) has been studied for the effectiveness of reducing the occurrence of WSL during orthodontic treatment by Geiger et al.[32] The study used 236 patients who were given a sodium fluoride rinse and instructed to rinse daily with 10 ml of solution before bedtime and after brushing for the duration of their treatment. WSLs were clinically evaluated after debonding the appliances. No pre-treatment WSL evaluation was performed. Therefore, the number of WSLs that developed during treatment could not be determined. In addition, self-reports of compliance revealed that only 13% of the patients followed the instructions provided. Another 42% of the patient's reported using the rinse every other day, and the remaining 45% reported rinsing less than once every other day. Compliant patients had significantly fewer lesions than the non-compliers, indicating that fluoride rinses can reduce the number of WSLs in orthodontic patients.[32]

Recently, MI Paste and MI Paste plus have been used to aid in the remineralization of WSLs. Casein phosphopeptide-amorphous calcium phosphate is the active ingredient which is thought to keep calcium, fluoride, and phosphate at the tooth surface for a longer period of time and provide deeper remineralization of the WSLs.[84] MI Paste Plus combines fluoride with the MI paste to increase available fluoride. Huang et al performed a study using a MI Paste Plus group, a fluoride varnish group, and a home-care only control group.[84] Over an 8 week period, no differences were found between the MI Paste Plus group, the fluoride varnish group, and home care only group.[84] However, patients were not

monitored for use of the products given to them during the study which could contribute to the lack of difference from the home care only group.

For patients who do not comply, professionally applied topical fluoride varnish may be a better option. These 5% NaF varnishes contain approximately 22,600 fluoride ions that remain in contact with enamel for greater periods of time. Studies have shown that the amount of demineralization around orthodontic brackets is reduced when fluoride varnishes are used. [85] Todd et al performed a study using 36 extracted canines and premolars with bonded orthodontic brackets that were divided into three groups: control with no fluoride application, a placebo group with a non-fluoridated varnish, and a group that received a fluoride varnish application.[26] The teeth were then exposed to a carious challenge for 1 hour twice a day for 37 days with mechanical tooth brush simulation. The average depth and area of demineralization was determined following the carious challenge period. Results showed that the lesions were the greatest in area and depth in the placebo group. The fluoride varnish group's lesions were the shallowest and smallest in area, with 50% less demineralization than the control group.

Another professionally applied fluoride is acidulated phosphate fluoride (APF). Since, calcium fluoride formation is increased in acidic environments, a fluoride gel in an acidic solution was created to provide more calcium fluoride to the enamel. Studies evaluating the efficacy of APF gel have determined that caries formation varies based on the caries risk category of the patient. High risk patients show the poorest results with APF gel. [86] One study found that weekly application of 1.2% APF gel for a month produced hyper-mineralization of the outer enamel layer indicating re-hardening of enamel or inhibition of demineralization. [58] However, when 1.23% APF was applied at more

likely application intervals, corresponding with average appointment times of 8 weeks, white spot lesion was not prevented. [87]

Caries Risk Assessment Methods

While many studies have attempted to prevent WSL with treatments or oral hygiene modifications, few studies have attempted to prevent white spot lesions using risk assessment methods. Since WSL are the initial stage of caries development, methods used for caries risk assessment and prevention could aid orthodontists in determining high risk WSL patients.

Risk Assessment Forms

Caries risk assessments have been developed to identify patients with increased caries risk factors. Risk assessment forms were developed to assess risk factors and caries preventative factors. Common risk factors increase a patient's likelihood of developing caries. The factors normally evaluated include caries lesions and/or restorations within the last 36 months, poor oral hygiene, frequent snacking, low socioeconomic status, and the presence of dental/orthodontic appliances. [6, 8, 9] The presence of these risk factors indicate that the patient may be at a higher risk of developing caries.

A patient's diet and frequency of carbohydrate consumption have also been shown to change the rate of demineralization. Areas that have a high carbohydrate exposure and low salivary flow are common sites of demineralization. Carbohydrate intake causes a decrease in the oral pH due to bacteria breaking down the carbohydrate and the resultant production of acid. Salivary function helps the pH to return above the critical level of 5.5. However, this process requires approximately 20 minutes. [88] The frequency of intake has been shown to be more harmful than the total amount of carbohydrate intake. [89] Increased

frequency of carbohydrate intake subjects the enamel to longer periods of acidic insult, leading to increased amounts of demineralization. [53] In addition, sucrose is thought to be the most detrimental form of sugar for oral plaque and caries formation. All dietary sugars diffuse into plaque and are converted into lactic acid or stored as intracellular polysaccharides by bacteria. Sucrose causes the production of extracellular and matrix polysaccharides that can increase the colonization of microorganisms and stickiness of plaque. [49]

Risk assessment forms evaluate the patient's caries protective factors. Protective factors are methods or situations that decrease demineralization or increase remineralization, creating a caries defensive mechanism. [9] Common protective factors include residing in a fluoridated water community, utilizing fluoride toothpaste, gels, or rinses, professionally applied fluoride varnish, and regular dental visits. [6, 8, 9] Increased numbers of protective factors reduce a patient's risk of developing carious lesions and help offset caries risk factors. Using these forms, dentists are able to evaluate the overall risk assessment for caries in patients and determine if preventative measures should be taken.

Salivary Characteristics

To further evaluate a patient's likelihood of developing caries, research has begun to evaluate differences in the patient's oral environment that could be contributing to this disease process. Most studies evaluate saliva or plaque characteristics. [90, 91] Commonly researched characteristics include salivary or plaque microbial composition, saliva buffer capacity, oral pH, saliva consistency and amount, and biofilm activity level. [10]

It has been shown that patients with more acidic saliva are more likely to develop white spot lesions than those patients with less acidic saliva. [92] However, it has also been

shown that no correlation exists between salivary pH and caries susceptibility. [10] Due to conflicting results and the fact that salivary pH always follows the salivary flow rate, ranging between 5 and 8.17 with the lowest pH at night and in the morning, pH should not be used to predict caries susceptibility. [11]

In contrast to the lack of evidence linking pH and increased caries, salivary buffer capacity has been shown to be one of the best indicators of caries susceptibility. [10] Salivary buffer capacity, defined as the quantitative measure of resistance of pH changes, is indicative of the patient's response to acid challenges. [10, 11] Every time carbohydrates are ingested, the patient's salivary buffer system is activated to neutralize the acid that bacteria produce byproducts. The faster a patient's buffer system can return an acidic environment to a normal environment, the less time the patient is in the demineralization state. The typical amount of time required for a patient's buffer system to return the oral environment to neutral pH is approximately 20 minutes, but it can take up to one hour. [88] Studies show that the amount of bicarbonate in a patient's saliva is an indicator of caries risk, with caries-free patients exhibiting higher levels of bicarbonate than their caries-active counterparts. [93]

The function of human saliva, aside from aiding in digestion, is to provide 1) calcium and phosphate to replenish the mineral content of teeth, 2) caries-resistant proteins and antibodies, and 3) electrolytes for buffering the pH of the oral environment. [10] However, these effects require saliva to flow throughout the oral cavity in adequate amounts both during rest and active carbohydrate ingestion. Resting saliva has a higher mucoid composition than the more serous fluid composition of stimulated saliva, which allows for increased clearance of ingested materials. [46] Together, these salivary

components work to resist caries development. Studies have shown that commonly affected sites of demineralization during orthodontic treatment are the maxillary incisors, where little salivary flow occurs. [3] In addition, xerostomic patients have a higher caries incidence than matched non-xerostomic patients. [94] Therefore, testing for low salivary flow rate can aid in diagnosing a patient's caries susceptibly. [10, 12] Low salivary flow may lead to a lengthened amount of time spent in the demineralization state, with increased dental caries as a result. [12] An individual's flow rate can be affected by diurnal variation, diet, age, sex, certain diseases, and medications. [11] Normal salivary flow rate is 0.3 ml/min for unstimulated whole saliva, and 1.5 ml/min for stimulated saliva. [13] Generally, less than 0.7 ml of stimulated saliva per minute is considered inadequate. [11] If a patient's salivary flow remains low over an extended period of time, then the patient could be at an increased risk for caries. [11] Some studies have shown that orthodontic patients' salivary flow rates increase following orthodontic appliance placement which assists in sugar clearance from the increased retentive intraoral surfaces during treatment.[95] An increase in salivary flow also creates an increase in salivary pH and salivary buffer capacity, which combat demineralization.[37]

Oral Flora

In addition to salivary function, the oral flora environment of orthodontic patients has been shown to be related to caries development. Several studies have shown that a difference exists in the microbial composition of patients with dental caries and those without dental caries. [14, 15] Higher levels of streptococcus mutans and lactobacilli have been shown to increase the risk of developing dental caries. [16] Streptococcus mutans specifically have been found to be associated with white spot lesions, while lactobacilli

have been found to be associated with advanced carious lesions. [96] Lang et al. found that smooth surface lesions without cavitation on first permanent premolars had S. mutans colonies present. [17] In addition, the proportion of S. mutans increased 10-12% 6-9 months prior to smooth surface lesion detection. [17] In the cases where S. mutans levels decreased from 20% to 2-5%, the lesions remineralized. [17] The finding that streptococcus mutans are more common in individuals with caries is rational because the strep mutans strain of bacteria exhibits highly cariogenic properties. [18] In addition, saliva samples of orthodontic patients show increases in overall oral bacteria counts after the placement of orthodontic appliances. [19] Several studies have found an increase in caries-causing streptococcus mutans after orthodontic appliance placement. [20, 21] Following orthodontic treatment cessation and bracket removal, the streptococcus mutans levels appear to return to normal levels, indicating that the appliances cause an increase in the bacteria due to their plaque trapping qualities. [20] While the bacterial levels may return to normal after orthodontic treatment, the WSLs that can be created by these bacteria are harder to remove.

Another salivary test that can be performed to determine a patient's oral microbial flora environment is biofilm activity. Tests using this method measure the adenosine triphosphate (ATP) bioluminescence of saliva. This technique has been used for many years to monitor bacterial activity levels in situations such as food manufacturing and wastewater treatment plants. [10] ATP bioluminescence tests are based on the fact that active bacteria in the oral cavity survive in the acidic environment due to their ability to pump hydrogen ions out of their cells and maintain a more neutral intracellular pH. [10] However, this ability requires a large expenditure of ATP. [10] By measuring ATP levels in an individual's saliva or plaque, a determination of overall bacterial load and bacterial activity

can be made. [10, 22] The higher the bacterial activity measured by these quick chairside tests, then the higher the caries risk categorization of that patient.

White Spot Lesion Risk Assessment

Orthodontic Treatment Risk Factors

Since white spot lesions represent the initial stages of dental caries, it stands to reason that caries risk assessment forms and salivary characteristics that have been proven to increase the probability of developing caries could also be used to help identify which orthodontic patients are at high risk of developing white spot lesions. In fact, many of the risk assessment forms used today indicate that patients in orthodontic treatment are at increased risk to develop caries.[6, 8] Orthodontic appliances have been shown to increase the risk of WSLs due to the method of appliance placement and preparation. Teeth must be prepared for orthodontic bonding by acid etching the tooth to allow for bracket adhesion. Teeth that have been acid etched show approximately 34% more decalcification than teeth that have not been acid etched. [59] In addition, excess cement along bracket margins have been reported as major sites for plaque accumulation. [97] Plaque on the cement adjacent to bracket bases reaches a mature status approximately 2-3 weeks following placement, while plaque on gingival enamel nearby remained in the immature status. [97] Other treatment related factors have been investigated as well. For example, Chapman et al investigated risk factors that contributed to the incidence and severity of white spot lesions. [34] The variables evaluated included treatment duration, number of emergencies, clinical outcome, number of practitioners performing treatment, type of bracket, patient demographics, and patient oral hygiene. The results showed that patients' age at treatment start, poor pretreatment oral hygiene, unfavorable clinical outcome,

Caucasian race, and poor hygiene notations during treatment were positively correlated with developing white spot lesions. Julien et al also investigated the effect of treatment length on WSL. Studies show that treatment time more than 36 months was positively correlated with increased WSL development. [2, 5]

Risk Assessment Forms

In addition to treatment related factors, patient characteristics that are protective and risk factors that influence WSL susceptibility have also been investigated. Patient pre-existing factors investigated include gender, age, socioeconomic status, and diseased first molars.[33] These studies have shown that boys, younger patients, and patients with diseased first molars developed greater demineralization during orthodontic treatment than their counterparts. [33] Julien et al evaluated patient pretreatment characteristics that could be correlated with white spot lesion development. They found that patients were more susceptible to white spot lesions if they had a lack of fluorosis, poor oral hygiene, declining oral hygiene during treatment, and pre-existing white spot lesions. [2] Brown et al evaluated orthodontic treatment factors in addition to utilizing the ADA Caries Risk Assessment form to assess if WSL development could be determined using this method. The ADA Caries Risk Assessment form is a well-known system which uses three general categories, contributing conditions, general health conditions, and clinical conditions, to evaluate and determine a patient's caries susceptibility score. This study showed that the risk of developing WSL is higher for patients exhibiting ADA caries risk factors, poor oral hygiene, and poor gingival health. [5]

Oral Flora

While several studies in orthodontics have investigated the risk factors associated with white spot lesion development, few have assessed oral flora in relation to orthodontic treatment. Bloom and Brown performed a study on 23 adolescent patients prior to and following orthodontic treatment start. The study concluded that the total oral bacterial count was increased following orthodontic bracket placement and that patients who received additional appliances besides braces showed even larger increases in total bacteria counts.[19] Other studies have confirmed that strep mutans levels are higher following orthodontic appliance placement. [20, 21] One study evaluated the levels of strep mutans during retention and found that the levels returned to normal after orthodontic appliance removal. [20] This suggests that the increased plaque trap and oral hygiene difficulties orthodontic appliances present could be the culprit of the increased cariogenic bacteria levels.

To date, no studies have evaluated the ATP bioluminescence in orthodontic patients to determine if caries risk assessments can be used to determine which patients will develop WSL. The present study will attempt to build upon the studies that have provided risk factors correlated with WSL development by incorporating more risk factors and evaluating the cariogenic bacterial counts and cariogenic bacterial activity levels. Our expectation is that orthodontic patients who have developed WSL will exhibit increased streptococcus mutans and lactobacilli along with an increased ATP bioluminescence.

2. MATERIALS AND METHODS

This prospective case control study included 50 orthodontic patients recruited at the Texas A&M University Graduate Orthodontic Clinic. The control group consisted of 25 patients who did not develop new WSLs or increase the severity of existing WSLs during orthodontic treatment. The experimental group (cases) consisted of 25 patients who developed new WSLs or increased the severity of existing WSLs during orthodontic treatment. The determination of new WSLs or increased severity/size of WSL was made using intraoral photographs taken at initial and final records.[2]

All of the patients had to be between the age of 11-17 years and in full orthodontic appliances, have diagnoseable initial intraoral photos of all teeth from first molar to first molar, and have been scheduled for debond during the data collection period. Patients with fixed retainers on the lingual of the upper anterior teeth, those taking multiple daily medications, those with special needs that would hinder oral hygiene, and those with generalized WSLs and/or fluorosis in the gingival third of teeth were excluded from the study. Patients with fixed retainers on the lingual of the lower anterior teeth, isolated WSLs in the gingival third, or generalized fluorosis in the middle to incisal third of teeth were allowed to participate. Mean ages of the controls and cases were 16.07 ± 0.88 years and 15.71 ± 1.43 years, respectively. There was no statistically significant difference (p=.290) in age between controls and cases. Average treatment times were 2.43 ±0.51 years for the controls and 2.43 ±.67 years for the cases.

Data Collection

Pre-Procedure Protocol

Both parent/guardian and patient consents were acquired prior to inclusion (IRB #2016-0563-CD-EXP). Patients were required to refrain from brushing their teeth, eating, or drinking for one hour prior to saliva collection. On the day of data collection, saliva collections occurred immediately prior to appliance removal to avoid contamination from composite removal or rinsing.

Unstimulated Saliva Data Collection

Salivary ATP Bioluminescence

To determine cariogenic mutans streptococcus activity levels, ATP bioluminescence was evaluated following the CariScreen (Carifree; Albany, Oregon) guidelines. Fazilat et al found that ATP bioluminescence diagnostic tests done using the Cariscreen system are valid and have a strong statistical association with bacterial number in plaque and saliva samples, including numbers of oral streptococci. [22] However, to the authors knowledge, the cariscreen has not been used in orthodontic patients. Two Cariscreen swabs were removed from the plastic protective tube using gloved hands. One swab was firmly swiped along the lingual surface of the lower anterior teeth from mandibular canine to canine and the other swab was swiped along the lingual aspects of the upper anterior teeth from maxillary canine to canine. These teeth were chosen because the plaque levels on the lingual of the lower anterior teeth have been shown to be highly correlated with total mouth plaque accumulation and have higher levels of plaque than other teeth. [98, 99] The palatal surfaces of the upper anterior teeth were chosen because they

have been shown to have one of the lowest plaque accumulation throughout the mouth. [98, 99] Swabs were kept refrigerated at 35-46 degrees Fahrenheit prior to use. Care was taken during swab collection to avoid touching any soft tissues including lips, cheeks, tongue, and gingiva to allow for optimal results.

The swabs were evaluated using the CariFree CariScreen for oral bacterial load. [22] The CariScreen assigns a score to the sample based on luminescence registered when reagents in the swab combine with the sample. Possible scores range from 0 to 9,999 relative light units (RFUs), with scores under 1,500 RFUs being considered healthy and those above 1,500 RFUs indicating an increased risk of decay. After sample collection, the swab was placed back inside the plastic protective tube and the liquid snap bulb was broken and squeezed to release the liquid contents. The tube was shaken vigorously for 10 seconds and placed into the CariScreen meter. The lid was closed, the meter replaced back in the meter stand, and evaluation began to provide a reading for the patient's swab. This process was repeated for the other swab obtained from the patient.

Stimulated Saliva Data Collection

Stimulated saliva was also collected. The patient was asked to chew on a paraffin pellet for 3 minutes to stimulate salivary production. The saliva was expectorated into a sterile collection cup and used to determine salivary flow, buffer capacity, and bacterial levels.

Flow Rate

The amount of saliva produced was recorded to determine salivary flow rates. Salivary flow rates less than 0.7 ml/min were considered inadequate. [100] Patients with salivary flow rates above 0.7 ml/min were categorized as normal.

Buffer Capacity

Salivary buffer capacity was measured using the Saliva Check Buffer (GC America; Alsip, Illinois). Previous studies using the Saliva Check Buffer have found that the strip test correlates at 95% with the gold standard buffering capacity method, Ericsson method.[101] A saliva buffer strip with three color-changing squares was used. Saliva was pipetted onto each of the three squares of a buffer strip for each patient and allowed to process for 2 minutes. After the two minutes, each square color was determined to be either red, blue, green or a combination of those colors. Each square was scored numerically using the manufacturer's conversion table and summed ranging from 0 to 12. Buffer capacity was determined based on the total score with 0-5 indicating very low, 6-9 indicating low, and 10-12 indicating normal or high buffer capacity.

Bacterial Levels

Salivary bacterial levels were evaluated using the Saliva Check Mutans (GC America, Alsip, Illinois), following the manufacturer's protocol. Saliva check mutans test has been proven to have satisfactory sensitivity (88%) and specificity (90%) when patients were compliant with refraining from eating, drinking, or performing oral hygiene measures for one hour prior. [102] [103]A standard amount of saliva was pipetted into a collection container. One drop of reagent #1 was added, and the container tapped 15 times over 10

seconds to mix the reagent and saliva. Four drops of reagent #2 were then added to the container and shaken until the saliva mixture changed to light green color. Using a pipette, a measured amount of saliva was collected and dispensed onto the sample window at the end of the test device. The test device was allowed to sit for 15 minutes at room temperature, after which the test strip was read. An indicator line that appeared in the control window was used to confirm that each test was performed properly. A line in the test window indicated that salivary levels of streptococcus mutans were high ($>5x10^5$ Colony forming units (CFU)/mL). No line in the test window indicated a low level of salivary streptococcus mutans ($<5x10^5$ CFU/mL). High or low salivary levels of streptococcus mutans were recorded in the patient's records. Throughout the research on salivary bacterial levels, a cut-off of $\geq 5x10^5$ has been used to indicate high levels of cariogenic bacteria. [104-107]

Survey

Separate surveys were administered to the parents and patients to assess the patients risk of WSL development. Surveys were used to help assess any known caries risks factors the patient exhibits. [6, 8, 9] The patient questionnaire included three demographic questions, one question on their oral hygiene routine and one question on dietary habits/frequency of carbohydrate intake. The parent questionnaire included five questions pertinent to the patients' oral health, including frequency of dental visits, use of fluoridated toothpaste/rinse/gel or professionally provided fluoride varnish, exposure to fluoridated drinking water, and recent caries activity/restorations (last 3 years).

Intraoral Photographs

White spot lesions, fluorosis and oral hygiene were evaluated using the patients' initial and final intraoral photographs. Maxillary and mandibular teeth from first molar to molar were evaluated. The presence of WSLs was determined visually using photographs taken perpendicular to the anterior and posterior segments. Determination of WSLs was done using a visual evaluation. Any isolated white spot on a tooth was determined as a WSL. Final photographs were evaluated for white spot lesions using the same procedure. The final photographs were compared to the initial photographs to determine if the WSLs were new or had worsened (enlarged or increased severity). Photographs were placed side by side and a WSL was determined to have increased in size or severity by visual examination only (Figure 1&2). If a WSL appeared the same as the initial photograph, then no WSL formation was determined to have occurred. It the WSL had increased in size or severity, then the WSL was determined to have formed during treatment. Fluorosis was evaluated on the initial photographs only due to possible enamel desiccation from appliance and composite removal when viewed on the final radiographs. Fluorosis was deemed as either not present, isolated to a few teeth in the incisal third, or generalized in the incisal third (Figure 3). Oral hygiene was evaluated in both pretreatment and posttreatment photographs. Since the final photographs were acquired immediately after appliance removal, different criteria of good, fair, and poor oral hygiene were applied to initial and final radiographs (Table 1) (Figure 4). [2]

Statistical Analysis

To ensure standardization of the procedures, one research technician performed all of the salivary collections and testing. Once all data were collected, they were coded and entered into SPSS (IBM SPSS Statistics Inc., Chicago, IL) for statistical testing. Significance level was set at 0.05 ($p<0.05$). Chi square tests were used to determine group differences in survey questions data (excluding age), intraoral photo evaluation data, and saliva check mutans salivary data. Independent T tests were used to determine the age of patient at treatment start and time in treatment. Mann-Whitney tests were used to determine differences in all salivary data (excluding saliva check mutans).

3. RESULTS

Fifty patients were included in the study. Twenty-five patients who did not develop WSLs during treatment were deemed as controls. Twenty-five patients who did develop WSLs during treatment were deemed as cases. There were no pretreatment differences in WSLs between controls and cases (Figure 5). Twelve percent of the controls had 6 or more WSLs on maxillary teeth, compared to only 4% of the cases. Approximately 8% of the controls and cases had 6 or more mandibular pretreatment WSLs. There were no significant between-group differences in number of patients with pretreatment WSLs (p=.252) or in the number of pre-treatment WSLs on the maxillary (p= 0.303) or mandibular (p= 0.765) teeth.

Data Categories

Survey Question Data

Demographics

There were no statistically significant gender, age, or race differences between controls and cases (Table 2). Average ages of the controls and cases were 16.07 ± 0.88 years and 15.71 ± 1.43 years, respectively. The majority of participants self-identified as White/Caucasian, followed by Hispanic. Females made up slightly more than 50% of the participants in both groups.

Risk Factors Data

The cases reported eating sugary foods significantly (p=.001) more often than the controls (Table 2). Only 4% of the cases reported eating sugary foods only with meals, compared to 44% of the controls. None of the other risk factors showed significant

between-group differences, including regular dental check-ups, prescription or OTC fluoride use, fluoridated water, recent caries, and frequency of brushing. Ninety-two percent of the controls and 88% of the cases reported brushing two to three times a day. The majority of both the controls and cases, 96% and 80% respectively, were seeing a dentist regularly. However, only 56% reported that they are receiving professional fluoride applications or prescription fluoride products. Twenty-four percent of patients in both groups said they are not receiving fluoride professionally; another 20% in both groups were unsure about their fluoride information. While no statistically significant difference was found between cases and controls for toothpaste fluoridation, only 68% of cases reported using fluoride toothpaste compared to 92% of controls.

Intraoral Photo Evaluation

There were no statistically significant between-group differences in pretreatment fluorosis, pretreatment oral hygiene, posttreatment oral hygiene, or presence of lower fixed retainers (Table 3). No fluorosis was found in 48% of both controls and cases, isolated fluorosis was found in 16% of controls and 28% of cases, and generalized fluorosis was found in 36% of controls and 24% of cases. The majority of patients in both controls and cases, 64% and 52% respectively, had pretreatment oral hygiene categorized as good. However, only 12% of cases and controls had good posttreatment oral hygiene, indicating a decline in oral hygiene during treatment. The decline in oral hygiene during treatment was highly significant ($p=.001$). The greatest proportion of controls had fair post-treatment oral hygiene and the greatest proportion of cases had poor post-treatment oral hygiene. There also was no statistically significant between-group difference in oral hygiene change

from initial to final records (p=.631). A slight decrease in oral hygiene was noted in 52% of controls and 44% of cases. Approximately 4% of the cases and none of the controls showed improvements in oral hygiene during treatment. A large decrease in oral hygiene, based on oral hygiene decreasing two categorizes, was seen in 12% of controls and 20% of cases. There was no statistically significant difference in the number of maxillary or mandibular teeth affected by WSLs (p=0.115) (Figure 6). Only 12% of cases had no maxillary WSLs posttreatment, while 48% had no mandibular WSLs. Forty-eight percent of cases had one maxillary WSL and 24% of cases had one mandibular WSL. Six or more WSLs were found in 16% of the maxillary and mandibular teeth. Only 38% of patients with pre-existing WSLs went on to develop WSLs, while 62% of patients with pre-existing WSLs did not develop new WSLs. For patients who did not have pre-treatment WSLs, 59% of patients developed WSLs and 41% of patients remained WSL-free. The maxillary anterior segment was most commonly affected with 26% of teeth showing a WSL post-treatment. The mandibular posterior segment resulted in 18% of teeth being affected, followed by the maxillary posterior segment at 15% of teeth, and finally the mandibular anterior segment at 11% of teeth. The most commonly affected tooth was the maxillary canine at 38% with a WSL following treatment. Maxillary laterals were second with 28% of teeth. Maxillary and mandibular first molars were almost equally affected at 26% and 24%, respectively. However, mandibular premolars were affected more frequently than maxillary premolars with 15% of mandibular premolars and 9% of maxillary premolars. Mandibular canines were the most commonly affected mandibular anterior tooth at 20% while the lower central incisor was least commonly affected of any tooth at 6%.

Salivary Data

There were no significant differences between controls and cases in the amount of saliva, buffer capacity, upper cariscreen swab, lower cariscreen swab, and saliva check mutans (Table 4 and Figures 7, 8, 9). Both cases and controls showed adequate amount of salivary production at all percentiles (Table 4). While no difference was found between controls and cases for buffer, both groups showed lower than normal buffer capacity. Buffer capacity is considered normal at the 10-12 range. The median buffer for controls in this groups was 7 and for cases was 6. Even at the 75th percentile, both cases and controls did not enter the normal buffer capacity range. High cariogenic bacterial levels have been established in the literature as greater than 1500 CFU/mL.[104-106] Ninety-six percent of both controls and cases were positive for high levels of S. Mutans; only 4% of the controls and and 4% of the cases were negative. Spearman's correlations showed that upper and lower cariscreen swab numbers were highly correlated (R=0.633;p=0.01). Spearman's correlations also showed that upper cariscreen swab numbers decreased significantly as age increased (R=-0.281;p = .048). No other significant correlations were found between upper cariscreen swab, lower cariscreen swab, buffer, amount of saliva, and age. When cariscreen data was divvied into high (>1500) and low (<1500), no statistically significant differences were found between cases and controls for maxillary or mandibular ATP bioluminescence levels (Figures 7 and 8). For the maxillary cariscreen, 36% of controls and 44% of cases showed high levels of ATP bioluminescence. For the mandibular cariscreen, 44% of controls and 60% of cases had high levels of ATP bioluminescence. There was a trend for cases to have higher maxillary and mandibular cariscreen scores at all percentiles than the controls (Table 4). At the 50th percentile, only the lower cariscreen

swab for cases was considered in the high range above 1500. The remaining swab were all indicated as low to normal at the 50th percentile.

4. DISCUSSION

Whether or not pre-existing WSLs increase the likelihood of developing new WSLs depends on the teeth being evaluated. The current study showed no significant relationship between pre-existing and new WSLs. The patients with pre-existing lesions were no more likely to develop WSLs during treatment than those without pre-existing lesions. In fact, only 38% of patients with pre-existing WSLs developed new WSLs, whereas 59% of patients without pre-existing WSLs developed WSLs during treatment. Based on digital records and photographs of 885 orthodontic patients, those with pre-existing WSLs were 3.39 times more likely to develop new WSLs; 87% of patients who had pre-existing WSLs developed new WSLs during treatment.[2] However, Lovrov et al showed that only 47% of their 53 patients with pre-existing WSLs developed new WSLs.[108] The differences between studies could be due to the teeth that were evaluated. The present study, as well as the study by Lovrov and coworkers,[108] evaluated maxillary and mandibular teeth from first molar to first molar, while Julien et al only evaluated the maxillary and mandibular anterior six teeth. Including the posterior teeth is important because the mandibular first molars commonly develop WSLs at an early age. As patients continue to develop their oral hygiene skills, the WSLs on the lower molars remain, but the likelihood of developing more WSLs decreases. Therefore, WSLs on posterior teeth do not increase the risk of developing new WSLs. In contrast, WSLs on anterior teeth could be a risk factor for developing new WSLs, but since only two of the present study's patients had anterior WSLs pre-treatment, this relationship could not be evaluated.

WSLs affect the maxillary and mandibular teeth equally. No significant difference in WSLs was found in the present study between maxillary and mandibular teeth. However, almost half of the cases had no mandibular WSLs after treatment, while only 12% of cases had no maxillary WSLs posttreatment. Julien et al, who only evaluated the anterior segments, found that maxillary teeth were 2.5 times more likely to be affected by WSLs than mandibular teeth.[2] While the present study also showed that the maxillary anterior teeth are more affected than mandibular anterior teeth, the opposite was the case for the posterior teeth (i.e. the mandibular posterior teeth are more affected than the maxillary posterior teeth). When the posterior segments are included, the present study as well as others[3, 23] show no differences in WSLs between the maxillary and mandibular. The mandibular premolars are affected at higher rates than the maxillary premolars causing the mandibular posterior segment to be affected by WSLs more than the maxillary posterior segment.[3]

With respect to specific teeth affected by WSLs, the maxillary canines are the most commonly affected teeth, followed by maxillary laterals, maxillary first molars, mandibular first molars, and mandibular canines. The WSLs affected both the left and right sides of the mouth equally. Previous research confirms that the maxillary lateral incisors are the most commonly affected teeth, followed by the maxillary canine, probably due to the lack of salivary exposure to this area as well as increased plaque retention due to the crown contours requiring close proximity of the brackets to the gingiva.[2, 3, 23, 34] The degree to which the maxillary and mandibular molars are affected depends on lack of adequate

band sealer creating access for saliva and plaque without proper oral hygiene access.[3, 36,109, 110]

S. mutans levels do not accurately predict which patients develop WSLs. The present study showed that the Saliva Check Mutans test could not differentiate between patients who developed WSLs because almost all of them (96%) tested positive for high bacterial counts, regardless of whether they developed WSLs during treatment or not. It has been well established that the placement of orthodontic appliances changes the oral environment and increases levels of cariogenic bacteria. [19-21] This explains why the patients tested in the present study all had high levels of cariogenic bacteria. The threshold of the Saliva Check Mutans test was set for dental rather than orthodontic patients and it was too low to distinguish any differences that may exist between bacterial loads for cases and controls in this study.

ATP bioluminescence with Cariscreen also does not accurately predict which patients develop WSLs. When cariscreen data divided patients into high (>1500) and low (<1500) groups, no statistically significant differences were found between the cases and controls for either maxillary or mandibular ATP bioluminescence levels. There was a tendency for cases to have higher maxillary and mandibular cariscreen scores, but there was so much variability among patients that statistical differences could not be established. Larger samples sizes would have been necessary to establish statistically significant group differences. Some of the variability could be due to the fact that ATP bioluminescence tests determine bacterial activity levels and not bacterial load. Bacterial activity levels change throughout the day based on sugar consumption, oral hygiene, and other factors. As

sugar is consumed, bacterial activity levels increase as salivary bacteria produce acid and expend ATP. During times when bacteria are not exposed to sugar, bacterial activity levels are lower. While patients were required to refrain from eating, drinking, or performing oral hygiene for at least one hour prior to the appointment, compliance could also have been a confounding factor. If the patients brushed their teeth before going to their appointments and over-estimated the amount of time that had lapsed since they brushed, low ATP bioluminescence readings would have been expected.

Buffer capacity is lower than normal among orthodontic patients. The median buffer capacity in the present study was 7 for controls and 6 for cases, indicating a low buffer capacity. A buffer capacity of 10-12 is considered to be normal.[111] Studies evaluating buffer capacity in orthodontic patients have shown mixed results. Some have shown that orthodontic treatment has little or no effect on the salivary buffer capacity.[112, 113] Other studies have reported that orthodontic treatment might decrease buffer capacity slightly, but sample sizes were too small to establish statistically significant differences.[114, 115] Our study does not have the power to determine whether orthodontic treatment does reduce buffer capacity, but all data in our study indicates that a correlation could exist.

Increased sugar consumption increases the development of WSLs. The cases reported eating sugary foods significantly more often than the controls. Dental caries risk factor assessments have shown that repeated exposure to sugar contributes to WSL development by causing longer and more frequent acidic insults to the enamel.[53, 89] Therefore, it is important to inform patients about these risks and suggest reducing between meal snacks and avoiding sugary drinks.

Ideally, this study would have tested patients with actual bacterial culture tests prior to treatment, at 4-week intervals after appliance placement, at appliance removal, and following orthodontic treatment. This would have allowed for better evaluation of differences in pre-treatment bacterial levels and it would have allowed for determination of any differences in the actual bacteria counts between cases and controls. The tests used in the present study did not provide enough information to answer this question. With exact bacterial counts, quantitative assessments could be performed to determine if patients with WSLs have increased levels of cariogenic bacteria. In addition, larger sample sizes are required due to the various confounding risk factors that are difficult to control (e.g. oral hygiene, frequency of sugar intake, fluoride use). However, since 96% of the orthodontic patients tested in this study were positive for high levels of S. mutans which have been found to be high enough to increase risk of developing WSLs, further testing to quantify the exact numbers of WSLs may not provide any further evidence into why some patients develop WSLs while others do not. As further research into what causes dental caries is developed, like salivary or patient characteristics, those variables should be evaluated in orthodontic patients as well.

5. CONCLUSIONS

1. ATP bioluminescence with Cariscreen and S. Mutans levels with Saliva Check Mutans do not accurately predict which patients had developed WSLs.
2. Whether or not pre-existing WSLs increase the likelihood of the developing new WSLs depends on the teeth being evaluated.
3. WSLs affect the maxillary and mandibular teeth equally.
4. An increased frequency of sugar consumption positively correlates with increased development of WSLs.

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APPENDIX A

Figure 1. Comparison of intraoral photographs of anterior segment from initial (picture A) to final (picture B) for determination of new WSL formation (blue arrows) during treatment.

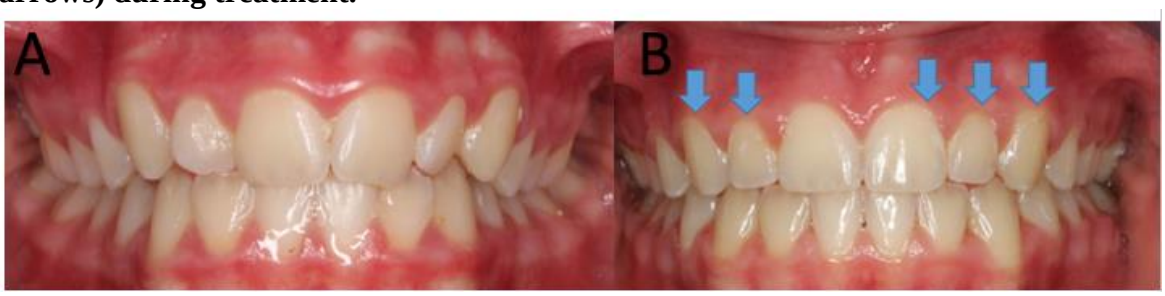


Figure 2. Comparison of intraoral photographs of posterior segment from initial (Picture A) to final (Picture B) for determination of new WSL formation (blue arrows), no change in WSL (yellow arrow), and increased severity/area of WSL (green arrow).

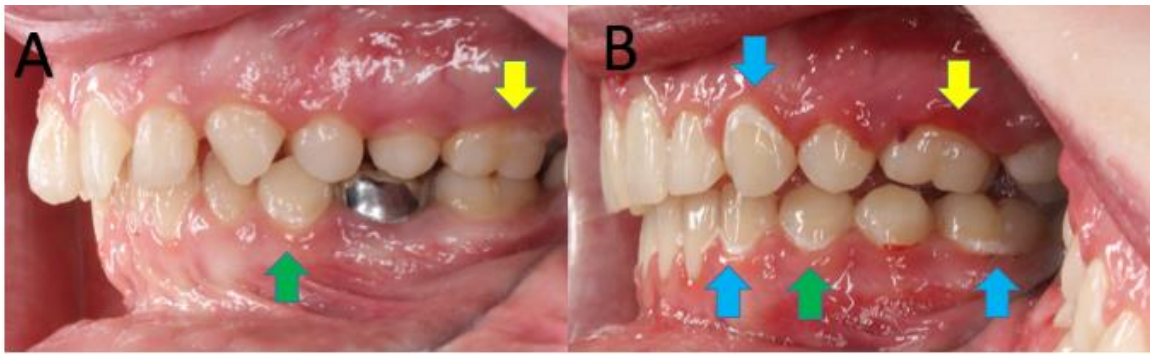


Figure 3. Pre-treatment photographic examples of fluorosis scores: No fluorosis (picture A), Isolated fluorosis with 1-2 teeth affected (picture B), or Generalized fluorosis with 3+ teeth affected (picture C).

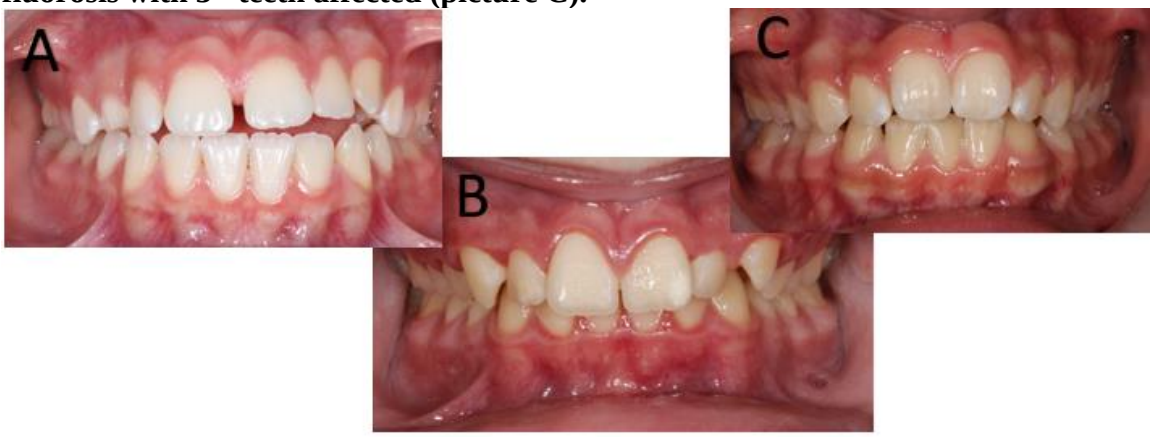


Figure 4. Example of significant decline of OH during treatment with good pre-treatment OH (picture A) and poor final OH score (picture B).

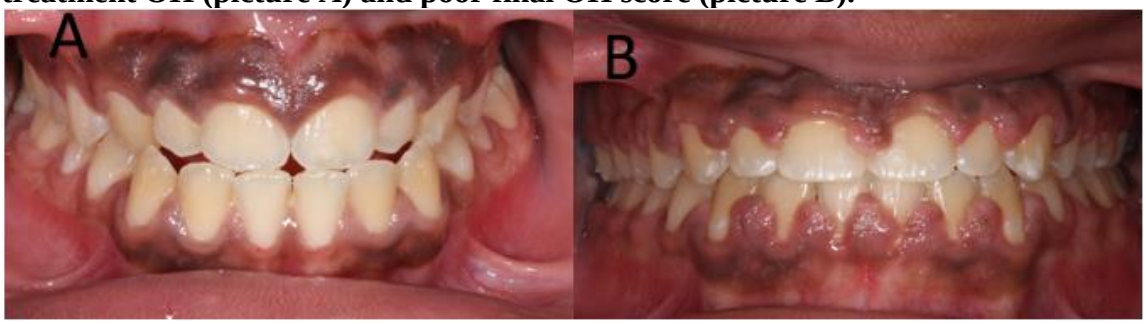
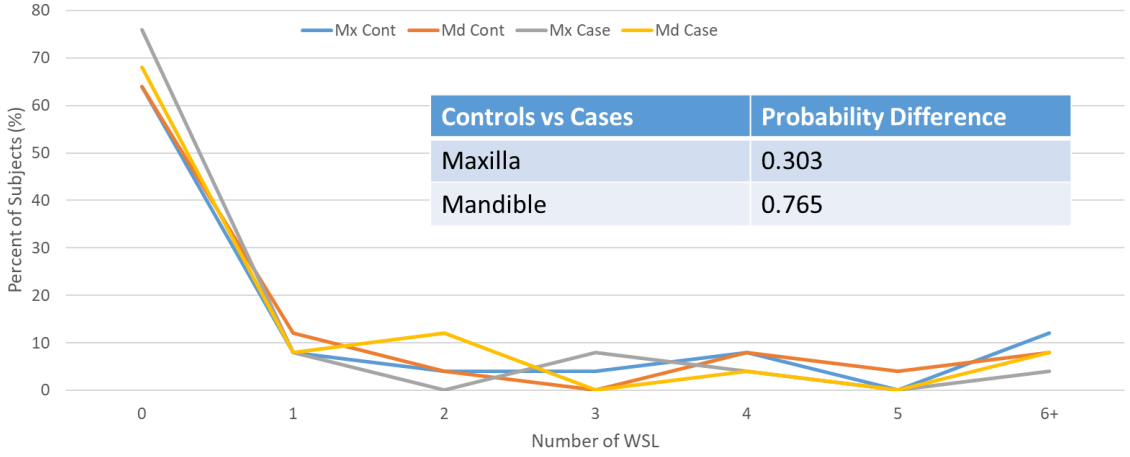


Figure 5. Number of pre-treatment WSLs on maxillary and mandibular teeth of controls and cases, along with probability of between group differences.



Controls vs Cases	Probability Difference
Maxilla	0.303
Mandible	0.765

Figure 6. Number of post-treatment WSLs on maxillary and mandibular teeth of cases, along with probability of within group differences.

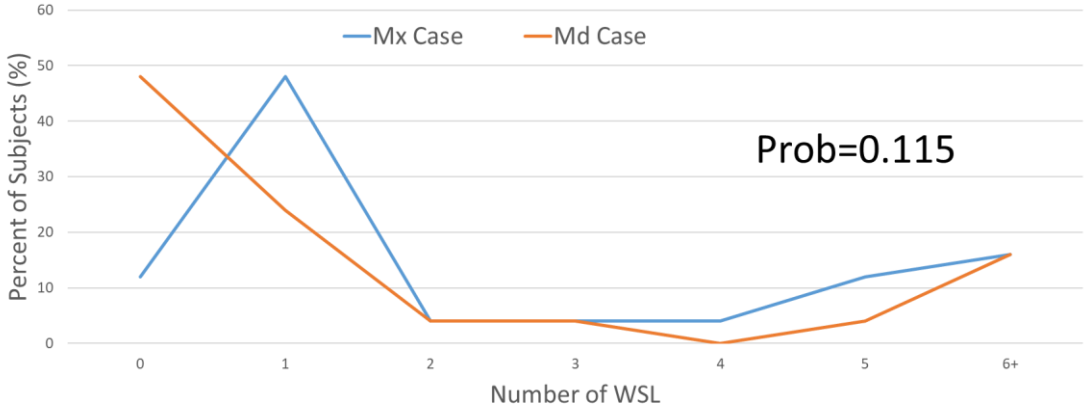


Figure 7. Percent of controls and cases with low and high maxillary cariscreen ATP bioluminescence levels.

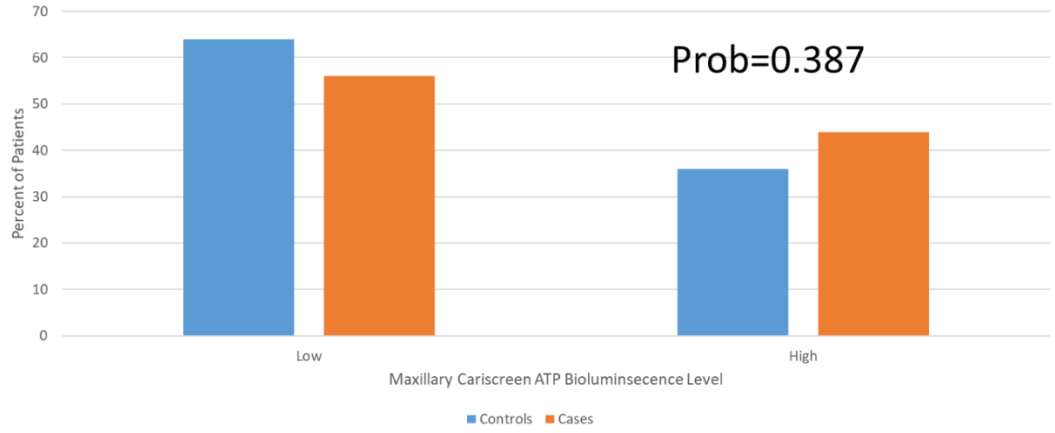


Figure 8. Percent of controls and cases with low and high mandibular cariscreen ATP bioluminescence levels.

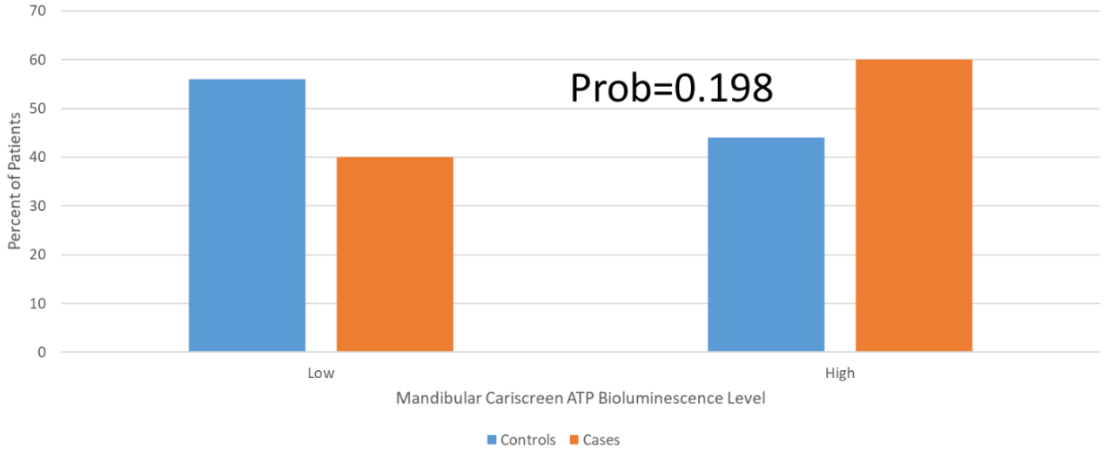
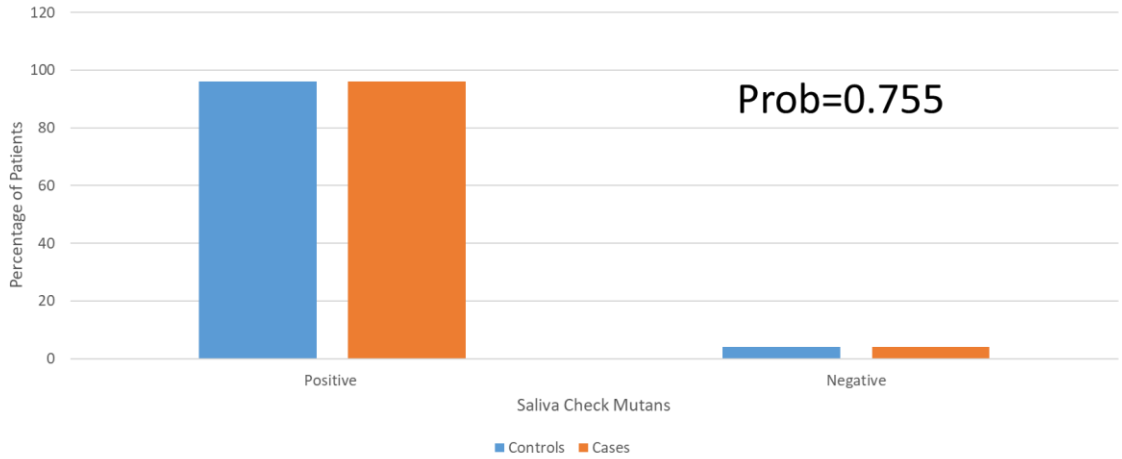


Figure 9. Percent of controls and cases with high and low salivary bacterial levels using saliva check mutans test.



APPENDIX B

Table 1. Criteria used for evaluating pre-treatment and posttreatment oral hygiene status.

Oral Hygiene	Pre-treatment Status	Post-treatment Status
Good	Adequate plaque removal, no plaque visible, no inflammation or gingivitis	Adequate plaque removal, no plaque visible and bleeding only due to gingival trauma during debond appointment
Fair	Less than ideal plaque removal, some plaque or inflammation visible in isolated areas	Less than ideal plaque removal, some plaque or inflammation visible in isolated areas
Poor	Inadequate plaque removal, plaque visible, inflammation present, or gingival hypertrophy generalized throughout mouth	Inadequate plaque removal, plaque visible, inflammation present, or gingival hypertrophy generalized throughout mouth

Table 2. Survey data of controls who did not develop WSLs and cases who did develop WSLs during treatment taken at orthodontic appliance debond appointment.

	Response	Controls	Cases	Prob
Parent Survey Questions				
Does your child see the dentist regularly (at least twice per year) for dental check-ups?	Yes	96	80	0.189
	No	4	20	
Does your dentist apply a topical fluoride tx or prescribe fluoride rinse/toothpaste?	Yes	56	56	1.000
	No	24	24	
	Unsure	20	20	
Does your child's toothpaste contain fluoride (OTC or prescription)?	Yes	92	68	0.067
	No	4	4	
	Unsure	4	28	
Does your child drink fluoridated water?	Yes	36	36	0.776
	No	40	32	
	Unsure	24	32	
Has your child had cavities and/or fillings within the previous 3 years?	Yes	28	20	0.236
	No	56	76	
	Unsure	16	4	
Patient Survey Questions				
Patient's gender	Female	56	52	1.000
	Male	44	48	
Race of patient	White/Caucasian	48	60	0.182
	African American	8	4	
	Hispanic	40	16	
	Asian	4	12	
	Other	0	8	
How often do you brush?	2-3 times a day	92	88	1.000
	1 time a day	8	12	
	Less than once a day	0	0	
How often do you eat sugary foods?	Only with meals	44	4	0.001
	1 to 2 times a day	52	68	
	3 or more times a day	4	28	

Table 3. Pre-treatment and post-treatment intraoral photo evaluation of controls who did not develop WSLs and cases who did develop WSLs during treatment.

Photo Variables	Score	Controls	Cases	Prob
Pre-treatment fluorosis	None	48	48	0.492
	Isolated	16	28	
	Generalized	36	24	
Pre-treatment oral hygiene	Good	64	52	0.470
	Fair	24	40	
	Poor	12	8	
Post-treatment oral hygiene	Good	12	12	0.664
	Fair	52	40	
	Poor	36	48	
Change in oral hygiene during treatment	Slight Increase	0	4	0.631
	No Change	36	32	
	Slight Decrease	52	44	
	Large Decrease	12	20	
Lower fixed retainer	Present	80	72	0.742
	Not Present	20	28	

Table 4. Salivary data of controls who did not develop WSLs and cases who did develop WSLs during treatment taken at orthodontic appliance debond appointment.

Variable	Unit	Controls			Cases			Prob
		Percentiles			Percentiles			
		25	50	75	25	50	75	
Amount of saliva	ml	3.1250	4.7500	7.0500	3.2500	4.5000	7.1250	.954
Buffer	β	5.500	7.000	8.000	6.000	6.000	7.000	.245
Upper cariscreen swab	RFUs	243.000	693.000	3749.500	337.500	1123.000	3162.500	.628
Lower cariscreen swab	RFUs	314.500	1187.000	3045.000	534.500	2492.000	6473.500	.204