Long-term changes of white spot lesions after orthodontic treatment

Dmitry Shungin

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Tutor: Ronny Fors

Epidemiology and Public Health Sciences
Department of Public Health and Clinical Medicine
Umeå University.
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2. Abstract

Background: fixed orthodontic appliance disbalances oral environment, enhancing plaque formation around appliances elements and initiating process of enamel demineralization. Coupled with poor oral hygiene this can result into formation of early caries areas known as white spot lesions (WSL). Both quantitative and qualitative methods are introduced to assess longitudinal changes in WSL following orthodontic treatment and quantitative methods are shown to be more precise and reliable. Quantitative studies on short-term examination of WSL after orthodontic treatment showed increase in WSL after treatment followed by decrease within several months, but not to the pretreatment values. There is a shortage of long-term quantitative studies of WSL which are crucial for assessment of long-term potential risks during orthodontic treatment planning.

Aim: to quantitatively assess area changes of WSL during 12 years of follow-up after orthodontic treatment in comparison to pretreatment status.

Materials and methods: In 30 patients WSLs areas on scanned photographic slides of teeth were evaluated at the beginnig of treatment, at debonding, 1-, 2- and 12 years of follow up after treatment. The upper lateral incisors and the lower canines were studied in each patient. Differences in WSL between the abovementioned time periods and before treatment assessment as well as differences between upper and lower dentitions were tested with Wilcoxon matched-pairs signed ranks test (one tailed).

Results: the sum of areas of white spot lesions were compared with the before treatment readings as a baseline. The sum of areas of WSL before treatment was significantly lower then the sum of WSL areas recorded just after treatment (p<0.01). This was also the fact for sums of WSL areas recorded after 1 year of follow-up (p<0.01) and 2 years of follow-up (p<0.01). The sum of areas of WSL before treatment was significantly higher than the sum of areas of WSL after 12 years of follow-up after orthodontic treatment (p<0.01).

When compared in pairs, the sum of areas of WSL of maxillary teeth was higher than those of mandibular teeth for each measurement sessions (p<0.01).

All actual $p$ values were not changed when they were adjusted with Bonferroni procedure.

Conclusion: The sum of areas of WSLs, induced by orthodontic treatment, does not reach pretreatment values within period of 12 years from debonding. The sum of areas of WSLs on the maxillary teeth is higher than that on mandibular teeth at all measurement sessions (before treatment, at debonding, after 1, 2 and 12 years from debonding).
3. Plain English glossary

*Malocclusion* refers to the misalignment of teeth and/or incorrect relation between the teeth of the two dental arches.

*Bonding* is a procedure of gluing of elements of fixed orthodontic appliance (braces, coils) to the teeth.

*Debonding* is a procedure of removing of elements of fixed orthodontic appliance (braces, coils) from the teeth.

*Remineralization* is the process of restoring minerals in the form of mineral ions in dental enamels.

*Demineralization* is the process of removing minerals, in the form of mineral ions, from dental enamel.

*pH* is a measure of the acidity or alkalinity of a solution. Solutions with a pH less than seven are considered acidic, while those with a pH greater than seven are considered basic (alkaline).
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5. Introduction

During last decades orthodontic treatment has become an integral part of modern life. The survey, conducted in 2003 in UK, showed that approximately 14% of 12-year-old children underwent orthodontic treatment in 2003, while only 5% received orthodontic treatment in 1983[1]. Furthermore, there is a rise in the number of adults undergoing orthodontic treatment, especially among those who were not treated during adolescence[2]. There is evidence that this tendency will mature resulting in even more remarkable increase in number of both adults and children seeking for orthodontic interventions[3].

Studies dealing with expectations after orthodontic therapy figured out that patients and their parents (in case patient is a teenager) expected orthodontic treatment not only to improve their oral health, but also to enhance self-esteem[4]. It is important to underline, that neither patients nor their parents expect improvements in general health after orthodontic therapy, while both parents and adolescents believe that orthodontic treatment can improve social life and self-image[5].

Being a major part of social life, career is also shown to be related with facial attractiveness. There are studies indicating that attractive people are paid more for exactly the same job than less attractive people[6].

Facial appearance plays crucial role in development of self-image[7,8]. Those people who are unsatisfied with their facial appearance tend to be less self-confident and self-esteem than those who are satisfied[8,9]. Furthermore, those dissatisfied with their face, point at their teeth as the major dissatisfying characteristic of their facial appearance[8], and females have more negative emotions about their unstraightened dental arches than males[10,11], especially by spacing, crowding or rotations of teeth[12].

With proper diagnostic and treatment planning contemporary fixed orthodontic appliance can deal with majority of dentofacial problems withing 15 to 22 months[13] and significantly improve both static and dynamic (smile and mimics) facial esthetics[2].

Apparently, as well as any other type of treatment, with all its advantages, orthodontic treatment has some risks. The following complications of orthodontic treatment are described[14-18]:

- relapse, or situation when teeth to different extend move back to their before-treatment positions;
- local demineralization of dental enamel, or white spot lesions;
- enamel wear against metal and ceramic braces during eating or speaking;
- resorption of tooth’s root during their movement;
- periodontal reactions;
- allergy to orthodontic components extraoraly or intraoraly;
- reactions of tempromandibular joints (TMD).

Clearly, all these possible sources of potential damage of orthodontic treatment must be taken into consideration during the development of treatment strategy. This study deals with one of most common complication of orthodontic appliance treatment – local demineralization of dental enamel. Besides being a serious oral health problem as a dental caries lesion, its cosmetics effects may also erode patient attitude towards treatment results.
6. Background

6.1 Contemporary orthodontics.

In general, orthodontics is defined as “the area of dentistry concerned with the supervision, guidance and correction of the growing and mature dentofacial structures. This definition includes conditions that require movement of teeth or correction of malrelationships and malformations of related structures by adjusting relationships between and among teeth and facial bones by applying forces or by stimulating and redirecting the functional forces within the craniofacial complex.”[19] Clearly, severity of various types of orthodontic problems differs. Some types of malocclusion bias basic physiological functions, for example patients with severe forms of open bite have problems with biting food, and for such patients orthodontic treatment is obligatory for improvement of their physiological malfunction[20]. Meanwhile, in many cases orthodontic problems have minor or no functional impact, but only esthetic impacts on patient’s well being[2].

Orthodontic treatment consists of two major parts: active treatment and retention period[21].

During active treatment period actual movement of tooth happens and the orthodontic appliance is systematically presented in the mouth. Depending on weather patient can or can not take appliance away from the mouth as a part of treatment protocol, the orthodontic appliance can be either removable or fixed.

Fixed appliance is the dominating type of modern orthodontic treatment and is presented by braces, bands and arch wires (Figure 1). Braces and bands are fixed on the tooth surfaces with special bonding materials, while arch wires are put into braces’ slots and fixed there with ligatures. Arch wires are pre-fabricated in such way that they resemble the form of well-aligned dentition and if tooth is wrongly positioned, force of ligated arch wire will move this tooth to correct position.

Removable appliance is presented by different types of acrylic plates (Figure 2) and activators and usually used in young children as the first stage of active orthodontic treatment, usually followed by treatment with fixed appliance.
During retention period of orthodontic treatment tooth are hold in the aligned position which was achieved during active treatment stage[14]. The rationale for this is that it takes a lot of time for all orofacial tissues to adapt to the new position of teeth[15,16], and patient must wear special devices (retainers) to keep teeth in this position. Retainers can also be either fixed or removable, but as far as their aim is just to hold teeth rather than to move them, retention appliances have small sizes.

As stated above, the benefits of treatment with an orthodontic appliance have to be weighted against the possible risk of complications. On one hand, some complications are relatively not difficult both to deal with and to study. For example, enamel wear against metal and ceramic braces could be avoided by thorough positioning of braces and regular checking of tooth surface during routing visits during orthodontic treatment period[13].
On the other hand, such complications as relapse after orthodontic treatment, reactions of tempromandibular joints, allergy to orthodontic components or local demineralization of dental enamel are difficult to predict and require well designed longitudinal studies. According to several research reports and systematic reviews, there is a shortage of long-term studies about nearly all of these complications[22-25], while there are a pool of short-term studies, that can misguide both clinicians and patients.

As an example, a survey of the literature shows that those studies stating that orthodontic treatment can contribute to the development of temporomandibular joint dysfunction have low level of scientific significance and are predominantly presented by case reports[25]. In contrast, 20-year follow-up study has reported a tendency for a lower incidence of TMD among patients, who had undergone orthodontic treatment, compared with matched groups of untreated patients[26].

Most demineralisations related to orthodontic appliance treatment do not end up as cavities, but many will show up as white spots, clearly seen on the tooth surface and affects facial esthetics of patient. Although a number of preventive methods are available, the outcome is still dependent on patient cooperation and oral hygiene. There are some studies reporting that areas of dematerialized enamel, that frequently occur during orthodontic treatment[27-30], tend to disappear with time[31], but there is a shortage of longitudinal studies on this topic. The problem with these white spots is that they are clearly seen on the tooth surface and (1) can affects facial esthetics of patient as well as they are (2) considered to be initial form of caries and can result into cavities, that require restorations.

It is reasonable that patients should discuss topic of having these two problems related with white spots, while treating another (malposition of teeth). This can not be done without longitudinal studies on white spots after orthodontic treatment.

To conclude, with all achievements of contemporary orthodontics, there are some risks that should be taken into consideration while planning orthodontic treatment, especially if it is done for cosmetic reasons. But such management of risk analysis can not be performed if the shortage of knowledge on some area of potential risk exists.
6.2 White spot lesions as a complication of orthodontic treatment.

Areas of local demineralization of enamel are reported to be a significant complication of orthodontic treatment with fixed appliance (Figures 3,4)[27-29]. Prevalence of these so called “white spot lesions” (WSL) in patients after orthodontic treatment varies from 15% to 91% [30], with the majority of studies reporting figures around 50-70%[27-29,31-34].

Figure 3. Multiple white spots on upper dentition after orthodontic treatment.

Figure 4. White spot on upper lateral right incisor (after orthodontic treatment).

The term “white spot lesion” is defined by Fejerskov et al[35] as “the first sign of caries lesion on enamel that can be detected with the naked eye” and used along side with terms “initial” or “incipient” lesions. Although these terms are meant to deal with description of caries as a dynamic process or, in other words, describe one of the first stages of caries development (Figure 5), such lesions may stay stable for many years. In this case they are called “arrested
lesions”[35] and are usually not treated and in majority of cases “healing” of these spots happens in form of natural abrasion of superficial enamel during tooth brushing and eating.

![Diagram](image)

Figure 5. Progression of mineral loss in relation to time. The angle of the line may vary and is related with cariogenic situation (oral hygiene, frequency of consumption of saturated sugars, fluoridation of water, etc.). “Time” may also vary from weeks to months and years. Note that white spot lesions (“enamel lesion”) are the first visible signs of caries, and the border between visible and invisible lesions can not always be clearly defined (adapted from Fejerskov et al[35])

High prevalence of white spot lesions after orthodontic treatment is explained by difficulties in performing oral hygiene procedures on teeth with braces and arch wires[32]. Caries lesions develop in sites where microbial associations have possibility to form a plaque that is not taken away or disrupted by mechanical forces (abrasion) during routine tooth brushing or flossing.

Microorganisms from dental plaque associations near or/and on elements of fixed orthodontic appliance trigger the process of lesion formation.[35] The process starts with dissolution of the crystals in the enamel, resulting into changes of its optical characteristics. As a result enamel becomes opaque (visible for naked eye or “white”), as far as sound enamel disperses the light less than porous enamel [36]. Due to differences in the refractive index of air (stated as 1.00), water (1.33) and enamels crystals (1.66), it is possible to make a deduction that those lesions that require air-drying to become visible (opaque) has lost less amount of minerals than a lesion which is visible without being air-dried. This fact is confirmed by histological studies, that detected a lower level of porosities and less deep penetration of the lesion into the enamel in lesions visible only after being air-dried in comparison to lesions which are visible without being air-dried[35].
Hypothetical sequence of events, leading to formation of white spot lesions during orthodontic treatment with fixed appliance was described by several authors[35,36].

The following factors contribute to the formation of white spot lesions:

1. **Microbial factors.**

A bacterial plaque that forms near elements of fixed orthodontic appliance is an example of microbial biofilm[37], where associations of microorganisms form three-dimensional structures in matrix of environmental remnants (food, saliva, extracellular material). Among various bacteria, constituting microflora of the dental plaque[38], mutans streptococci are shown to have significant role in the initiation and progression of dental lesions[39,40]. Several studies also reported an increase in proliferation of lactobacilli in patients undergoing fixed appliance therapy[41,42]. Major characteristics of these cariogenic microorganisms include[43]:

- ability to rapid conversion of fermentable sugars, in comparison with other plaque bacteria;
- ability to produce extracellular and intracellular polysaccharides, which contribute to the growth of the biofilm;
- ability to perform metabolism of sugar in low pH, when other microorganisms can not function, giving mutans streptococci and lactobacilli possibility to produce acid in presence of acid environment, which is crucial in caries etiology.

2. **Salivary factors.**

Saliva is shown to be an important factor that contribute to the process of loosing and gaining minerals within the enamel-biofilm system[35]. In general, saliva contains sufficient amounts of calcium and phosphate and is supersaturated with respect to components of enamel, making demineralization impossible under normal conditions (provided that saliva is not enriched with acids of diet, gastric or medicinal origins). It was shown that in order to make saliva unsaturated with respect to mineral structure of enamel, saliva’s pH must be below 5,5[35].

The bacterial plaque, which is present on or/and near elements of fixed orthodontic appliance, restricts access of saliva to tooth surface. As a result properties of plaque fluid differ from those of saliva. The most important difference is ability of plaque fluid for numerous short time (approximately one minute) pH fluctuations at the border between enamel surface and biofilm, usually initiated by sugar intake. These fluctuations lead to constant multiple short time increase and decrease of concentration of calcium resulting into multiple de- and
remineralization processes. Cumulative result of such changes is dissolution of the enamel minerals (hydroxyapatites and fluorhydroxyapatite).

Process of dissolution of subsurface hydroxyapatites (major mineral component of enamel) is triggered by these fluctuations in pH of plaque liquid, while fluorhydroxyapatites (major mineral component of surface of enamel) are less soluble and stay stable. Such conditions of lesion formation lead to production of fluorhydroxyapatite in the surface layer of enamel, with simultaneous dissolution of hydroxyapatites in subsurface layer[35].

Salivary flow rate is also important for WSL formation. It is shown that level of supersaturation of saliva with respect to mineral enamel component increases during stimulation of saliva’s flow rate, for example during chewing. Due to anatomical position of salivary glands, lower front regions of dental arches experience higher amount of flow resulting into smaller pH drop after exposure to substrates compared with plaque[37]. Gorelick et all[44], described higher incidence of white spot lesions after orthodontic treatment with fixed appliance in maxillary anterior teeth, which also can be attributed to salivary flow.

3. Oral hygiene and diet

Presence of orthodontic appliance in the mouth makes tooth brushing much more difficult, enhancing the process of plaque attachment (1) on the elements of orthodontic appliance as well as (2) in areas between teeth that brush can not reach[36]. In addition, fixed elements can prevent tongue and cheeks from removing small pieces of food from interteeth areas and supply those areas with saliva. As a result process of multiple plaque formation is taking place in the mouth increasing risk of caries initiation process, which was described earlier. That is why proper oral hygiene is crucial during orthodontic treatment with fixed appliance as well as diet.

Both frequency and amount of intake of sugary food have etiological importance for caries initiation process[45,46], especially if in presence of fixed elements there is a possibility for easier adhesion of such food to elements of fixed appliance and enhance caries process.


Along side with proper patient motivation for standard tooth brushing and dietary recommendations, different kinds of fluoride therapy are introduced and shown to be effective in reducing incidence of white spots lesions during fixed orthodontic treatment[32,47,48]. Fluoride supplement in form of mouthwashes or rinses turned out to be successful[47-50], but patients compliance is usually not satisfactory[32]. Fluoride-releasing electrometric ligatures are also tested as potential solution to the problem to iatrogenic demineralization[51].
Another possible way of bringing fluoride to the tooth surface during orthodontic treatment is to use fluoride-releasing bonding materials. Glass-ionomer cements are proved to have such property[52,53] and are currently intensively used in orthodontic practice. There is some evidence that these materials can decrease incidence of white spots after fixed appliance treatment when compared with acrylic-based materials[31,54,55]. Long term studies indicate beneficial effect of glass-ionomer cements by lower incidence of white spot lesions after orthodontic therapy[31].

To conclude, fixed orthodontic appliance disbalances oral environment, enhancing plaque formation around appliances elements and initiating process of enamel demineralization. Oral hygiene and dietary recommendations are crucial for inhibiting this process. Fluoride supplementation in different forms can also prevent formation of white spot lesions.
6.3 Methods of WSL detection and assessment.

There are several methods to detect and assess local demineralization of enamel.

Visual method is the most obvious and the most commonly used and clinicians eye is utilized as the major diagnostic tool. Areas of decalcification are detected by visual inspection of tooth surfaces for discoloration (white, brown), changes in texture (smooth or rough) and reflection (shiny or not)[35]. Ekstrand et al. [56] found correlation between some easy to detect features of lesions and their histological properties: white spot lesions, which are detected only after air-drying, tend to be located in the outer ½ layer of the enamel. The depth of a white or brown area of decalcification which is clearly seen without air-drying is extended to the inner half of the enamel and the outer one third of the dentin.

To compare visual evaluations of white spots made by different clinicians or/and to trace changes of spots in longitudinal studies several scales are described[44,57]. In orthodontic practice such scale method, based on visual evaluation, was introduced by Gorelick et al[44] and has become a standard method on visual assessment[31,34,58,59]. The major disadvantage of this qualitative method is that it is insensitive to small and moderate changes in white spot lesions and thus it can hardly be utilized in comparative studies, especially long-term ones.

Another way of visual investigation of WSL is based on the quantitative assessment of photo images of white spots by measuring its area. In general, photography is quite frequently used in clinical practice and is shown to be useful in assessment of enamel discolorations in epidemiological studies[60,61] and in description of WSL after orthodontic treatment[28,31,44,62].

By photographic image it is possible to calculate area of white spot with good precision even if slides are taken at slightly different angles[63]. Levels of validity, repeatability and agreement of such measurements are shown to be equal to more sophisticated and reliable methods of enamel demineralization assessment[64,65,66]. Both scanning of 35 mm captured slides and digital images can be utilized for purpose of measurement of enamel demineralization[67].

A number of quantitative optical methods exist for assessment of enamel demineralization. Among them are two laser fluorescence methods, which were utilized in studies on white spot lesions after orthodontic therapy[68, 69-72]. The first is called DIAGNOdent and is based on the principle that spectrum of carious tooth enamel exposed to red light induced fluorescence considerably differs from that of sound enamel[73]. Another is called quantitative light-induced fluorescence (QLF). With this method it is possible to measure a changes in mineral structure of enamel by detecting decrease in fluorescence intensity of enamel, when enamel is exposed to
violet-blue light[74]. Both methods are successfully used for identification of early caries, but require special equipment.

Yet another quantitative method of early caries lesions assessment, stereomicroscopic examination of tooth sections, is considered to be a “gold standard“[75], but this method requires extraction of teeth, which is not possible in majority of studies. Alongside with optical methods, sectioning allows assessment of depth of WSL, which can be important for complex assessment of WSL.

Quantitative methods of WSL assessment should be used in long-term studies of WSL because they are less subjective. Areas of demineralization can remain on the teeth for several years after the end of orthodontic treatment[35] and thus be a considerable cosmetic problem, but there is a shortage of conclusive long-term studies on changes in WSL after orthodontic treatment and qualitative methods could be insensible to detect them.

To conclude, quantitative methods are shown to be more precise and reliable that quantitative[57], and among quantitative, photographic technique is the only method that does not require sophisticated equipment and skills, and have comparable with other methods validity, repeatability and agreement[66,76,77]. There is a shortage of long-term quantitative studies on WSL.
7. Aim.

The aim of the study was: to quantitatively assess area changes of white spot lesions during 12 years of follow-up after orthodontic treatment in comparison to pretreatment status.
8. Materials and methods

Patient sample

The patient sample was made up by 60 consecutively recruited orthodontically treated in Umeå university clinic patients, who have been earlier described in a 2-year follow-up of white spot lesion changes[31]. Actual addresses of these patients were traced in the National Population Register and places of residence for 59 out of 60 patients were possible to trace. Forty one of these 59 patients were living within 50 km distance from the clinic and were invited by phone for a follow-up examination. Five out of the 41 patients could not be reached and another 6 more declined to participate in a clinical examination. Thus 30 (12 males, 18 females) out of 60 patients earlier assessed before orthodontic treatment, at debonding and 1, 2 years after debonding were examined and they formed the final sample of the study. This sample has earlier been used in a preliminary report of long-term quantitative changes of WSL[78].

Four teeth – the upper lateral incisors and the lower canines – in each patient were selected for evaluation, as they were previously reported to be most frequently affected by WSL after orthodontic treatment[44]. In three patients, treated only in the upper jaw, only upper laterals were evaluated.

Characteristics of the sample are presented in the Table 1.

Table 1. Number and age of participants at different examinations, time from debonding and number of slides analyzed at different periods of follow-up.

<table>
<thead>
<tr>
<th>Examination</th>
<th>Number of participants</th>
<th>Median age/Minimum-Maximum (decimal years)</th>
<th>Median time from debonding (decimal years)</th>
<th>Number of slides (equal to number of tooth surfaces) chosen for measurements of WSL area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>30</td>
<td>13.7/11.6-16.7</td>
<td>-</td>
<td>114</td>
</tr>
<tr>
<td>At debonding</td>
<td>30</td>
<td>15.5/12.5-18.8</td>
<td>-</td>
<td>112*</td>
</tr>
<tr>
<td>1 year follow-up after debonding</td>
<td>30</td>
<td>16.7/14.0-19.9</td>
<td>1.2</td>
<td>114</td>
</tr>
<tr>
<td>2 year follow-up after debonding</td>
<td>30</td>
<td>17.5/15.1-20.9</td>
<td>2.0</td>
<td>114</td>
</tr>
<tr>
<td>12 year follow-up after debonding</td>
<td>30</td>
<td>27.9/25.0-34.5</td>
<td>12.4</td>
<td>113**</td>
</tr>
</tbody>
</table>

* two slides were excluded because of poor quality
** one slide was excluded because of poor quality
Type of study: prospective cohort study, performed during 1988 and 2004.

WSL documentation

Photographic technique.

Color photos of teeth were taken according to standardized procedure with magnification equaled to 1:1 utilizing the camera set, consisted of Nikon F4 camera and Medical Nikkor Lens 120mm f/4.0 IF with incorporated ring flash (Nikon Corporation, Tokyo, Japan). Although it was impossible to use the same film during the whole period of study, attempt was made to use positive film with the same characteristics every time photos were taken.

To minimize reflections and glares on the enamel surface that could mask its original color two photos of each tooth, dried with air, were taken during every photo session: the first photo from the pair was taken parallel to the center of tooth surface and the second at 2-3 degrees angle.

Scanning.

All slides were first scanned using Epson Perfection 4990 Photo scanner (Seiko Epson Corporation, Japan) at 2400 dpi, 24-bit color (normal), and than saved in Tagged Image File Format (TIFF) on external hard drive (Packard Bell store and save 2500, 120Gb).

Randomization.

To ensure blindness during image analyzing stage, all pairs of tooth surface images were copied to another folder of removable hard drive and renamed according to the table of random numbers. Images from each pair were given the same names, except for prefixes “_1” and “_2”, so that the there were utilized exactly two times less random numbers than the total amount of images. These renamed images were copied to the hard drive of personal computer (IBM ThinkCenter) for analyzing. "Print screen” code of file renaming algorithm was stored separately in the sealed envelope by independent person and opened after all measurements were done.

WSL evaluation.

Image analysis.

Analysis of images was made by one investigator. Images were 600% enlarged and analyzed utilizing the FACAD 2.2 software (Ilexis AB, Sweden). First, both images of the same tooth were examined and the one without or if it was impossible, with minimal reflections was chosen. Than borders of white spots lesions, that corresponded to both first and second group of visual examination criteria by Ekstrand et al[79] were hand-outlined (Figure 6) by mouse (IBM
MU29j, International Business Machines Corporation, China). Area of identified white spot’s border was calculated by the software in pixel$^2$ and than transferred into SPSS 12 database (SPSS for Windows 12.0, SPSS Inc., Chicago, IL, USA).

Figure 6. Area of white spot lesions, defined by the hand on the digital picture with the mouse

*Calculation of outcome measure.*

For evaluation of longitudinal changes the sum of all WSL’s from four tooth surfaces of each patient was used.

For examination of differences between upper and lower dentitions, sum of area of WSL on upper and on lower dentition for each patients were utilized.

*Statistical Analysis.*

Possible differences between scanning resolutions of 2400 dpi and 3200 dpi, and repeatability of measurements interclass correlation coefficient was utilized.

In order to analyze changes in WSL during follow-up period and between upper and lower dentitions Wilcoxon matched-pairs signed ranks test (one tailed) was applied, $p<0.05$ was chosen for significant difference.

Sum of areas of WSLs on teeth surfaces before treatment was compared with the following evaluations (after debonding, 1-, 2- and 12 years of follow up after debonding). Differences in sum of areas of WSLs for upper and lower jaw were tested for each measurements session.

Statistical analysis was performed with SPSS 12.0 software (SPSS for Windows 12.0, SPSS Inc., Chicago, IL, USA).

*Ethical considerations.*

The original sample was formed in 1988-1989. Patients were assigned to the study group following informed concern. While processing of data for this report all researchers had no access to patients confidential information, except of name and personal number and all means
were taken not to disclose this information. Spreadsheets with patients names were stored separately from slides.
9. Results.

Methodological results.

A total of 1114 pairs of photo images were evaluated and 567 slides were chosen for WSL assessment, representing 567 vestibular tooth surfaces. The reproducibility of WSL area measurement technique, was tested on 25 slides with time interval of 3 weeks. The interclass correlation coefficient equals was 0.87, showing that the random error of repeated measurements was low.

The difference in scanning resolution of 2400 dpi versus 3200 dpi was tested on 25 slides before the actual scanning of 1114 slides was done. A high agreement between measurements of areas on slides, scanned with two resolutions was found (interclass correlation coefficient was 0.99)

*Interindividual variation of area of WSL.*

The sum of areas of WSL showed a huge interindividual variation and was not distributed normally (Figure 7.)
Figure 7. Distribution of sum of areas of WSL in different measurement sessions during 12 years of follow-up after orthodontic treatment. (A-before orthodontic treatment, B-at debonding, C-1 year after orthodontic treatment, D- 2 years after orthodontic treatment, E- 12 years after orthodontic treatment)
Changes in the sum of WSL areas during 12 year of follow up after orthodontic treatment.

The sum of areas of white spot lesions were compared with the before treatment readings as a baseline (Table 2). The sum of areas of WSL before treatment was significantly lower then the sum of WSL areas recorded just after treatment (p<0.01). This is also the fact for sums of WSL areas recorded after 1 year of follow-up (p<0.01) and 2 years of follow-up (p<0.01).

The sum of areas of WSL before treatment was significantly higher than the sum of areas of WSL after 12 years of follow-up after orthodontic treatment (p<0.01).

Sum of areas of WSLs for upper and lower jaws.

When compared in pairs, the sum of areas of WSL of maxillary teeth was higher than those of mandibular teeth for each measurement sessions (p<0.01) (Table 3).

All actual p values were not changed when they were adjusted with Bonferroni procedure.
Table 2. Sum of areas of white spots (maxillary lateral incisors plus mandibular canines) during 12 years of follow-up after orthodontic treatment (in square pixels) and p-values for differences between the measurements at four time periods in comparison to the before treatment values as a baseline.

<table>
<thead>
<tr>
<th>Area</th>
<th>Before treatment</th>
<th>At debonding</th>
<th>1 year follow-up</th>
<th>2 year follow-up</th>
<th>12 year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median area (25 percentile-75 percentile)</td>
<td>Median area (25 percentile-75 percentile)</td>
<td>p</td>
<td>Median area (25 percentile-75 percentile)</td>
<td>p</td>
</tr>
<tr>
<td>Sum of WSL</td>
<td>6488.8 (0.0-14922.9)</td>
<td>47264.5 (23561.0-115672.0)</td>
<td>&lt;0.001</td>
<td>18325.5 (8642.9-53517.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3. Sum of areas of white spots of maxillary lateral incisors and mandibular canines during 12 years of follow-up after orthodontic treatment (in square pixels) and p-values for differences between them for each measurement session.

<table>
<thead>
<tr>
<th>Sum of WLS areas</th>
<th>Before treatment</th>
<th>At debonding</th>
<th>1 year follow-up</th>
<th>2 year follow-up</th>
<th>15 year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median area</td>
<td>p</td>
<td>Median area</td>
<td>p</td>
<td>Median area</td>
</tr>
<tr>
<td></td>
<td>(25 percentile-75 percentile)</td>
<td></td>
<td>(25 percentile-75 percentile)</td>
<td></td>
<td>(25 percentile-75 percentile)</td>
</tr>
<tr>
<td>Maxillary incisors</td>
<td>5890.0 (0.0-15620.8)</td>
<td>&lt;0.001</td>
<td>31304.0 (15358.8-80901.3)</td>
<td>&lt;0.001</td>
<td>15290.0 (6048.8-33621.1)</td>
</tr>
<tr>
<td>Mandibular canines</td>
<td>0.0 (0.0-0.0)</td>
<td></td>
<td>3555.0 (1396.0-10758.8)</td>
<td></td>
<td>1345.0 (0.0-3826.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1236,5 (0,0- 4095,0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>201.0 (0,0-2705.3)</td>
</tr>
</tbody>
</table>
10. Discussion.

Similar to earlier studies[31,44], in the present study an increase in WSL on vestibular surfaces of teeth as a result of orthodontic treatment was found. A large variation between patients were detected, earlier also reported by van der Veen et al[72]. The possibility of WSL to decrease with time was evaluated in the present study.

Methodology of quantitative measurement of area of WSL.

Photographs and slides have been used in a number of studies on prevalence and long-term assessment of discolorations of enamel of different origins[31,34,58-62].

The method of quantitative assessment of WSL utilized in this study deals with defining borders of WSL on the digital image and then calculating its area. The major advantage of this method is that it allows utilization of conventional camera slides after being scanned, which can be very useful since conventional photography is a common procedure in dental practice and large slide collections exist.

One problem in the method used is whether an increase in scanning resolution will increase precision of measurements. In earlier studies particular resolution was utilized without prior evaluation of resolutions[64,65-67]. Two scanning resolutions were tested in present study for difference and the lower 2400 dpi resolution appeared to be almost equal to 3200 dpi, which is why 2400 dpi was chosen.

The agreement between repeated measurements was also high, which is in line with previous reports on photographic-based techniques for WSL measurements and adds to the evidence that such technique can be routinely utilized for WSL assessment[66,67].

Changes in WSL area.

The present study showed that pretreatment value of sum of WSLs areas for upper lateral incisors and lower canines is lower than the sum at debonding. Such a difference was also found not only for 1- and 2-years after debonding, but after 12 years after the appliance was removed. Although there are no previous data on such a long term evaluation, short term studies describe a regression in WSL, over time as compared to the time at debonding[31,44]. Accordingly, Ogaard and Ten Bosch[80] showed an exponential pattern of remineralization when studying experimentally induced lesions on teeth when followed during a month.

The majority of studies on WSL evolution have a qualitative nature. They report either the incidence of WSL during treatment and describe reduction in percent of WSL-free surfaces[28], or utilize index introduced by Gorelick et al[31,44]. Although qualitative approaches have a low sensitivity to minor changes in WSL, they were able to detect the same trend, as reported in the
present quantitative study. In accordance, Marcusson et al. on the same patient sample as in the present study, but using index by Gorelick et al[44], found a decrease of WSL after 2 years after orthodontic treatment, although pretreatment level was not reached[31].

Other quantitative approaches to WSL assessment, like quantitative light-induced fluorescence (QLF), were predominantly reported in \textit{in vitro} short-term studies of artificially induced WSL. In these investigations a regression of WSL after orthodontic treatment was also shown[69-71].

\textit{Sum of areas of WSLs for upper and lower jaws.}

A higher sum of areas of WSL on maxillary dentition than on mandibular, detected in the present study, was also found by Gorelick et al[44]. These findings could strengthen the theory that a higher incidence and severity of WSL on front upper dentition could be explained by peculiarities of saliva supply.

\textit{Interindividual variation of sum of WSLs areas.}

The great variation on size of area of WSL detected in this study, also described by van der Veen et al[72], can probably be explained by variation in oral hygiene patterns in different individuals and between sexes. None of the subject in present study received fluoride supplementation either during orthodontic treatment or after and only standard recommendations on oral hygiene were given to individuals. Fluorides have shown to be beneficial in preventing localized decalcification during the treatment phase with fixed orthodontic treatment[49,50,81,82]. Although possible advantages of fluoride supplementation during retention phase were not studied, the fact that sum of areas of WSL have not reached the pretreatment level, even after 12 years after the active phase of treatment was finished, suggest that strategies of fluoride supplementation both during and after orthodontic treatment should be examined deeper.
11. Conclusion.

The results of the present study indicate that sum of areas of WSLs, induced by orthodontic treatment, do not reach its pretreatment values within period of 12 years from debonding. The sum of areas of WSLs on the maxillary teeth is higher than that on mandibular teeth at all measurement sessions (before treatment, at debonding, after 1, 2 and 12 years from debonding).
12. Impacts of the study.

Conclusions of the present study suggest that both patients and doctors should consider WSLs as a potential complication of orthodontic treatment that, to some extend, can remain for up to 12 years on tooth surfaces. Patients should also be aware that maxillary frontal teeth have larger areas of WSLs than mandibular, which could influence their decision about treatment because upper frontal teeth play more important role in facial esthetics.

13. Future areas of study

2. Fluoride supplementation during and after orthodontic treatment.
3. Long-term study of influence of different bonding materials on severity of WSL


