THE STUDY OF GRAY-SCALE VALUE CORRELATED WITH INTERPROXIMAL CARIES LESION PROGRESSION BY DIGITAL INTRAORAL BITEWING RADIOGRAPHS

NIRAYA KORNSOMBUT

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Master of Sciences in Dentistry (Operative Dentistry) -
Type A 2
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By NIRAYA KORNSOMBUT

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science in Master of Sciences in Dentistry (Operative Dentistry) - Type A 2 of Naresuan University

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Title	THE STUDY OF GRAY-SCALE VALUE CORRELATED WITH INTERPROXIMAL CARIES LESION PROGRESSION BY DIGITAL INTRAORAL BITEWING RADIOGRAPHS
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ABSTRACT

Grayscale value is an adjunct for the caries detection in some dental radiographs, due to its distinguishability of initial proximal carious lesion. This study aims to correlate the gray-scale value of digital intraoral bitewing radiographs with progression level of carious lesions. The sixty samples were recruited from orthodontic patients. Sound, non-restored human premolars containing no restorative material and extracted for an orthodontic purpose were used in this study. Teeth with carious lesions no farther than middle one third of dentin on the proximal side were served as experimental teeth, while teeth without lesion were served as controls. Before extraction premolar tooth, patients fulfilling the inclusion criteria were allocated individually to take the radiographs under a standard bitewing condition by one examiner, the mean grayscale values were calculated. Using a hard tissue macrotome, ground sections, 1 mm thick, were serially and mesio-distally cut in a parallel direction to each anatomical crown's long axis and observed with a stereomicroscope. Intra-and inter-observer agreements were assessed with a Kappa statistic. Statistical scores and histological gold standard indicated the diagnostic ability. A Simple linear regression test was a statistical method that performs to summarize and study relationships between the size of approximal carious lesion and mean grayscale value. And the result presented a significant relationship between

the size of approximal carious lesion and the mean grayscale value. This indicated that 75.1% of the size of approximal carious lesion can be explained by that mean grayscale value with a 0.05 significant level. This is quite high so predictions from the regression equation are reliable. So that, it is possible to define a standard mean grayscale value for the detection of an initial caries in the future study.

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CHAPTER I

INTRODUCTION

Dental caries, in other words known as tooth decay, is one of the most prevalent chronic diseases of people worldwide. Continually, people are susceptible to this disease throughout their lifetime. (1) Dental caries is described as a localized chemical dissolution of tooth surface caused by metabolic events taking place in the dental biofilm covering the affected area. The acid is produced when sugars in foods or drinks react with bacteria present in the dental biofilm on the tooth surface. The acid leads to a loss of calcium and phosphate from the enamel, this process is called demineralization. (2) Dental caries is also defined as biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the demineralization and remineralization of tooth. The balance between pathological and protective factors impacts the initiation and progression of caries. It is a dynamic process and able to be modified by protective factors. (3) It can be arrested and potentially reversed in its early stages, but is often not self-limiting and without proper care, caries can progress until the tooth is destroyed.(4) It has been found recently that improved oral hygiene practices with fluoride in dentifrices, fluoride mouth rinses and frequently professional fluoride application have greatly reduced the prevalence of dental caries by arresting early caries lesions. There is no need to treat non-cavitated carious lesion by surgical removal and restoration. Should dentists detect this type of lesion early and give patient an intervention on perfect timing, it can be remineralized and arrested.(1)

Interproximal caries lesions progress between the contacting proximal surfaces of two adjacent teeth. They first appear clinically as opaque regions and are caused by the loss of enamel translucency at the outermost enamel between the contact point and the top of the free gingival margin. Interproximal caries on posterior teeth is usually difficult to identify on radiographs. The early and accurate diagnosis of a proximal caries lesion can lead to immediate preventive therapy, in order to avoid extensive tooth loss. Nowadays, concept of operative dentistry is changing from extensive way to minimal intervention, so early detection of proximal caries has become a major topic of interest. The more rapid detection of proximal caries the less the natural tooth loss. Information on surface-specific dental caries patterns is a useful source in deciding which preventive strategies is proper to use. (5) Visual and clinical examination in detection of early proximal caries lesion is challenging due to enamel thickness of marginal ridge and tight contact of adjacent teeth.

Various diagnostic methods in the management of dental caries have been used to determine the presence of caries and its extent, to monitor the course of caries progression, and to evaluate the efficiency of treatment. (6) Visual and clinical examination combined with bitewing radiography have been recommended as the methods of choices for proximal caries detection. Posterior bitewing examination should capture an image of the crowns of the teeth from the distal surface of the canine to the distal surface of the most posterior erupted molar without overlapping. Intraoral bitewing radiographs can be obtained by either film or digital sensors. Digital systems offer reduced radiation exposure, time saving, image enhancement and ease of image storage, retrieval, and transmission.

However, non-cavitated lesions, whose assessment is essential for future clinical trials, cannot be detected accurately by radiography.(7) The advantages of a digital system are to manipulate image contrast and brightness and to magnify images. Several studies showed that the diagnostic accuracy in detecting carious lesions was equivalent to or slightly higher than the conventional film system.(8)

Consequently, an initial carious detection is recommended by using digital image processing methods.(9, 10) There are some existing techniques such as a modification of the grayscale value range of all image pixels (contrast, density, and brightness) or a filtration of an image to emphasize or remove some certain features (smoothing, sharpening, and an edge enhancement). (11)

It is possible to improve the effectiveness of an initial caries detection by using grayscale differentiations. A previous *in vitro* study confirmed that a grayscale intensity of a digital radiograph was affected by the demineralization of enamel at a clinical manifestation of a carious lesion, when compared to the gold standard of microscopic features. The result suggested that the mean grayscale value of the non-cavitated lesion was significantly different from that of the cavitated one, with the insignificant difference from that of the sound enamel ($p > 0.05$). (11) However, some modifications of the research methodology are necessary to define a standard mean grayscale value for the detection of an initial caries.

The present study was preformed to identify the gray-scale value of digital intraoral bitewing radiographs for detecting interproximal caries lesions using ImageJ software. Histological examination of extracted human tooth was used as the gold standard.

Research Aim

To correlate the gray-scale value of digital intraoral bitewing radiographs with progression level of carious lesions.

Research Significance

The data from this study can be applied as a guideline for the general practitioners to differentiate dental carious lesions.

Research Scope

This was the *ex-vivo* study performing on the human premolar teeth of orthodontic patients at faculty of dentistry, Naresuan University, Phitsanulok, Thailand. Before human premolar tooth extraction, digital intraoral bitewing radiographs were taken, and histological examination were performed after extraction.

Keywords

Grayscale value Interproximal caries lesions, proximal caries, digital intraoral bitewing radiographs, digital radiograph, cavitated carious lesion, non-cavitated carious lesion

Research Hypothesis

The gray-scale value of digital intraoral bitewing radiographs is correlated with level of caries lesion progression by using ImageJ software.

CHAPTER II

LITERATURE REVIEW

Dental caries

Dental caries is a post-eruptive bacterial infectious disease defined by a demineralization process that involves the mineralized dental tissues. It is a high prevalent oral disease all over the world and the main reason of tooth loss. Socio-economic and demographic conditions are related to prevalence of dental caries among the population, including behavioral manners.(12) Dental caries is also defined as a complex, chronic, progressive, multifactorial disease intermediated by pathogenic factors that may cause tooth damage (regular exposure to dietary carbohydrates, dry mouth, and an acid-producing biofilm capable of fermenting sugars and producing a decrease in pH) and opposing protective factors that may protect teeth from harm (fluoride, sealants, normal salivary flow, and buffering capacity, and products such as antibacterial agents).(13) The disease progresses in both the crowns and roots of teeth, and it can occur in early childhood as an aggressive tooth decay that affects the primary teeth of children. The risk for caries consist of physical, biological, environmental, behavioral, and lifestyle-related factors such as high numbers of cariogenic bacteria, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, inappropriate methods of feeding infants, and poverty. (1)

Etiology of Dental caries

There are several important characteristics and major etiological factors regarding dental caries which the dentists should consider before negotiating the treatment to prevent the diseases. The first factor is acid-producing bacteria. An exposure of these bacteria to dietary fermentable carbohydrates will produce small chain organic acids that are small enough to diffuse into the tooth subsurface causing the dissolution of tooth mineral called demineralization.(13)

Microorganisms,particularly aciduric streptococci, lactobacilli, diphtheroids, yeasts, staphylococci, and certain strains of sarcinae, can process enough acids to demineralize tooth structure. The mutans streptococci (MS) has been concerned as the major and most virulent of the caries-producing organisms. Accordingly, MS has been targeted in a large share of research. (14)

Previous study showed an extensive review of the literature regarding the etiology of caries. Loesche concluded that the evidence suggests that MS, possibly *Streptococcus sobrinus*, and lactobacilli are human odontopathogens. He defined that aciduricity appears to be the most consistent characteristic of MS and is associated with its cariogenicity. He also noticed that other aciduric species, such as *S. sobrinus*, may be more critical in smooth-surface decay and are perhaps associated with rampant caries. Loesche concluded the review with the recommendation that treatment strategies that inhibit the colonization of MS may have a profound consequence on the incidence of caries in humans.(15)Accordingly, the requirements for the carious process may be depicted by the Venn diagram seen in Figure 1. (16)

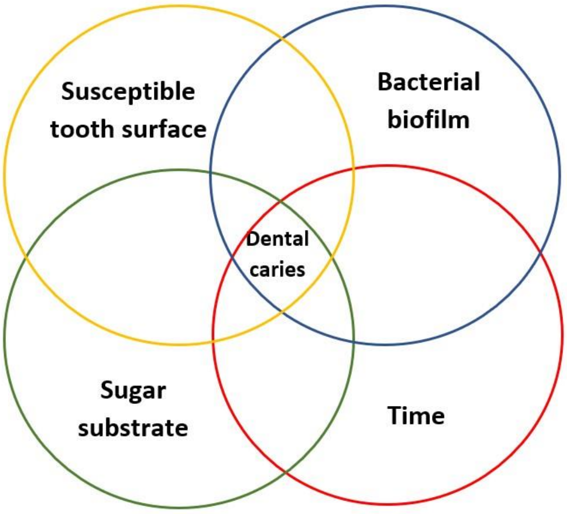


Figure 1 Venn diagram illustrating.
Venn diagram illustrating the requirements for caries to occur, modified from Ricketts et al. 2011. (16)

Nevertheless, this diagram is excessively simplistic and implies that the disease process and its progression are predictable. Clinically, some tooth surfaces that are frequently covered in plaque do not develop detectable caries whereas other tooth surfaces covered with plaque in the same mouth do.

The disease process, which is complex and dynamic, has been impacted by many other factors, such as dietary habits, fluoride, and saliva.

Saliva has benefits in neutralizing these bacterial acids and providing calcium and phosphate to diffuse back into the tooth subsurface. This process is called remineralization. The balance between these pathogenic and protective factors determines whether there will be a net demineralization or remineralization of the mineral in the teeth. (17)

Demineralization over time will possibly result in harmful damage to the tooth. Demineralization occurs at the molecular level where calcium and phosphate are lost from the subsurface and will not be visible by the naked eye in the earliest stages of demineralization. (13)

Continued demineralization from the earliest stage is not definite and lesion arrest is possible by simply disrupting the plaque biofilm on the surface of the tooth at regular intervals. Very early lesions which are not detected clinically may therefore not progress to clinically detectable white spot lesions and the carious process is better represented by Figure 2.(16)

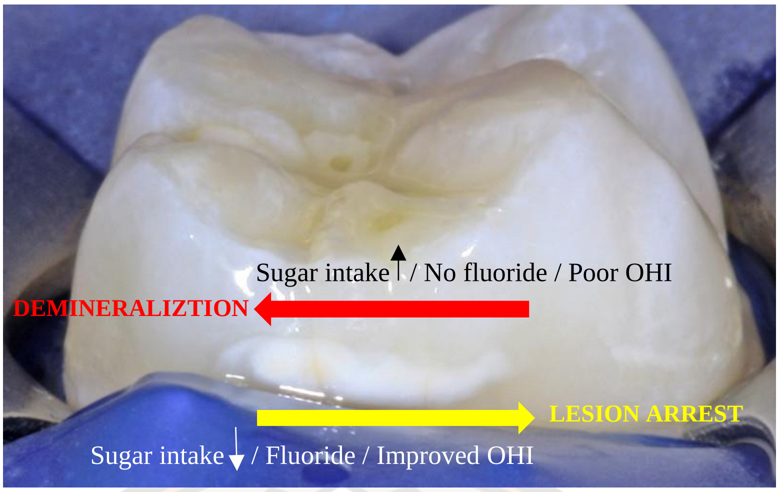


Figure 2 Dental caries diagram.
Diagram showing the dynamic nature of the carious process and how it can be influenced by some external factors, modified from Ricketts et al. 2011. (16)

It is often said that if an early white spot lesion develops, remineralization can take place. While complete remineralization and resolution of the lesion is unlikely, the clinically apparent remineralization of a white spot lesion may also be due to its surface abrasion following improved oral hygiene procedures. Remineralization of partially demineralized apatite crystals in the surface zone of an enamel lesion has been reported by Figure 3.

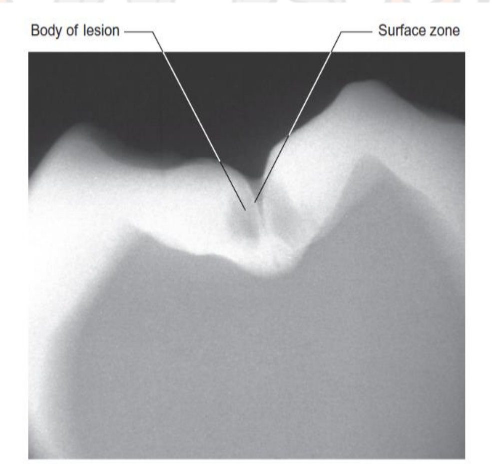


Figure 3 High-definition macro-radiograph of a section of a tooth.
This figure showing two enamel lesions on the bilateral walls of a fissure. The well-mineralized surface zone and body of the lesion can obviously be seen. (16)

Nonetheless, the relatively well-mineralized surface zone performs also as a diffusion barrier to ions, making it less likely that, in the underlying body of the lesion, supersaturation with respect to apatite will occur with subsequent mineral deposition. Prevention of the disease is therefore our primary aim. However, for many patients, this primary prevention fails, and lesions develop. Caries risk assessment and early caries detection are important so that further prevention can be targeted to those patients and lesions that are in need. In this situation, a method of monitoring the lesion is also essential to determine the effectiveness of the chosen preventive approach.(16)

Remineralization of incipient subsurface lesions may happen if the surface layer of the enamel remains intact. Saliva, which is supersaturated with calcium and phosphate and has acid-buffering capability, diffuses into plaque, where it neutralizes the microbial acids and repairs the damaged enamel. The time required for remineralization to replace the hydroxyapatite lost during demineralization is determined by the age of the plaque, the nature of the carbohydrate consumed, and the presence or absence of fluoride. (18)

Fluoride not only greatly enhances the rate of remineralization of enamel by saliva but also results in the formation of fluorohydroxyapatite during the process, which increases the resistance of the remineralized enamel to future attack by acids. Moreover, fluoride also has antimicrobial effects. (19)

However, if allowed to progress, incipient lesions will eventually cause visible changes that can be seen on the tooth. The visible changes seen on the teeth are sometimes called caries lesions. One of the earliest signs of a caries lesion appears as white-spot lesions, and if not arrested and reversed, the weak subsurface of the tooth will eventually breakdown and form what is commonly called a cavity or cavitation.(20) Cavity treatment usually involves surgical removal of tooth structure and dental restoration (fillings, crowns, root canals). However, the cavity is only a sign of the end stages of caries disease, fillings cannot treat the cause of the disease.

Thus, a caries risk assessment (CRA) is critical to identify the risk factors and protective factors for caries disease.(21) Once the CRA is completed, the clinician can manage risk factors using strategies that combine oral products and behavioral change to decrease the pathogenic risk factors and increase the protective factors. Current dental science supports caries management by risk assessment, in which risk factors for caries disease are identified and treated similar to the medical approach of treating risk factors for heart disease (22).

The approach to primary prevention should be based on common risk factors. Secondary prevention and treatment should focus on management of the caries process over time for individual patients, with a minimally invasive, tissue-preserving approach. (1)

The approximal white-spot lesion

The shape of the white-spot lesion is determined by the distribution of the microbial deposits between the contact facet and the gingival margin, which results in a kidney-shaped appearance. On the proximal smooth surface there will typically be an interdental facet area surrounded by an opaque area extending in the cervical direction. The cervical border of the lesion is formed according to the shape of the gingival margin (Figures 4, 5). It is often possible in such surfaces to see thin extensions of the opaque area, in buccal and lingual directions, running in parallel with the gingival margin. Some of these lesions will be active and others inactive owing to different efforts to control the microbial accumulations, for example with dental floss.

Figure 4 Active, non-cavitated early white-spot lesions.
Lesions on mesial surfaces of lower second molars are easily observed once the adjacent tooth is extracted. The shape of each lesion indicates the stagnant areas where the biofilm (dental plaque) remained undisturbed. In the most demineralized areas in the center of the lesions, the porous enamel has taken up stain. (Courtesy of Dr.Niraya Kornsombut)

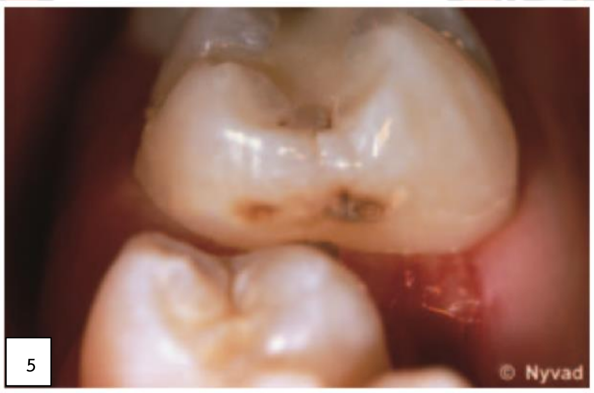


Figure 5 Active, discolored lesion on first molar with obvious small cavity.
Note that the cavity contains microbial deposits (dental plaque). (Courtesy of Professor Bente Nyvad) (23)

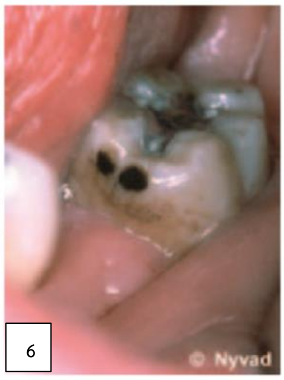


Figure 6 Figure 7 Severely inactive discolored lesions.
Lesions can straightforwardly be diagnosed once the adjacent tooth is extracted. (Courtesy of Professor Bente Nyvad) (23)

Figure 8 Approximal lesions
Lesions are difficult to detect by direct visual inspection. (Courtesy of Professor Bente Nyvad) (23)

Pathology of dental caries

The progression and morphology of the caries lesion vary, depending on the site of origin and the conditions in the mouth. The time for progression from non-cavitated caries to clinical caries (cavitation) on smooth surfaces is estimated to be 18 months ± 6 months. Peak rates for the incidence of new lesions occur 3 years after the eruption of the tooth. Occlusal pit-and-fissure lesions develop in less time than smooth-surface caries. Poor oral hygiene and frequent exposures to sucrose-containing or acidic food can produce non-cavitated ("white spot") lesions in 3 weeks. Radiation-induced xerostomia (dry mouth) can lead to clinical caries development in 3 months from the onset of the radiation. Caries development in healthy individuals is usually slow compared with the rate possible in compromised persons.(24)

The classical description of enamel lesion histology has been based on the incipient lesion positioned at the cervical margin of the interdental facet on the proximal surfaces. Typically, as described in the previous section, the lesion appears triangular in sections cut through the central lesion part. Carious dissolution follows the direction of the rods. Systematic measurements of enamel porosity along traverses following the rod direction make it possible to understand the morphogenesis of the conically shaped approximal lesion. (25)

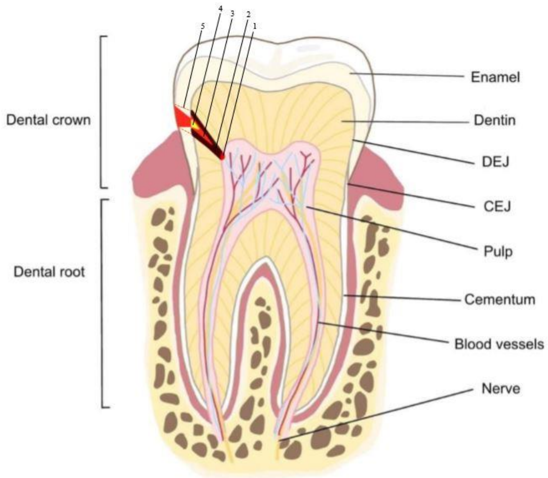


Figure 9 Illustration of progressive stages of lesion formation.
1: Reactive dentin; 2: sclerotic reaction or translucent (transparent) zone; 3: zone of demineralization; 4: zone of bacterial invasion and destruction; 5: peripheral rod direction, modified from Bjørndal et al. 1991. (25)

A typical lesion shows in Figure 10. A line is drawn which has been designated the central traverse, in the rod direction from the deepest point of lesion penetration to the surface. The highest degree of tissue porosity is always observed along this line, irrespective of lesion depth. Measurements of the surface-layer thickness where the central traverse crosses the surface disclose a gradual increase in surface-layer thickness in relation to lesion depth. Comparisons of surface-layer thickness within lesions showed that the peripheral part of the surface layer was always thinner than the central part, thus probably reflecting a less advanced stage of lesion progress in the lesion periphery.

Initiation spread and development of the approximal lesion are simple reflections of the specific environment created by the microbial communities on the enamel surface in the approximal space. On the other hand, if bacteria are offered similar growth conditions anywhere in the dentition by allowing biofilms to become established, for example beneath an orthodontic band positioned so that a space is created between the band and the enamel surface, then the metabolism in this biofilm produces lesions with the advancing front of the lesion running parallel to the outer enamel surface.(4)

Dentin reactions to caries progression

Enamel caries and dentin caries are described as two independent entities. This convention is to some extent explainable, as the two tissues differ markedly from each other in terms of both developmental origin and structure. The enamel is avascular and acellular and cannot respond to injuries, whereas the dentin and the dentinal cells, the odontoblasts, are integral parts of the pulpo-dentinal organ and thus considered to be a vital tissue possessing specific defense reactions to external insults. As will be remembered, the enamel is a microporous solid and hence it is understandable that stimuli from the oral cavity pass through the tissue into the pulpo-dentinal organ, even in intact enamel. With increasing porosity as a result of enamel demineralization it is to be expected that the underlying pulpo-dentinal organ reacts.

The most common defense reaction by the pulpo-dentinal organ is tubular sclerosis, which is deposition of mineral along and within the dentinal tubules, resulting in their gradual occlusion. Caries is another stimulus that accelerates tubular sclerosis, a process which requires the presence of a vital odontoblast (Figures 11, 12).

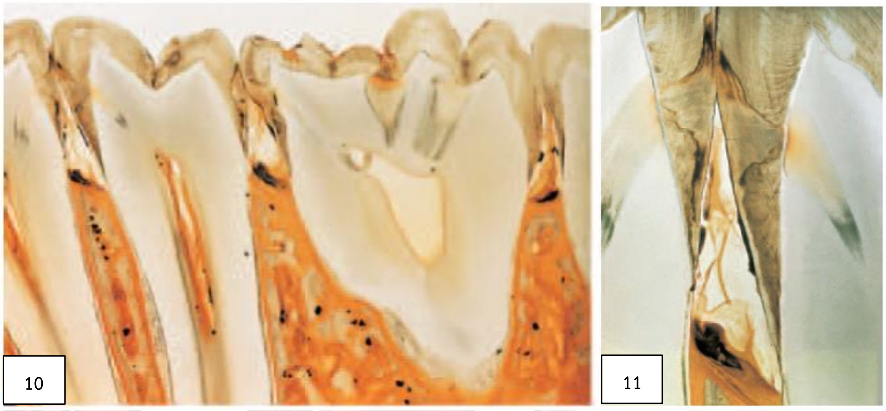


Figure 10 Figure 11 Histological ground sections in the mesiodistal direction through human mandibular premolars and molars.

In the approximal surfaces caries lesions extend at a varying depth towards the dentin. Note how reactions in dentin (the translucent zone) and pulp may appear even at these stages of lesion development, which may not be recorded in a rapid clinical examination and may also be missed in a bitewing radiograph.

Figure 12. This is a higher magnification of the approximal space between the premolars. This shows how the lesions penetrate in depth below the contact area. The approximal space appears partly empty because substantial shrinkage occurs during tissue preparation (the gingiva is edematous and swollen) and some of the microbial deposits are lost. (Courtesy of Professor T. Yanagizawa from the Hanagawa collection.)

The tubular sclerosis observed in conjunction with caries has been described because of either initial mineralization of the peritubular space followed by calcification of the odontoblast process, or an initial intracytoplasmic calcification followed by a secondary periodontoblastic mineralization. In addition to the presence of intratubular hydroxyapatite crystals, large rhombohedral crystals have often been observed and identified as whitlockite crystals. (26) (27)

At the light microscopic level, it is not possible to distinguish between the different form of sclerosis. In sections, the obturated dentinal tubules appear translucent because the mineral in the tubules makes the tissue more homogeneous, reducing the scattering of light passing through the affected tissue. Therefore, sclerotic dentin is often mentioned to as translucent (transparent) dentin or a translucent zone (Figure 13).

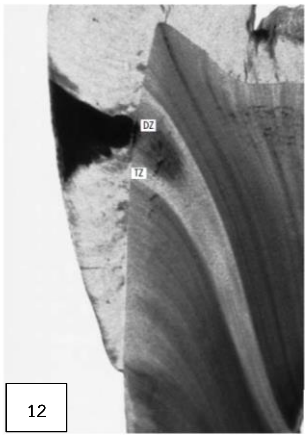


Figure 12 Ground section of active approximal lesion examined in transmitted light.

The triangular enamel lesion reaches the enamel–dentin junction with demineralization of the outer dentin (DZ) and sclerotic reactions (TZ) corresponding to the less advanced peripheral parts of the enamel lesion. (Courtesy of Professor Ole Fejerskov) (4)

Figure 13 Microradiograph of ground section through inactive approximal lesion.

Lesion has been arrested for many years. It can be seen in the enamel redeposition of some mineral, corresponding to the bottom of the cavity, unless the peripheral dentin demineralization remains unchanged after lesion arrest. (Courtesy of Professor Ole Fejerskov) (4)

Detection of dental caries

Hypocalcified enamel does not represent a clinical problem except for its esthetically objectionable appearance. The surface texture of a non-cavitated lesion is unaltered and is undetectable by tactile examination with an explorer. A more advanced lesion develops a rough surface that is softer than the unaffected, normal enamel. Softened chalky enamel that can be chipped away with an explorer is a sign of active caries. Using an explorer tip can cause actual cavitation in a previously non-cavitated area, requiring, in most cases, restorative intervention. Similar non-cavitated lesions occur on the proximal smooth surfaces, but usually are undetectable by visual or careful tactile (explorer) examination.

Non-cavitated enamel lesions sometimes can be seen on radiographs as a faint radiolucency that is limited to the superficial enamel. When a proximal lesion is clearly visible radiographically, the lesion may have advanced significantly, and histologic alteration of the underlying dentin probably already has occurred, whether the lesion is cavitated or not (Figure 15).(24)

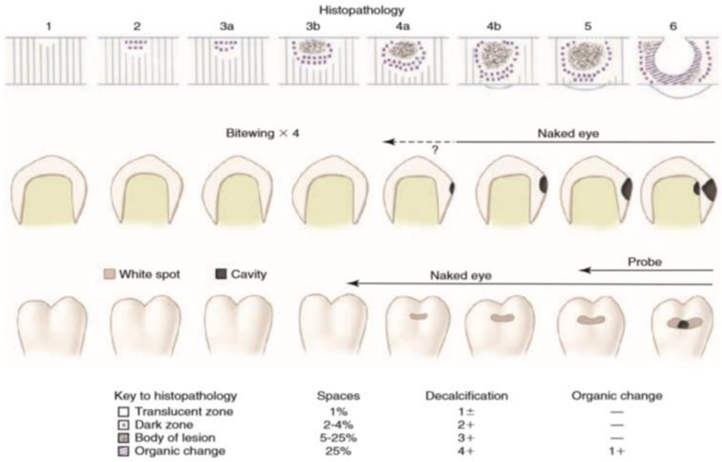


Figure 14 Schematic representation of developmental stages of enamel caries lesion correlated with radiographic and clinical examination.
Cavitation occurs late in development of the lesion and before cavitation remineralization is possible. (7)

Detection devices: The oldest device used for detecting carious lesions is the X-ray machine, besides the probe. Radiography is reliable for detecting carious lesions in approximal tooth surfaces but considered unreliable in occlusal surfaces, especially for diagnosing carious lesions in enamel and in the outer one third to one half of the dentine. (28)

Fibre-Optic Trans-Illumination (FOTI) develops to be a greatly reliable device for detecting carious lesions in approximal surfaces, especially in anterior teeth. (28)

On the other hand, an infrared laser fluorescence device (e.g., DIAGNOdent; Kavo GmbH, Bibberach, Germany) has been informed to be ineffective in detecting carious lesions in occlusal surfaces because it detects not only organic carious tissues and porphyrins from bacterial metabolism, but also other organic material such as plaque, calculus, stain and food remnants. Its validity is more compromised by the presence of enamel hypomineralization of origin other than that of dental caries. (28)

Quantitative light-induced fluorescence (QLF, Inspektor, Amsterdam, The Netherlands) also has similar drawbacks, which applies the fluorescence differences between sound and demineralized enamel to detect and quantify enamel carious lesions, however its reliability seems to be higher than the infrared laser fluorescence-based devices. (28)

A new system using light fluorescence technology (Sopralife, Acteon, Bordeaux, France) applies a different wavelength than QLF to detect carious lesions, in conjunction with a camera. Currently, the value of QLF systems for carious lesion detection in clinical practice seems to be limited. (28)

Other methods, for example electrical impedance (CarieScan PRO™, CarieScan Ltd, Dundee, Scotland) and photothermal radiometry (Canary System™, Quantum Dental Technologies, Toronto, Canada) have recently been developed. However more research is required before they can be advised. (28)

It has been presented that both X-ray and FOTI devices are suitable for carious lesion detection on approximal surfaces and that infrared laser fluorescence and light-induced fluorescence devices are not adequately reliable for assessing carious lesions in pits and fissures of occlusal surfaces. This also applies to the deciduous dentition, in which newer technology-based systems have not been supported to be reliable for the accurate detection of carious lesions on approximal surfaces.

Consequently, different techniques should be used for assessing carious lesions on occlusal and smooth tooth surfaces. One technique employs visible tactile methods. (28)

Carious lesion detection shifted back to visual-tactile detection methods in the second half of the 1990s because of the absence of a properly validated and reliable carious lesion detection device. The World Health Organization (WHO) had developed visual-tactile detection methods, which was based on a ‘yes/no’ clearly cavitated dentine lesion, yielding decayed, missing and filled (DMF) teeth scores. DMF score has been data. However, this data was very cut-off point, and the fact that caries prevalence and carious lesion development have declined in many industrialized countries. Accordingly, there were reasons for epidemiologists to subsequently include the assessment of enamel lesions in caries assessment indices. (28)

The International Caries Detection and Assessment System (ICDAS) was developed by a group of epidemiologists. This two-digit enamel and dentine carious lesion scoring system has recently received much attention. It was developed for use in epidemiological surveys, research, dental education and in practices. The index, when used in epidemiological surveys, has received some criticism, could not be applied correctly and was unable to properly allow the reporting of findings. (28)

Previously the launch of ICDAS, Nyvad published her ‘Nyvad-index’, which permits the assessment of enamel and dentine carious lesions as well as the activity/inactivity of enamel carious lesions. The index has been used recently in several studies and appears to be valid. (28)

The Pulpal Involvement Ulcerations Fistula Abscess (PUFA index) was introduced by Monse and coworkers with the intention of alerting the dental / medical / educational communities about the poor state of dentitions of children in the Philippines. A novel visual one-digit caries assessment index was reported recently. It includes non-cavitated and cavitated carious lesions, pulpally involved and abscessed teeth, as well as sealed, restored and lost teeth. (28)

In developing the index, experience gained from applying the ICDAS II and PUFA indices in the field were important. The index is termed Caries Assessment Spectrum and Treatment (CAST). It has been validated for face and content, while construct validity and reliability testing is on-going.(28)

Carrying out an oral investigation based on assessing teeth with cavitated dentine lesions only (DMF) should be considered a screening exercise. If the investigation is conducted for healthcare planning purposes, enamel carious lesions should be assessed as well, even if in clinical practice or when conducting an epidemiological survey. (28)

The ICDAS II and Nyvad-index may be suitable in a clinical practice setting, although the number of studies supporting this assumption is low. The same caveat applies to the recently developed epidemiological indices PUFA and CAST, which appear to be promising, but need further research. (28)

Caries lesion detection by the medical provider

The term caries has been used to describe various parts of the tooth that are bacterially infected, stained, white in appearance, and actually cavitated. To solve this problem, the profession has called for more universally accepted precise terminology.

The ADA published the Caries Classification System (ADA CCS) in 2015. This publication has two helpful tables that can visually train clinicians simply by comparing what they see when they look in the patient’s mouth with the tables. Figure 16 shows all possible appearances of the teeth at 3 different sites displaying what the tooth would look like from healthy to all stages of caries lesion progression based on the site where the clinician is looking. Figure 16 shows what a sound, initial, moderate, and advanced caries lesion would look at the chewing surface (occlusal surface), in between teeth via a dental radiograph (approximal) and the visible facial (cheek side) and lingual (tongue or palate side) surfaces. It also includes more precise nomenclature should the clinician so choose to use it. (13)

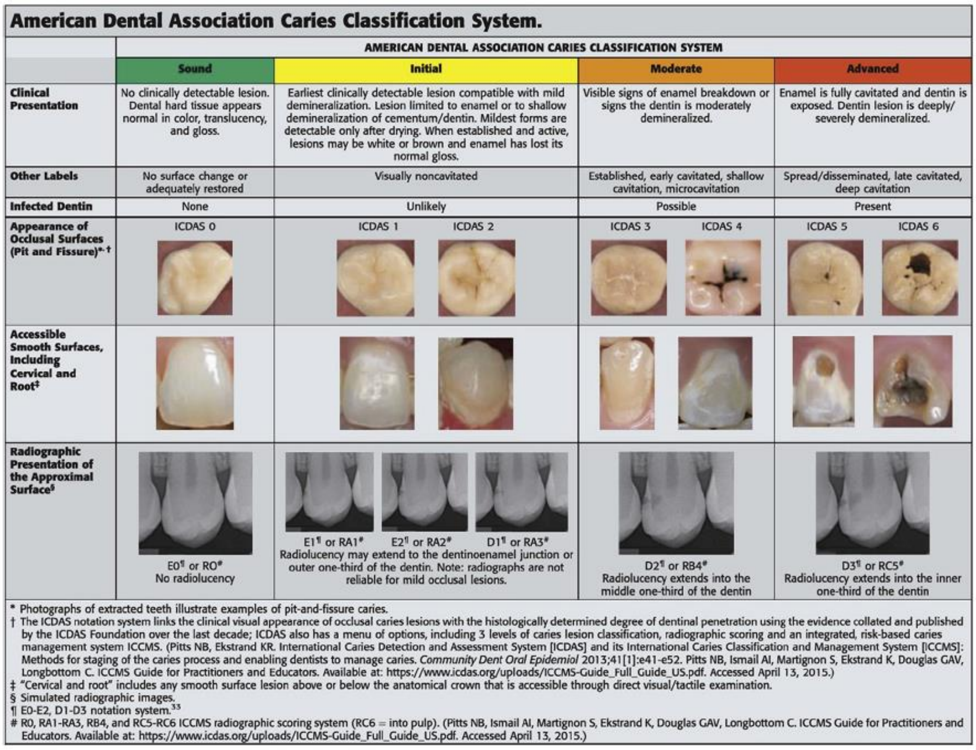


Figure 15 American Dental Association Caries Classification System.
(20)

Table 1
Characteristics of active and inactive lesions

	Caries lesion activity assessment descriptors	
Activity assessment factor	Likely to be inactive/arrested	Likely to be active
Location of the lesion	Lesion is not in a plaque stagnation area	Lesion is in a plaque stagnation area (pit/fissure, approximal, gingival)
Plaque over the lesion	Not thick or sticky	Thick and/or sticky
Surface appearance	Shiny; color: brown-black	Matte/opaque/loss of luster; color: white-yellow
Tactile feeling	Smooth, hard enamel/hard dentin	Rough enamel/soft dentin
Gingival status (if the lesion is located near the gingiva)	No inflammation, no bleeding on probing	Inflammation, bleeding on probing

From Ekstrand KR, Zero DT, Martignon S, et al. Lesion activity assessment. Monogr Oral Sci 2009;21:63–90; with permission.

Figure 16 Characteristics of active and inactive lesions.
This figure describes how the lesion would look if it is active (progressing) or inactive (arrested). An inactive (arrested) caries lesion does not need to be treated. (20)

Detection of lesions on contacting approximal surfaces

Detection of lesions on contacting approximal surfaces (i.e., the sides of adjacent teeth that are touching each other) of posterior teeth is also a challenge, and the inadequacy of clinical visual and tactile methods is the reason that use of ionizing radiation for bitewing radiographs is still sanctioned. However, the same systematic review of high-quality studies showed that, for approximal surfaces, radiographs had an overall sensitivity of 50% and a specificity of 87%. (29)

Thus, using conventional clinical and radiographic methods, the dentist will detect only about half the lesions present and, could misclassify sizeable numbers of sound surfaces as decayed. Radiographs are not very helpful for anything but advanced dentinal lesions on occlusal surfaces (sensitivity 39%, specificity 91%). The consequence of diagnostic errors depends on the treatment strategy used.(1)

There is a previous study that compares the visual caries classification system ICDAS II with conventional (CR) and digital radiography (DR) for diagnosis of non-cavitated caries on free proximal surfaces and examines the potential of micro-computed tomography (MCT) to substitute histological examination for the in vitro caries assessment. The result is Visual examination (ICDAS II) reached significant higher sensitivity (0.92–0.96) and negative predictive value (0.9–1) than radiography. Also, the radiographic modalities presented significantly higher specificity (0.93–1) and positive predictive values (0.92–1) than the ICDAS II criteria. The overall accuracy performance of radiographic modalities was related to the diagnostic threshold. MCT did not agree with histological validation at each disease severity scale. (30)

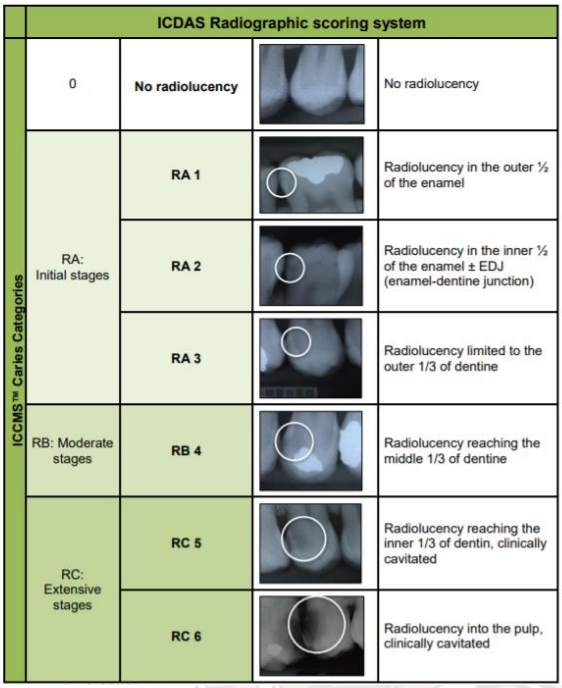
Staging coronal caries lesions radiographically

Radiographic information adds significantly to clinical findings in terms of finding lesions at different stages of progression. Radiographs help estimate the depth of caries demineralization into enamel and dentin. Depth is not always associated with the presence of cavitation, particularly on approximal surfaces. Clinical investigations in a country with low caries progression rates revealed that, on average, 32% of radiographically visible lesions that extended into the outer third of the dentin manifested cavitation; in contrast, 72% of lesions extending into the inner 2/3 of the dentin were cavitated. Clinically cavitated lesions or lesions with obvious dentine radiolucency (deeper than the outer 1/3) on the occlusal surface are heavily infected in the dentin beneath the enamel dentin junction. For establishing whether a lesion has progressed or not, two radiographs with a time lapse between are required. If radiographs are available, the first step is to grade coronal caries lesions on posterior teeth according to the scores in Figure 17.

The International Caries Classification and Management System - ICCMS™ - deliberately incorporates a range of options designed to accommodate the needs of different users across the ICDAS (International Caries Detection and Assessment System) domains of clinical practice, dental education, research, and public health. The ICCMS™ system seeks to provide a standardized method for comprehensive caries classification and management but recognizes fully that there are different ways for implementing such systems locally. ICCMS™ builds on the evidence based ICDAS system for the staging of caries. It also maintains the flexible approach of the ICDAS "wardrobe" which provides several approved options for categorizing the disease according to local and/or specific needs, preferences and circumstances.

The International Caries Classification and Management System - ICCMS™ - is linked to ICDAS. While ICDAS provides flexible and increasingly internationally adopted methods for classifying stages of the caries process and the activity status of lesions, ICCMS™ provides options to enable dentists and the dental team to integrate and synthesize tooth and patient information, including caries risk status, in order to plan, manage and review caries in clinical practice.

The ICCMS™ classifies posterior tooth surfaces radiographically. Both the reproducibility and accuracy of this scoring system has been reported to be substantial to excellent. The evidence indicates that the radiographic penetration depth, at which one can reliably predict that the tooth surface is cavitated and dentine is heavily infected, is in the region of radiolucency deeper than the outer third of the dentine. This corresponds to scores 4, 5 and 6 in the ICCMS™ radiographic scoring system. With faster caries progression rates, cavity formation can also be expected in cases scored as 3 in the above system. It must be appreciated that different conventions exist in different countries for classifying the severity of lesions where operative care is required. More evidence is needed to reduce international variation on this issue.(31)



ICCMS™ Caries Categories	ICDAS Radiographic scoring system		
0	No radiolucency		No radiolucency
RA: Initial stages	RA 1		Radiolucency in the outer ½ of the enamel
	RA 2		Radiolucency in the inner ½ of the enamel ± EDJ (enamel-dentine junction)
	RA 3		Radiolucency limited to the outer 1/3 of dentine
RB: Moderate stages	RB 4		Radiolucency reaching the middle 1/3 of dentine
RC: Extensive stages	RC 5		Radiolucency reaching the inner 1/3 of dentin, clinically cavitated
	RC 6		Radiolucency into the pulp, clinically cavitated

Figure 17 ICDAS/ICCMS™ radiographic scoring system.

Bitewing radiograph for detection of caries lesions

Bitewing radiography still be used in individual caries diagnosis and clinical trials for detecting demineralization produced by microbial activity on the tooth surface (caries lesion). Radiography takes a part in monitoring lesion development over time, since remineralization procedures can arrest or reverse progression of the lesion and lead to changes in mineral quality and quantity. The evidence for dental caries is not restricted to the levels of surface cavitation, and radiography can add information about many of the clinical stages of the caries process at approximal surfaces and the more advanced stages on occlusal surfaces. The most appropriate technique, low-dose receptors, and selection criteria must be considered.(32)

For a posterior bitewing examination, the recommendation is that it should capture an image of the crowns of the teeth from the distal surface of the canine to the distal surface of the most posterior erupted molar. The clinician must use two #2-sized dental films or one long bitewing film (size #3) in each side of the mouth to cover this area in an adult patient with erupted third molars. (32)

The benefit of 1 long film over 2 conventional films is that the patient receives less radiation. In contrast, significantly more overlapping surfaces, cone cuts, and missing surfaces have been found in bitewing examinations undertaken with 2 long films compared with examinations with 4 conventional films. (33)

Type of radiographic systems

There are two main types to the radiographic system. The first one is Conventional system (Analog) that refers to old-fashioned film and requires developing and fixing with chemicals. The second one is Digital system which can be divided into two subtypes (Direct digital and Indirect digital). Direct digital system refers to sensors that send a digital image directly to a computer and is also knows as

DR or digital radiography. Indirect digital uses plates that are run through a scanner to obtain the digital image which is then sent to the computer. This is also known as CR or computed radiography.

Digital radiography

Digital radiographic receptors are brought into use in the dentistry so, projection errors and retakes should be considered. There are two basically different concepts for digital bitewing radiography: solid-state sensors (Charge-Coupled Device [CCD] and Complementary Metal Oxide Semiconductor [CMOS] technology), where a cord connects the receptor and the computer, and storage phosphors, which appear like film and must be processed (scanned) after exposure. (32)

Conventional versus Digital radiography

The solid-state sensors are suboptimal for bitewing examination, since not only is the effective radiation field smaller than a #2-sized film, but also the problem lies in their thickness and the cable that must come out of the subject's mouth. General practitioners who work with these sensors complained that they had to double the exposures compared to conventional radiograph technique. (32)

Versteeg et al. reported that technical error in periapical radiography retakes associated with a CCD sensor (28%) was higher than that found with conventional film (6%). (34) Thinner, cordless sensors with larger effective image areas may be available in the future. For field studies, a sensor system connected to a portable computer might be preferred, since the image can be displayed immediately after exposure. In addition, no processing has to be performed, either in wet chemicals as for film, or through scanning as for storage phosphors. (32) Other advantages of the digital systems include reductions in radiation dose and time savings. Unlike conventional films, images obtained from digital systems can be easily manipulated postprocessing (rotation, contrast enhancement, etc.).(35) Meanwhile, the digital systems are based on re-usable receptors, the possibility for cross-contamination should be considered. (32)

Castro et al. suggested that direct digital radiography could be approved for clinical use due to radiation exposure is reduced compare to conventional film. Overall diagnostic accuracy of conventional film and direct digital imaging was comparable in the detection of approximal caries. (35) In accordance with Wakoh et al., they found that direct digital imaging had a shorter working time, reduction in processing errors, and more convenient image storage. (36)

However, both of direct digital and conventional radiography performed unsuccessfully in the detection of enamel lesions. Further investigation is needed to identify an imaging system or enhancement mode to improve the detection of incipient carious lesions.(36)

System	Pros	Cons
Analog film	When done properly, best image quality Least expensive to set-up by far Nothing expensive or delicate to suffer break down	More time and labour intensive On-going expense for film, chemicals, mounts… Environmental issues with chemicals Many opportunities for processing errors, inconsistency in image quality and damage to films
Digital	No chemicals to buy or dispose of No processing errors (though can have setting errors) Less labour – faster 'processing' Speeds learning process Easier sharing of information (print, email, VIN) Small reduction in radiation dose/exposure	Image quality quite variable from brand to brand (basically proportional to cost) Wide range of software packages, some much better than others – confusing options Wide range of prices Requires lots of computer infra-structure and some computer competence

Figure 18 Pros and Cons of Conventional (Analog) film versus Digital film. (37)

Direct digital versus Indirect digital radiography

Intraoral dental imaging has a shift from conventional films to digital modalities over the last few decades. Several advantages of digital imaging have been described: a shorter working time, reduction in processing errors, and more convenient image storage. Two main options of digital radiography are available: direct digital sensors (DDSs) and indirect photostimulable phosphor plates (PSPs).(35) Both types of DDSs charge-coupled devices or complementary metal oxide semiconductors use wired or wireless systems that generate electronic images for immediate display on a monitor. This maximizes workflow unlike conventional films and PSPs, for which additional chemical or scanning processes are required. (35) Nonetheless, the phosphor plates are available in sizes like film. The holders available for bitewing examinations with phosphor plates appear like those for film, while the sensor holders still leave much to be desired.(32)

A previous study comparing two direct digital sensors and two phosphor plate systems for bitewing examinations showed that the first group had more positioning errors. Direct digital sensors most often did not display the most anterior surfaces in a bitewing examination.(38) Although, direct sensors provide less radiation than other technologies. But due to their bulky characteristic, they can be more difficult to position intraorally, thus increasing the chance of image retakes. (35) Moreover, the solid-state sensors are suboptimal for bitewing examination, the problems include smaller effective radiation field, and their thickness, and the cable that must come out of the subject's mouth. (32)

System	Pros	Cons
Direct Digital (DR)	Faster time from exposure to image on screen (but may not be faster to obtain a whole-mouth study) Can leave sensor in mouth to compare image with sensor position and make adjustments to improve positioning	Limited sensors sizes which are thick and rigid (too big for small patients, too small for large patients) Sensors very expensive so damage is a big issue More time spent 'priming sensor' and manipulating software (probably variable between programs)
Indirect Digital (CR)	Sensors for ScanX available in sizes 0 to 4 Sensors are 'cheap' so no catastrophe if animal bites one ScanX I/O can service multiple dental teams simultaneously ScanX Duo less expensive than the more expensive DRystems	More physical 'processing' required to place sensors in scanner Ongoing costs of protective sensor envelops (not a big deal). Need to remove sensor from mouth to get image on screen 4-slot ScanX I/O more expensive than cheaper DR systems

Figure 19 Pros and Cons of Direct digital film versus Indirect digital film.
(37)

Photostimulable phosphor plates (PSPs)

Photostimulable phosphor plates (PSPs) systems are an indirect intraoral imaging modality. Phosphor plates are exposed intraorally and then fed into a scanner to create a digital image. The lack of a cable and the thinner receptor of the PSPs makes them easier to position and better tolerated by patients with small mouths, tori, and strong gag reflexes (39)

The previous study has shown that diagnostic ability of visual inspection, film, charge-coupled device (CCD) sensor, photostimulable phosphor (PSP) sensor and cone beam CT in the detection of proximal caries in posterior teeth performed similarly. (5)

From this information, this study has decided to choose Indirect digital film (PSP) due to no different in diagnostic ability, image enhancement tools and reduced radiation exposure.

Nowadays, digital system for caries detection tools can be analyzed by many methods such as Manual method, Semi-automated and Automated image analysis systems. One of the advantages with digital receptors is the ability for post-processing (image enhancement). Image enhancement is one the example of Manual method for analyzed caries lesion. However, image enhancement have little effect on the validity of detection of demineralization compared to original un-enhanced images.(40)

Automated image analysis systems for Detection of Approximal Caries

Challenges to increase the accuracy and reproducibility of dental caries diagnosis have resulted in the development of automated image analysis systems. Subsequently the automated caries detection program uses digital images for analysis, exposure to radiation is still a concern.(41) However, there is at present little evidence that computer-aided expert programs actually help the clinician in the detection and treatment planning of dental diseases.

Wenzel et al. concluded that the automated caries detection program is less accurate than human observers in detecting approximal caries lesions. (42) In addition, a previous study on developing a neural network started with the design of a network model, then by training and validating this neural network, but the interpretive skills needed to correctly identify dental caries either by visual examination or radiographic interpretation depends on many factors. (41) More recently, Valizadeh et al. designed computer software for detection of approximal caries in posterior teeth and found that the designed software was able to detect a significant number of dentin caries and acceptable measuring the depth of carious lesions in enamel and dentin when compared to the results of histological analysis. However, the software had limited ability in detecting enamel lesions. (43)

Location	Number of Proximal Surfaces With Caries	Number of Surfaces Detected With Software	Percentage of Surfaces Detected With Software	ICC	95% confidence interval for ICC
Enamel Lesions	25 surfaces	15 surfaces	60%	0.609	0.159-0.849
Dentin Lesions	95 surfaces	93 surfaces	97%	0.937	0.906-0.958

Figure 20 Statistical indices for enamel and dentin lesions.
(43)

Cone beam CT for Detection of Approximal Caries

Cone beam CT is an advanced 3-dimensional radiographic modality, which seems much more accurate than intra-oral modalities for displaying cavitation in approximal surfaces. Wenzel et al. found its accuracy seems to exceed conventional modalities in non-restored teeth. Teeth without restorations included in a CBCT examination should be assessed also for approximal surface demineralization and cavitation. (40)

Akdeniz et al. also concluded that the CBCT technology seems to offer advantages over conventional 2-dimensional techniques for determining the depth of small proximal carious lesions in non-restored dentitions. However, restorations, particularly of dense materials, such as amalgam, can cause artefacts that make caries diagnosis difficult resulting in false positive diagnosis. (44)

In contrast, Tsuchida et al. assessed the accuracy of a limited cone-beam volumetric imaging system in detecting incipient proximal caries. They found that limited CBVI system, 3D Accuitomo, could not enhance the accuracy in detecting proximal carious lesions due to mechanical improvements, such as the reduction of noise, the enhancement of image contrast, and the reduction of artifact, would be required. (45) Accordingly, from previous study, there are several disadvantages with CBCT, such as radiation dose, costs and imaging artefacts. CBCT cannot be advocated at current as a primary radiographic examination with diagnosing cavitated carious lesions.

Based on these conflicting reports, Bitewing radiography is still state-of-the-art in diagnosing carious lesions in clinically in accessible approximal surfaces. (40)

Pixel grayscale value of radiograph

A grayscale image is one in which the value of each pixel is a single sample representing only an amount of light, and it carries only intensity information. Grayscale images, a kind of black and white or gray monochrome, are composed of shades of gray. Grayscale images are different from one-bit bi-tonal black and white images which, in the context of computer imaging, are images with only two colors: black and white (also called bilevel or binary images). Grayscale images have many shades of gray in between.

For computing, although the grayscale can be computed through rational numbers, image pixels are usually quantized to store them as unsigned integer, to reduce the required storage and computation. Some early grayscale monitors can only display up to sixteen different shades, which would be stored in binary form using 4-bits. Nonetheless, nowadays grayscale images proposed for visual display are commonly stored with 8 bits per sampled pixel. This pixel depth allows 256 different intensities (i.e., shades of gray) to be recorded, and also simplifies computation as each pixel sample can be accessed individually as one full byte. However, if these intensities were spaced equally in proportion to the amount of physical light they represent at that pixel (called a linear encoding or scale), the differences between adjacent dark shades could be quite noticeable as banding artifacts, while many of the lighter shades would be wasted by encoding a lot of perceptually-indistinguishable increments. Therefore, the shades are instead typically spread out evenly on a gamma-compressed nonlinear scale, which better approximates uniform perceptual increments for both dark and light shades, usually making these 256 shades enough to avoid noticeable increments.

Technical uses frequently require more levels, to make full use of the sensor accuracy (typically 10 or 12 bits per sample) and to reduce rounding errors in computations. Sixteen bits per sample (65,536 levels) is often a convenient choice for such uses, as computers manage 16-bit words efficiently. The TIFF and PNG image file formats support 16-bit grayscale natively, although browsers and many imaging programs tend to ignore the low order 8 bits of each pixel. Internally for computation and working storage, image processing software typically uses integer or floating-point numbers of size 16 or 32 bits. (46)

Challenges have been made to analyze the gray shade distribution inside the lesion in digital images to help distinguish between lesions with and without cavitation. Although gray shade values were significantly lower (areas appearing darker in the image) in carious dentine, no differences could be observed between gray values in cavitated and non-cavitated dentinal lesions.(40)

Meanwhile, there was another previous study that evaluate the performance of a quantitative method, based on pixel gray value measurements, for monitoring caries remineralization. A quantitative analysis was undertaken using Image Tool software. There was a statistically significant difference between the mean pixel values for demineralized, unchanged and remineralized lesions.(47)

ImageJ

ImageJ is an image analysis program for grayscale value measurement that was created at the National Institutes of Health. It is in the public domain, runs on a variety of operating systems and is updated frequently. The ImageJ website has instructions regarding the use of the program and links to useful resources. (37) This program is provided for free use under the GPL v3 license2.It is also user-friendly for both experts and non-experts in image analysis.

From all the above, an investigation of an initial carious lesions (Enamel caries) still cannot be effectively enhanced. Meanwhile, a digital radiograph will be a promising option for an improvement of the detection of an initial caries. In a digital radiography system, an image is made up of a large number of picture elements (pixels). The location of each pixel is identified by a row and column coordinate within the image matrix. The value referred to a pixel represents the intensity (grayscale value) of the image at that location. The grayscale value is correspondent to an X-ray intensity. However, the digital dental radiograph is relied on an amount of the dental tissue demineralization at a 40% mineral loss, its diagnostic ability at the detection of proximal caries is greater than a visual inspection. (48)

In addition, novel digital image processing methods should be utilized for an initial carious detection for example by modifying the grayscale value range of all image pixels (such as contrast, density, and brightness) and by filtrating an image to emphasize some certain features and to a removal of other features (such as smoothing, sharpening, and an edge enhancement). While there has been a consensus result for an initial caries detection, it will be possible to improve the efficacy of an initial caries detection by using only some grayscale differentiations.

A previous study has shown that mean grayscale value of the non-cavitated lesion was significantly different from the cavitated lesion, with a slight difference from the sound enamel. Some modifications of the research methodology are necessary to define a standard mean grayscale value for the detection of an initial caries. (11) Continuously, there was another unpublished study has shown that mean grayscale of non-cavitated lesions was significantly different from that of cavitated lesions by using ImageJ software.

In order to forward this topic, the present study was conducted to set standard of grayscale value and to determine a confirmation that a grayscale intensity of a digital radiograph that affected by the demineralization of enamel at a clinical possibility in the distinction of a carious lesion. This investigation undertook the digital intraoral bitewing radiographs at premolar area in oral cavity and utilized ImageJ software to analyze the grayscale value, compared to microscopic features (a histological gold standard).

CHAPTER III

RESEARCH METHODOLOGY

Ethical submission

Prior to the start of this study, the proposal has been approved by the Ethics Committee of Naresuan University. Patients who sign a consent form were accepted. Then, patients were assigned into registration number in order to minimize the risk of bias in this study.

Population and Samples

The study was conducted in the Narasuan University, Phitsanulok, during 2019. The study samples were recruited from orthodontic patients in the Narasuan University who have age over 15 years old. Sound, non-restored human premolars containing no restorative material and extracted for an orthodontic purpose were used in this study. Proximal sides of teeth with carious lesions no farther than middle one third of dentin were served as samples. Additionally, patients who volunteer in this study must have fully erupted premolar teeth with upright position and proper alignment with adjacent teeth.

Sample size calculation

In the calculation of sample size, Pearson Correlation coefficient (0.42) between mean grayscale values and the histological result based on previous studies was used. (11) The study was designed to have 80 percent power of test, assuming tests conducted at an overall 5-percent statistical significance level. Sample size was calculated using G*Power version 3.1.9.2 (Franz Faul, university kiel, Germany). An attrition rate of the sample size was set at about 20%. Total sample size number calculated was 60 samples.

Radiographic Examinations

Before extraction premolar tooth, patients fulfilling the inclusion criteria were allocated individually to take the radiographs under a standard bitewing condition by one examiner. Bitewing projection covered the center of premolar teeth and partial of mesial and distal adjacent teeth. An X-ray device (XCP-DS, DentSply Sirona, York, PA, USA) was horizontally positioned with a 35-cm focus-receptor distance. Photostimulable phosphor (PSP) plates sized #2 film exposing at 70 kV, 15 mA and 0.5 seconds were used to image each set of teeth and held in place with XCP-DS holder during exposure. Teeth were radiographed at a 10-degree positive vertical angle using the bitewing technique (film-holder with a bitewing). Photostimulable phosphor (PSP) plates were processed rapidly after exposure in accordance with the manufacturer’s instructions. The radiographs were exported in an 8-bit grayscale image with a *.tiff file format.

Teeth were included if the radiographs are rated from no radiolucency to RB4 (Radiolucency reaching the middle 1/3 of dentin) according to ICCMS™.

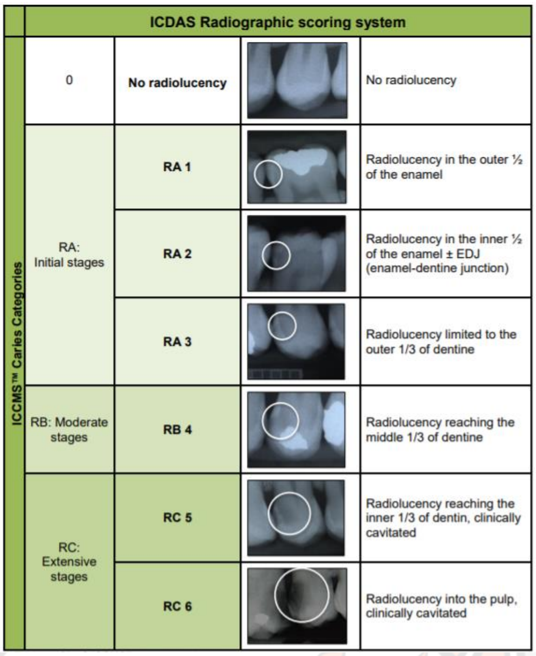


Figure 17 ICDAS/ICCMS™ radiographic scoring system.

All radiographs were examined without overlapping between the adjacent teeth. All groups were rated by one observer.

Intra-and inter-observer agreements

For inter-observer agreements, one observer and one specialist with highly experience worked independently of one another to calibrate the measurement before the experiment was performed. Intra-and inter-observer agreements were assessed with a weighted kappa coefficient no less than 0.81 (excellent agreement). For intra-observer agreements, repeat measurements were performed 3 times in a randomized order which each time was performed two weeks separately.

Ground section preparation

After extraction, all teeth were stored in a 0.1% thymol solution. Total 60 proximal sides of teeth with carious lesions no farther than middle one third of dentin were served as samples. Before ground section preparation, all samples were marked at the center of carious lesions and cut through their anatomical crown's long axis at the center of carious lesion in the first place. Then, all samples were cut through their anatomical crown's long axis serially and mesio-distally using a hard tissue macrotome (Isomet 5000; Buehler, Lake Bluff, IL, USA) under water irrigation for the histological validations of the caries states, ground sections, obtaining a final thickness of 1 mm.

Stereomicroscopy (SM)

Before subjecting the ground sections to examination by the stereomicroscope, ground sections were dried by paper towel for 15 seconds. A high-resolution stereomicroscope (Olympus SZX16, Tokyo, Japan) at a 1.25 magnification with reflected light was used to analyze ground sections. Moreover, a carousel was selected and controlled in darkfield background. Reflected light units including the ring light illuminator and the adjustable dual mounted combination light illuminator were chosen and fixed in the same position. An arbitrary adjustment of the coarse and fine focusing knob was performed by one observer to accommodate viewing processes before an observation. The observer evaluated the ground sections within a 15-minute period per viewing session in the same closed room and the same normal light. Six ground sections were performed in each time of viewing session to prevent fatigue of the observer. The criterion for caries diagnosis was the opaque-white to dark-brown discoloration in the caries' susceptible area. Images were saved in JPEG format from the microscopic views and transferred to the Olympus cellSens Standard software for determining the exact size of lesions.

To measure and define the size of carious lesion in this study, the most external border of the lesion was drawn and connected to the deepest border of the lesion. The Square micrometer or square micron (µm²) was a unit of measurement used this study. (Figure 21) In addition, enamel thickness of all ground sections was measured for further analysis.

Figure 21 Measuring the size of carious lesion in the histological specimen.

Grayscale evaluation

Images of each tooth were randomized, and mean grayscales of pixels were measured from the carious lesions by an investigator. The images were evaluated by one observer on an LCD medical monitor (MDNG2121; Barco, Kortrijk, Belgium) set at a screen resolution of 2*106 pixels and an 8-bit grayscale image using a standard software (ImageJ; National Institute of Health, Bethesda, MD, USA) with a 30-minute period per viewing session in a quiet room. Six images were performed in each time of viewing session to prevent fatigue of the observer. All images were examined for presence of proximal caries without overlapping between the adjacent teeth. An arbitrary adjustment of the brightness and contrast was performed by one investigator to accommodate viewing processes before an observation.

A region of interest (ROI) was selected to cover an extension of the lesion by visualization of an examiner. Each borderline of ROI was drawn by polygonal function in ImageJ software. A mean grayscale was computed from the grayscale values containing in each ROI area with the ImageJ software. Subsequently, a correlation of the mean grayscale values and the histological result were analyzed.

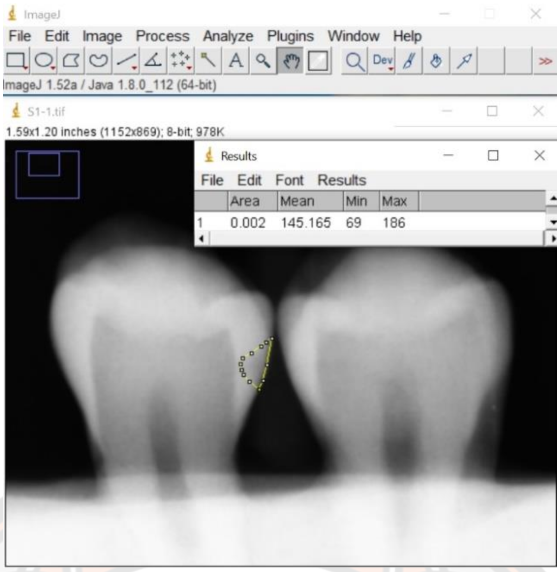


Figure 22 An example of the selections of regions of lesions in a tooth and mean grayscale calculations using an ImageJ® software.

Statistical Analysis

The mean grayscale values of the subtraction images and the size of carious lesion in the histological specimens were calculated for every image and specimens. A Simple linear regression test was a statistical method that performs to summarize and study relationships between two continuous (quantitative) variables.

Consequently, correspondences between the mean grayscale values of the subtraction images and the size of carious lesion in the histological specimens were calculated using a Simple linear regression test with a 0.05 significant level. All computations were performed with the statistical software (SPSS 20.0, SPSS Inc., Chicago, IL, USA).

CHAPTER IV

RESULTS

The study samples were recruited from orthodontic patients in the Naresuan University. Sound, non-restored human premolars containing no restorative material and extracted for an orthodontic purpose were used in this study. Patients who had fully erupted premolar teeth with upright position and proper alignment with adjacent teeth were radiographed with the bitewing technique. Proximal sides of teeth with carious lesions no farther than middle one third of dentin were served as samples. The samples were collected completely 60 carious lesions from 34 patients in this study. The mean grayscale values of all digital radiographs (8-bit) were computed from the grayscale values containing in each ROI area with the ImageJ software and recorded.

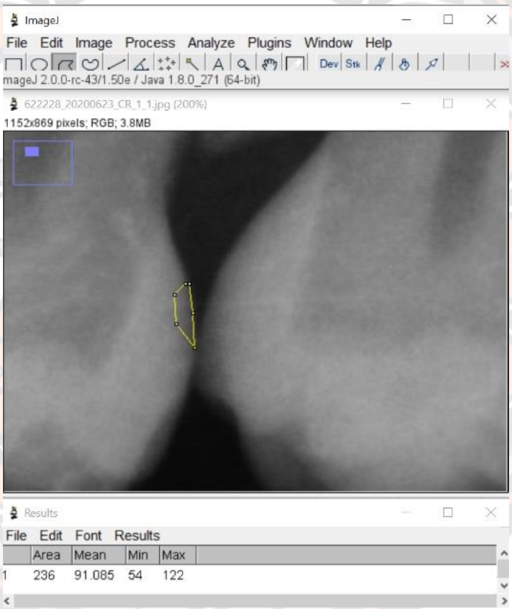


Figure 23 An example of the selections of regions of lesions

An example of the selections of regions of lesions in orthodontic patient and mean grayscale calculations using an ImageJ® software.

From histological analyses of teeth with proximal carious lesions, the sizes of carious lesions were measured 3 times separately and recorded with the Olympus cellSens Standard software. All data were put in the statistical software and analyzed. Intra- and inter-observer kappa coefficient of mean grayscale values were assessed with a weighted kappa coefficient 0.96 and 0.87, respectively. Furthermore, intra- and inter-observer kappa coefficient of histological examinations were assessed with a weighted kappa coefficient 0.98 and 0.88, respectively.

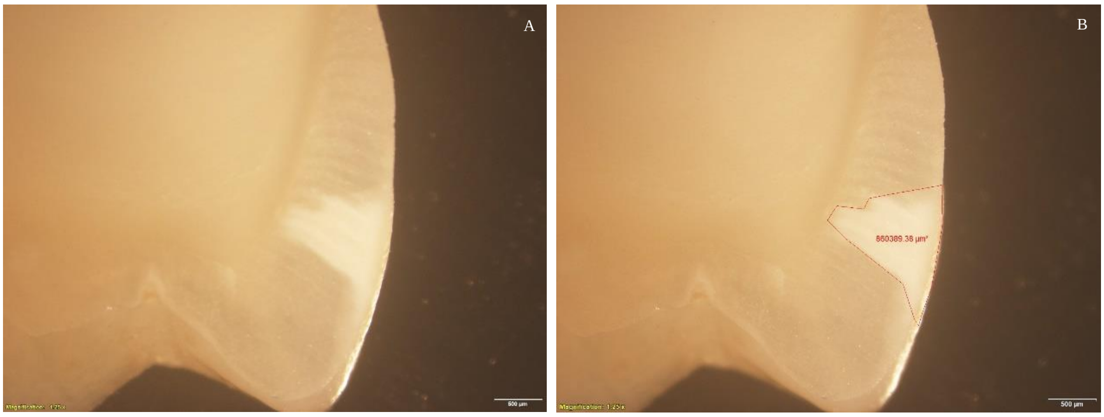


Figure 24 Measuring the size of proximal carious lesion in the histological specimen of orthodontic patient by the Olympus cellSens Standard software. A. Original radiograph B. All borders (red lines) of carious lesions were drawn and connected (µm²)

Simple linear regression was carried out to investigate the relationship between size of proximal carious lesion and mean grayscale value of digital radiograph. The scatterplot showed that there was a strong positive linear relationship between the two, which was confirmed with a Pearson's correlation coefficient of 0.867. Simple linear regression showed a significant relationship between size of proximal carious lesion and mean grayscale value ($p < 0.001$). The slope coefficient for mean grayscale value was 5082.89 so actual size of proximal carious lesion increases by 5082.89 μm^2 for each pixel of mean grayscale value of digital radiograph. The R^2 value was 0.751 so 75.1% of size of proximal carious lesion can be explained by mean grayscale value of digital radiograph. The scatterplot of standardized predicted values versus standardized residuals, showed that the data met the assumptions of homogeneity of variance and linearity, and the residuals were approximately normally distributed.

Model Summary[c,d]

Model	R	R Square[b]	Adjusted R Square	Std. Error of the Estimate
1	.867[a]	.751	.747	318366.12720

Figure 25 Model Summary

a. Predictors: Mean grayscale value

b. For regression through the origin (the no-intercept model), R Square measures the proportion of the variability in the dependent variable about the origin explained by regression. This cannot be compared to R Square for models which include an intercept. c. Dependent Variable: size of carious lesion d. Linear Regression through the Origin

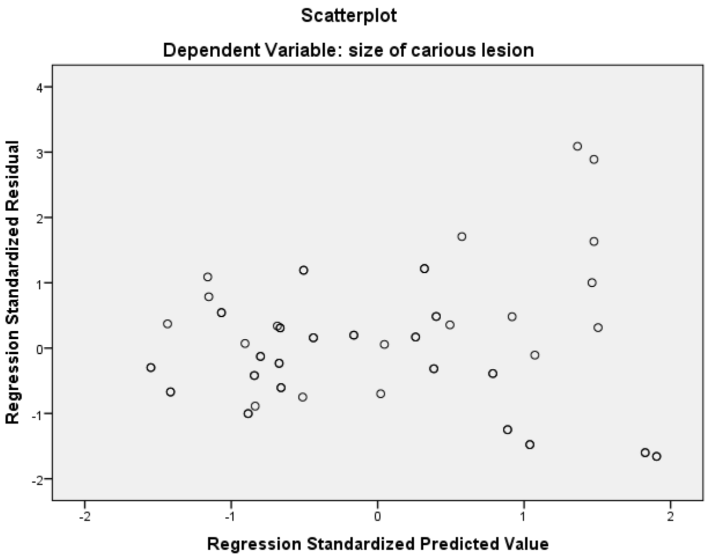


Figure 26 Homoscedasticity

This figure shows there is no pattern in the scatter. The width of the scatter as predicted values increase is roughly the same, so the assumption has been met.

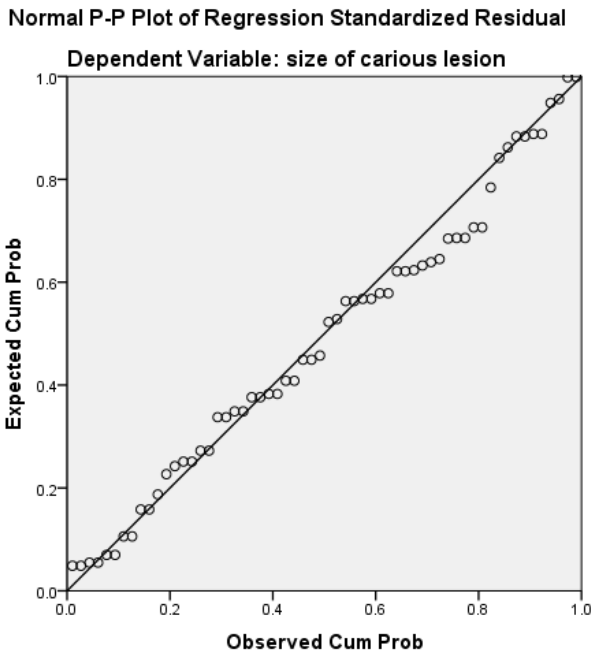


Figure 27 Normality of residuals

This figure shows the residuals are approximately normally distributed.

CHAPTER V

DISCUSSION

Diagnosis of caries is mostly based on direct visual examination and intra oral radiography. Proximal carious lesions commonly occur and can be detected by noticing discoloration or roughness at the site as well as by radiography. Although discoloration and proximal surface roughness may indicate caries, detection of carious lesion by direct observation is difficult. In recent years, methods of detection of early carious lesions have evolved considerably, starting with the identification of the earliest mineral changes and then to controlling the demineralization process using non-operative procedures. In addition to the identification of risk factors and the early detection of lesions, it is essential to evaluate the extent of each lesion (non-cavitated vs cavitated) and its status (active or arrested) to establish a monitoring procedure and predict the outcome, similar to any other diseases. Such a systematic approach should lead to a care plan for caries, based on prophylactic measures with minimal use of restorative measures, resulting in the better patient oral health. The detection of carious lesions at an early stage is necessary in order to implement preventive and interceptive treatment strategies. In daily practice, the diagnosis of initial lesions is not always simple, it is often subjective and based on the clinician's clinical experience. For this reason, the search is on for more specific and sensitive tools, using new technologies, to help the practitioner diagnose initial caries lesions as precisely as possible. (49)

During the recent years, there are many ongoing researches on dental image analysis using image processing techniques such as image enhancement, feature extraction, and image segmentation. The database provided is applicable for the performance evaluation of any image analysis techniques. New software tools are under development by researchers and manufacturers to improve the diagnostic outcome of digital radiography. Three-dimensional visualization of dental structures, as done with cone beam computed tomography (CBCT), is expanding rapidly, and it would not have been possible without the availability of digital images. CBCT technology seems to offer advantages over conventional 2-dimensional techniques for determining the depth of small proximal carious lesions in non-restored dentitions. Although, there was previous study presented that restorations, particularly of dense materials, such as amalgam, can cause artefacts that make caries diagnosis difficult resulting in false positive diagnosis. (44) Also, several studies assessed the accuracy of a limited cone-beam volumetric imaging system in detecting incipient proximal caries. They found that limited CBCT system and could not enhance the accuracy in detecting proximal carious lesions due to mechanical improvements, such as the reduction of noise, the enhancement of image contrast, and the reduction of artifact, would be required. (45) An area that is continuously under development is the application of artificial intelligence (AI) to the interpretation of digital radiographs. Many years ago, AI was expected to solve all diagnostic problems. The conventional wisdom was that computers would be able to collect information independently and solve diagnostic problems without intervention by the clinician. Today, this optimism is reduced. Today's systems leave the initiative with the clinician and support only in performing the diagnostic task. There was previous study showed that the designed software was able to detect a significant number of dentin caries and acceptable measuring of the depth of carious lesions in enamel and dentin. However, the software had limited ability in detecting enamel lesions. (43) Since, none of the new tools designed to enhance and facilitate caries diagnosis is yet proven, there is still no absolute substitute for the traditional clinical examination and radiographic bitewing examination. Therefore, bitewing radiographs are still the method of choice for early detection of carious lesions, especially on proximal surfaces. (49)

Intraoral radiography is one of the most used methods for dental caries diagnosis, especially for proximal dental caries that is hard to visually inspect. Radiography is 88% more efficient for detection of proximal caries compared to direct observation. (50) Since digital intraoral radiography introduced to dentistry, the use of digital radiography is increasing. A major goal of digital radiography is to restore and improve the diagnosis of a digital image by enhancement of valuable information and suppression of noise. A successful digital radiography in dental practice can be achieved from enhanced digital images compare with observer performance from radiographs only. In addition, the technique should be easy to apply.(51) Moreover, Insufficient processing of conventional images may affect interpretation. The time-consuming processing of conventional radiographs, lower patient exposure to ionizing radiation in digital radiography and the possibility of changing the contrast and density after exposure in digital radiography have resulted in increasing popularity of digital compared to conventional radiography. (52)

Radiographic diagnosis is based upon the amount of demineralization needed to create a change in radiographic density, with a minimum of 40% mineral loss needed for radiographic visibility.(53) The mineral content of enamel and dentin loss are recorded on the image receptor as an increase in radiographic density. This increase in radiographic density is observed as a sign of a carious lesion by the clinician. Exposure parameters, type of image receptor, image processing, display system, viewing conditions, and experience of the clinician eventually are various factors which can affect the ability to detect carious lesion precisely.(54) Advanced procedures benefited from digital radiography, such as digital subtraction radiography and computer-aided recognition of image features are available as well. However, in the previous studies, there were none of the modalities which can offer both of high sensitivity and high specificity, and none of the modalities obviously exceed the others in the detection of proximal carious lesion.(1) Combination different types of caries detection modalities can probably achieve the optimal diagnostic result.(5) For early detection of initial carious lesions, radiography is preferred to clinical examination. Although conventional radiography has a low sensitivity for detection of enamel lesions, it is still the method of choice. In the recent years, due to advances in PSP receptors in digital radiography, and the lower patient radiation dose in this method, this receptor has been frequently used in intra-oral radiography. (55)

With some of advantages of a digital system, information from radiographic images is collected more easily, reduced radiation dose and in a more objective way, which will improve the performance of the diagnostic process. Digital images captured by a sensor are displayed on the monitor in shades of gray. The grayscale displayed by the monitor is comprised of 256 distinct visible shades of gray even though some systems can contain up to 64,000 values. The human eye can only distinguish up to 32 shades of gray; this fact will be important later when the concept of image processing is presented. Each pixel in the digital image has a number ranging from 0 to 255. The radiation is registered when the sensor captures it.(56) Dental professionals rely on the contrast of a radiograph to be able to detect disease processes; the longer the scale of contrast in the image, the easier it is to make a diagnosis. With the ability to enhance gray scales using the software that accompanies the sensor in an x-ray digital imaging system, a clinician may be better able to diagnose even subtle changes that may have been missed previously with conventional films.(57) The digital radiograph have a higher degree of accuracy over traditional radiographs. When the images are represented directly on the computer screen and stored, the dental experts can then examine the images easily. The images are very clear and easy to manipulate for better viewing even by a non-computer expert. Digital imaging makes the diagnosis and treatment process reliable with more accuracy. An image in digital radiography is composed of a set of cells called pixels. Each pixel is defined by an x-coordinate, a y-coordinate, and a grayscale value. The grayscale value of radiography is analogous to a X-ray intensity, which high X-ray intensity at carious lesion location is displayed as low brightness during an exposure of a digital sensor.(11) The effective radiographic depth assessments on proximal surfaces in radiography is strongly related to actual lesion depth, and for small lesions validity is poor.(58) The apparent depth can also vary as a function of X-ray beam angulation (59) and radiographic density.(34) Variations in perceived, although not actual, lesion depth can lead dentists to erroneously believe that caries has progressed or regressed and result in unnecessary restorative intervention or delay in treatment.(44)

Since taking a radiograph with different exposure settings can affect the absorbed dose. It may also decrease contrast by using higher voltage (kVp) and decreases amperage (mAs). (50) Today, 60 and 70 kVp are more commonly used. Previous study shows radiography using photostimulable phosphor plate (PSP) receptor with 70 kVp is recommended for detection of initial enamel caries. The sensitivity of PSP receptor with 70 kVp for enamel caries was higher than film and charge-coupled device (CCD). For detection of non-cavitated and cavitated caries in dentin, PSP with 60 kVp was more appropriate. (55) Thus, this present study was designed to use PSP receptor and set 70 kVp for the optimal diagnostic value of digital radiography for detecting proximal caries.

A histological investigation was achieved as a reference test or a gold standard to regulate an accurate assessment of the extent of any carious lesions present.(60) Thus, histological observation served as the validating criteria for the presence of carious lesions. Several image enhancements have been tested already to improve initial caries diagnosis from digital images. but their effect on diagnostic accuracy has not yet been determined. (51) Accordingly, in this study the accuracy of the diagnosis of small approximal carious lesions from digitized radiographs and from enhanced image modality was compared with the accuracy obtained from histological validation.

The very high intra-observer kappa coefficients found in the present study suggest excellent intra-observer agreement and excellent inter-observer agreement among experienced observers for all detection methods. Cohen suggested the Kappa result be interpreted as follows: values ≤ 0 as indicating no agreement and

0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement. (61) According to this study, intra- and inter-observer kappa coefficient of mean grayscale values were assessed with a weighted kappa coefficient 0.96 and 0.87, respectively. Intra- and inter-observer kappa coefficient of histological examinations were assessed with a weighted kappa coefficient 0.98 and 0.88, respectively. For inter-observer agreements, one observer and one specialist with highly experience worked independently of one another to calibrate the measurement before the experiment was performed. Intra-and inter-observer agreements were assessed with a weighted kappa coefficient no less than 0.81 (excellent agreement). For intra-observer agreements, repeat measurements were performed 3 times in a randomized order which each time was performed two weeks separately. This study was attempted to reduce the risk of bias which could be occurred from one examiner, by collecting and defining code to samples. Also, one examiner performed grayscale evaluation of samples and later performed histological assessment of samples at least two weeks after grayscale evaluation according to wash out period.

The statistical analysis of mean grayscale values is performed with simple linear regression. Simple linear regression quantifies the relationship between two variables by producing an equation for a straight line of the form $y = a + \beta x$ which uses the independent variable (x) to predict the dependent variable (y). The independent variable (x) is mean grayscale value which uses to predict the dependent variable (y) which is the size of approximal carious lesion in this study. This is defined as the line which minimizes the sum of the squared residuals. A residual is the difference between an observed dependent value, and one predicted from the regression equation. The coefficients table contains the coefficients for the regression equation and tests of significance. The B column in the coefficients table, presents the values of the gradient and intercept terms for the regression line. The gradient (β) is tested for significance. If there is no relationship, the gradient of the line (β) would be 0 and therefore size of approximal carious lesion in every tooth would be predicted to be the same mean grayscale value. The significant value against mean grayscale value is less than 0.05 and so there is significant evidence to suggest that the gradient is not 0 ($p < 0.001$).

The key information from the table below is the R2 value of 0.751. This indicates that 75.1% of the size of approximal carious lesion can be explained by the model containing only mean grayscale value. This is quite high so predictions from the regression equation are reliable. It also means that 24.9% of the variation is still unexplained so adding other independent variables could improve the fit of the model. In summary, there is a significant relationship ($p < 0.001$) between the size of approximal carious lesion and the mean grayscale value, with a 75.1% increase in the size of approximal carious lesion.

Practitioners and manufacturers frequently use the reduction of the radiation dose that the patient receives as a reason to implement digital radiography. There are several reasons why the dose reduction is not as large as often suggested. The most important reasons are dose per exposure, an increase in the number of radiographs made and an increase in the number of remakes made. Sometimes manufacturers claim a dose reduction of 90 percent compared with film for digital sensors. The reality, however, is that the reduction compared with the current standard of F-speed film is somewhere between 0 percent and 50 percent. Storage phosphor plate systems, with their wide exposure latitude, carry the risk of using even more radiation, as the image quality will not alert the practitioner to possible overexposure in the same way in which a film image would. Dentists indicate that the decision to make a radiograph is reached more easily with a digital system. In a Dutch study, 55 percent of clinicians using storage phosphor plates and 65 percent of clinicians using solid-state systems reported making more radiographs than they had with film. It takes some time for the practitioner to get used to the positioning of the sensor inside the patient's mouth. It is obvious that this will result in a more frequent need to remake an image. After some time, however, when the dentist and other members of the team are trained in making digital radiographs, they still have a tendency to decide sooner to repeat an exposure than they would have with analog radiographs. This was reported in one study by 60 percent of clinicians using phosphor plates and even by 80 percent of those using solid-state systems. This probably is because it does not require much time to make another radiograph, consequently, the threshold for remakes is lower. In the present study, the bitewing technique was used when the radiographs were taken from the patients. The +8- to +10- degree angulations for projecting bitewing radiographs may influence the appearance of the proximal dental caries. However, earlier studies have revealed that a few degrees of horizontal and vertical angulations have no impact on quantitative evaluations of periodontal disease and root canal length, nor therefore to dental caries diagnosis. (62, 63)

According to a mean grayscale measurement and histological validation, this study was designed to define a region of interest (ROI) covering an extension of the lesion by visualization of an examiner. Each borderline of ROI was drawn by polygonal function in ImageJ software. A mean grayscale was computed from the grayscale values containing in each ROI area with the ImageJ software. From histological validation, to measure and define the size of carious lesion in this study, the most external border of the lesion was drawn and connected to the deepest border of the lesion. The design of measurement in this study was related to previous study of gray-scale value of cavitated and non-cavitated carious lesion which had a problem in deviation of measurement a mean grayscale value by selecting ROI from a fixed geometric shape. (11) The deviation of measurement a mean grayscale value from previous pilot study might be caused by a fixed geometric shape which involved area of carious lesion and involved area of occlusal embrasure. This area of occlusal embrasure was lower brightness than an area of carious susceptibility, so the mean grayscale might be deviated. Since ROI in this study for the measurement of a mean grayscale value was newly designed to cover just only an area of carious susceptibility, a method of measurement in histological validation should be related.

The initial carious lesion is managed through non-operative care using remineralization therapy, involving behavioral changes and promotion of mineralization over demineralization, typically by using fluoride containing products. Remineralization is aimed at stopping progression of the lesion or ideally, reversing it. As part of the mineralization therapy, management should involve reviewing the dietary and oral hygiene behaviors (plaque control) of the patient, followed by education, and encouraging behavioral changes. Fluoride can increase the rate and magnitude of remineralization of initial lesions. When an initial lesion is found, long-term monitoring combined with managing the caries risk factors is an option to consider. Surgical intervention can be considered only if the initial lesion advances.

Diagnosis of initial approximal caries and determining the proper treatment plan for them are the most common problems encountered in clinical dentistry. Even experienced dentists have not absolute accuracy and expertise in diagnosing proximal caries on a dental radiograph. Thus, it is not unusual if different dentists have different judgments about the same radiographs. This is because of their different diagnoses of presence of the lesions on the radiographs. This difficulty in diagnosis is due to the fact that the human eye generally can differentiate about 40 shades of gray compared to the maximum information from the shades of gray in digital radiographic images, which provide displays 256 shades of gray. (64) Although, caries detection determined by dental radiographs is highly accurate for proximal dentine caries lesions, digital radiography has some limitations and is unable to reveal the first stage of caries. (65) Moreover, designed computer software for detection of approximal caries in posterior teeth software is able to detect a significant number of dentin caries, but the software still has limit ability in detecting enamel lesions and underestimates the size of demineralized area. (43)

More advanced image analysis tools are available with different types of imaging. Several software studies have been published that describe the diagnostic importance of grayscale value. This procedure allows practitioners to distinguish small differences between subsequent radiographs that otherwise would have remained unnoticed because of over projection of anatomical structures or differences in density that are too small to be recognized by the human eye. Additionally, grayscale value is quite simple and user-friendly. ImageJ software corresponds pixels of image from digital bitewing radiograph, and it uses the outcome to calculate a mean grayscale value of approximal caries. From ImageJ software, digital image processing methods should be utilized for an initial carious detection for example by modifying the grayscale value range of all image pixels (such as contrast, density, and brightness) and by filtrating an image to emphasize some certain features and to a removal of other features (such as smoothing, sharpening, and an edge enhancement). Also, ImageJ software is used to improve the efficacy of an initial caries detection by using only some grayscale differentiations. A previous study has shown that mean grayscale value of the non-cavitated lesion was significantly different from the cavitated lesion, with a slight difference from the sound enamel. (11) Continuously, there was another unpublished study has shown that mean grayscale of non-cavitated lesions was significantly different from that of cavitated lesions by using ImageJ software.

In summary, considering the above findings from this study, the research methodology was designed to detect initial carious lesions using digital intraoral radiograph by in vivo study, this can be representative of diagnostic accuracy obtained in the real clinical condition. And the result presented a significant relationship between the size of approximal carious lesion and the mean grayscale value. This indicated that 75.1% of the size of approximal carious lesion can be explained by that mean grayscale value. This is quite high so predictions from the regression equation are reliable. The further study is necessary to define the range of mean grayscale value for initial caries detection.

This study has some limitations which is the experiment was performed by one examiner. Although, research methods were designated to reduce the risk of bias, research bias might be occurred. Also, this study was an in vivo study. Therefore, the influences of many factors such as malalignment of teeth and environment of oral cavity could have been affected. In the future, software for digital radiography will include tools to optimize contrast and brightness automatically for specific diagnostic tasks or some devices which help to detect initial carious lesion easier. Thus, the practitioner could use a single image, and thus a single exposure, to assess more than one diagnostic issue. However, the further study should be a greater number of observer or evaluator and large sample size may probably establish the result.

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