Diagnosis determination of chronic periapical abscess case using imaging radiography software and microbiological examination in female patients

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ABSTRACT

Introduction: Diagnosis determination of chronic periapical abscess can be achieved by subjective, objective, and other examination such as radiographic and microbiological examination based on clinical finding and differences intensity at the periapical area with healthy bone suspected to be chronic abscess periapical. A microbiological examination performed to obtain the predominant bacteria that involved to determine an adequate medicament to succeed endodontic treatment. This study was aimed to compare intensity abscess lesion segmentation image reconstruction and surrounding bone for determine the diagnosis of periapical abscess more accurately and to determine the characteristic of bacteria colony from a periapical abscess tooth. Methods: The study sampling method was consecutive sampling (6 female) consisted of inclusion criteria patient with a periapical lesion in anterior maxillary that diagnosed based on clinical signs and symptoms. CBCT 3D scan of the maxillofacial region taken from of patients was measured image intensity periapical lesion and surrounding bone using semi-auto segmentation with ITK-SNAP 3.6.0. Samples from the root canals taken using three sequential sterile paper points. The samples were plated, and microorganisms were then isolated and identified by the colony characteristics. Results: There was a significant difference between the mean content of lesion intensity of (n = $6,256.710 \pm 81.930$ and 636.022 ± 79.981 lesion boundary. The two-tailed p-value = 0.0050, this difference was considered to be very statistically significant. The mean of group lesion minus group surrounding bone equals to 163.715. 95% confidence interval of this difference from 75.542 to 251.888. Conclusions: Demineralisation of periapical bone can be determined by voxel intensity value in CBCT 3D radiograph using semi-auto segmentation ITK SNAP, could be recommended as a quantitative interpretation for detection periapical lesion and chronic periapical abscesses is a poly-microbial disease.

Keywords: Chronic periapical abscess, endodontic, microbiology, radiograph CBCT

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INTRODUCTION

Prevalence of pulp and periapical disease is quite high in Indonesia. According to the list of basic tabulations data, pulp and periapical disease ranked 11th out of all diseases with a total of 30.06% for outpatient diseases in Indonesia in 2006.¹ Pattern of 10 major diseases in outpatients in hospitals in Indonesia in 2009, pulp and periapical disease had an increase in position, from the 9th position to the 8th position of all diseases with the number of cases in 2009 as many as 122,467 cases, in men as many as 54,004 cases while women as many as 68,463 cases and in 2010 as many as 208,888 cases.¹,²

Study in the US shows that female patients with chronic periapical abscess are greater than male patient who came to emergency installation.³ Female patients are more aware with their dental and oral health care. Their motivation and needs are higher than male patients. Riskesdas 2013 stated that female patients received more dental treatment (33.4%) compared to male patients (28.6%).⁴ Based on that study, we interested to take the female patient as a sample in this study.

A chronic apical abscess is an advanced infection of pulp necrosis continuation from chronic apical periodontitis, which is a condition of bacterial invasion into periapical tissue, with formation of pus. Succeed therapy for this case depends on the clinician's understanding of the etiology of the disease and root canal treatment. The prevalence of periapical abscesses based on sex, shows women have a higher prevalence than men. Hormonal fluctuations such as puberty, menstruation, and pregnancy, make the oral environment significantly more cariogenic for women than men. Women are the shape of the nation's next generation, so their health must be considered.

Earliest changes that can be detected in periapical abscesses is loss of bone density, the periodontal ligament widen at the apex of the tooth and subsequently involves the resorption of the surrounding bone. An area with highest bone resorption is usually the centre of the periapical under the apex, with a sclerotic pattern at the edges of lesion. Radiolucent area showed at bone loss with diffuse trabecular patern. Any changes in thickness, continuity, the density of these

features can show various pathological disorders. Cross-sectional population studies reported the prevalence of apical periodontitis with root canal treatment are 64.5%. Early detection of periodontal and periapical diseases and assessment in accordance with bone conditions is very important for diagnosing a disease, planning treatment and the prognosis of disease.⁶

Radiograph examination is the common way to determine the pathological process in jaw bone included chronic periapical abscess. Measurements of image intensity grey values is an easy feature of most graphic analysis software for digital radiograph. The image intensity grey values are related to the absorption of x-rays, the radiolographic density of a certain tissue.7 ITK-SNAP is an open source medical image processing application that fulfils a specific need of biomedical imaging research by providing a combination of manual and semiautomatic tools for extracting structures in 3D image data of different modalities and from different anatomical regions8, allows the user to load multiple image volumes in a variety of common file formats, including DICOM from CBCT 3D Scan. Measurement of radiographic density using ITK SNAP with segmentation ROI lesion that representing voxel count, volume, and image intensity seems to be accurate, that visualize the pathological process to diagnose the periapical lesion.

This study evaluated measurements of bone density of different anatomic conditions, as measurement intensity represents as a valid method for clinical practice and differences intensity at the periapical area that suspected chronic abscess periapical hopefully can represent bones characteristic that can help in diagnosing the disease and to determine the characteristic of bacteria colony from periapical abscess lesion. This study was aimed to compare intensity abscess lesion segmentation image reconstruction and surrounding bone for determine the diagnosis of periapical abscess more accurately and to determine the characteristic of bacteria colony from a periapical abscess tooth.

METHODS

The type of this research was observational with a cross-sectional approach. The study sampling

method was consecutive sampling (6 female) taken at Universitas Padjadjaran Dental Hospital, with inclusion criteria were patient women with periapical lesion in anterior maxillary that diagnosed based on clinical signs and symptoms. Exclusion criteria in this study were patients who had a metabolic systemic disease that manifested in the jaw bone and fractures of the jaw. Ethical clearence statement with registration number 377/Un6.C.10/PN/2017 approved by the Health Research Ethics Commission of the Facuty of Medicine Universitas Padjadajaran.

CBCT scans of the maxillofacial region taken from patients who had been seen in the Radiology Department of Universitas Padjadjaran Dental Hospital, as consulted from the Endodontic Department of Universitas Padjadjaran Dental Hospital for reasons unrelated to this study, such as chronic periapical abscess diagnostic consideration for an endodontic treatment plan. Patients according to the criteria selected by history taking and clinical subjective and objective examinations that had been done from endodontic department. The data collection technique carried out in accordance with the inclusion criteria, by asking for ethical approval, preparing an informed consent sheet, working on root canal treatment and bringing samples to the microbiology laboratory to examined bacteria colony.

All the CBCT image data sets were obtained using Picasso Trio (VATECH Co. Ltd, Gyeonggi-Do, Republic of Korea). All data sets were acquired with voxel size of $0.202 \times 0.202 \times 0.186$ mm; spatial resolution fields of view of 416 x416 x 256; a tube voltage of 85 kV; and tube current of 5 mA.

Intensity measurement using ITK-SNAP in the following way, determine a region of interest periapical lesion area, segmentation ROI using segment 3D. Classification of different intensity using pre-segmentation classifier then updated the segmentation then intensity and statistic were measured. Boundaries of the alveolar bone also segmented with same procedure with periapical lesion area.

Subjective examination obtained from history taking, all patients examined were found complaining of pain while chewing, no throbbing pain and other pain and the average tooth was sick after filling, although pain experience could

not be explained by patients in detail. Teeth sensitive with percussion and palpation, negative in vitality tests and from objective examination show fistula in the gum. Clinical examination lead to a diagnosis of chronic periapical abscess.

A periapical lesion was defined as a periapical radiolucent area that was contacted with the apex of the root at least twice of the width of the periodontal ligament space. 7,9 The measurement of image intensity was performed with ITK-SNAP 3.6.0 (US National of health) that's used for a general-purpose interactive tool for image visualization using manual or semi-automatic segmentation, that's one of the most studied problems in the field of biomedical image analysis. 10 ITK-SNAP stands for providing an interactive platform for segmenting anatomical structures in 3D images both manually (by painting outlines on 2D cross-sections of a 3D image) and semi-automatically (by manually setting the parameters and initial seeds for two active contour algorithms.11 The CBCT images were evaluated using semi-auto segmentation by 2 examiners who had more than 5 and 11 years of clinical experience using CBCT imaging.

Observer calibration carried out for more than 1 person (equalisation of perception, preparation techniques and adjustments between observers). After calibration, a trial was carried out first. The goal was that the results of the study have the same consistency (reliability) between observers, have the same measurement results even if done several times, because of semi-automatic segmentation using classifier presegmentation processing.

The microbiological sampling was performed in several steps. Start with rubber dam installation, access opening, root canal initiation with K-file number 10, working length measurement with apex locator, and dried the root canal with a paper point. Taking microbiological samples from the root canal using a sterile paper point, then cultured on the blood agar plate and incubated 24 hours at 37°C. The tools used in this study were basic tools, K-file (Dentsply), apex locator and the materials used are Paper point (Dentsply), Chlorhexidine Gluconate (Minosep), NaOCl 2.5%, cotton roll, cotton pellets, latex rubber dam, Gutaperca ProTaper and ISO Gutaperca. Pus sample transfer into amies transfer medium.

Blood Agar Base (OXOIDCM0055B), Bacto Casitone, Bacteriological Agar, and Basic Fuchsin for subculture colonies.

Samples from 6 root canal with a diagnosis of chronic periapical abscess were studied using culture technique in facultative anaerob

conditions after subcultured were done on blood agar plate, incubated at 27°C for 24 hours thus various forms of bacterial colonies were obtained. Colonies are a large number of bacterial cells on solid media, which are visible to the naked eye which can be used to identify bacteria.

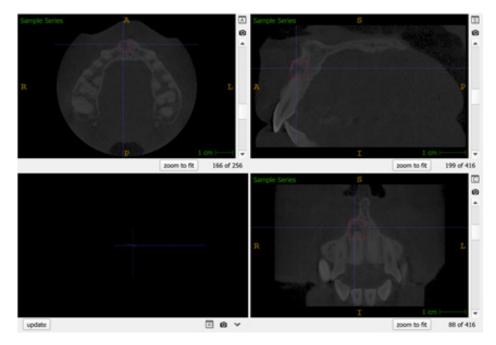


Figure 1. ROI selection auto segmentation using ITK SNAP

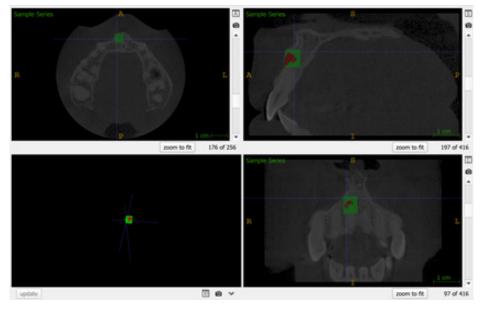


Figure 2. Segmentation 3D using ITK SNAP

Based on the characteristics shown in Table 1, the colonies of the various colonies were obtained. The elevation properties of the colonies were clearly observed from the side when the plate was held parallel to the eye.

RESULTS

Results of this research are presented in tables below. The results showed the region of interest in defect voxel area and surrounding bone

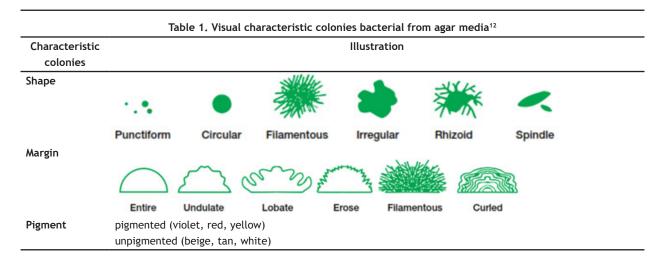


Table 2. Mean, minimum, maximum and standard deviation of the image intensity measurements of periapical lesion

Periapical lesion							
Mean	Min	Max	Standard deviation				
256.710	140.229	382.232	81.930				

Table 3. Mean, minimum, maximum and standard deviation of the image intensity measurements of surrounding bone

Surrounding bone							
Mean	min	max	Standar Deviation				
636.022	523.212	752.041	79.981				

have different intensity. Overall the intensity measurements showed results with mean and standard deviation of intensity for periapical lesion about 256.710 \pm 81.930 and 636.022 \pm 79.981 for surrounding bone (Table 2). Image

intensity was higher in the measurements of the surrounding bone (min 523.212, max 752.041) as shown in Table 3 than in the measurements of the periapical lesion (min 140.229, max 752.041) shown in Table 2.

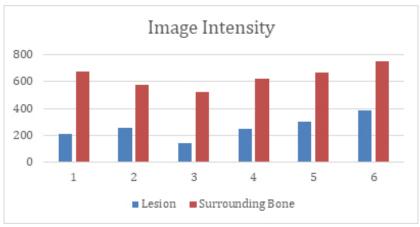


Figure 3. Image intensity of periapical lesion and surrounding bone

A value of means that the defect region has the same mean X-ray absorption like the surrounding bone, influenced by the presence or thickness of cortical bone and the density of cancellous bone. The regions of interest (ROI) had to be selected by semi-auto segmentation and classified with the voxel image intensity. The two-tailed p-value = 0.0050, this difference was considered to be very statistically significant. The mean of group lesion minus group surrounding

bone equals to 163.715, with 95% confidence interval of this difference from 75.542 to 251.888.

The pus sample was aseptically plated on Muller-Hinton agar plate and incubation was carried out under facultative anaerobe conditions at 24°C for 48 hours. The shape of colonies on the plates was then noted. The morphology was confirmed with a Gram staining. The following figures are the photograph of each of the samples mentioned above.

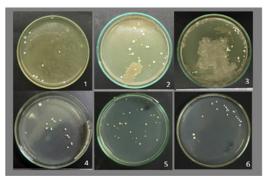


Figure 4. Photograph of bacterial colonies on Muller Hinton agar plate for each sample

examination was done by Gram staining and determined the bacterial shape, colour and formation. The macroscopic and microscopic characteristics showed as the following table.

Each colony was analyzed by macroscopic and microscopic examination. The macroscopic examination was analyzed by colonies characteristics in Table 1, and microscopic

Sample	Macroscopic			Microscopic			
	Shape	Margin	Color	Diameter	Shape	Gram	Formation
ı	Circular	Entire	White	1 - 2 mm	coccus	(+)	Staph
	Circular	Serrate	White	3 - 4 mm	coccus	(+)	Strept
	Circular	Entire	Yellow	1 - 2 mm	coccus	(+)	Staph
	Circular	Entire	White	1 - 2 mm	coccus	(+)	Staph
	Circular	Entire	White	1 - 2 mm	coccus	(+)	Staph
II	Circular	Entire	White	2 - 3 mm	coccus	(+)	Staph
	Circular	Entire	Yellow	< 1 mm	coccus	(+)	Staph
	Circular	Entire	Yellow	< 1 mm	coccus	(+)	Staph
	Circular	Entire	White	2 - 3 mm	coccus	(+)	Staph
III			White		coccus	(+)	Staph
	Circular	Entire	White	2 - 3 mm	coccus	(+)	Staph
	Circular Entire	Fatina	White	2 - 3 mm	coccus	(+)	Strept
IV		Entire	White		coccus	(+)	Staph
	Circular	Entire	Yellow	2 - 3 mm	coccus	(+)	Staph
٧	Circular	Entire	White	< 1 mm	coccus	(+)	Staph
M	Circular	Entire	White	2 - 3 mm	coccus	(+)	Staph
VI	Circular	Entire	White	3 - 4 mm	coccus	(+)	Staph

Table 4. Colony characteristics

Table 4 shows that there are so many varies of colonies characteristics in each sample.

DISCUSSION

The results in this study, only three patients had the clinical examination over drained sinus on their oral mucosa. A chronic periapical abscess is not usually associated with pain and is typically characterized by the clinical sign a draining sinus on the oral mucosa or occasionally on the facial skin¹³, and three patients on this study show a draining sinus on their oral mucosa. Periapical abscess based on occurrence divided into an acute

and chronic periapical abscess. A chronic periapical abscess is an inflammatory reaction to pulp infection and pulp necrosis that occurs gradually with or without discomfort with an intermittent discharge of pus through the associated sinus tract. Endodontic abscess formation is an inflammatory reaction to pulp infection and necrosis. 14 Chronic periapical abscess is a condition that arises due to continuously bacterial infection which forms abscess lesions then drain into the mucosa, an inflammatory reaction to pulp infection and necrosis characterized by gradual, slight or painless onset. 14-16 To determine a diagnosis of chronic periapical abscess, we should do another examination, such as radiographic and microbiological examination, not only from this clinical examination.

The ITK-Snap calculations result in this study based mean voxel intensity value of surrounding bone at 636.022 ± 79.981 and the centre of the lesion radiolucent images mean voxel intensity value 256.710 ± 81.930 that show at Table 2 and 3. As a visual interpretation of chronic periapical abscess showed radiolucent areas of periapical lesions with a diffuse radiopaque border of surrounding alveolar bone that indicate decreased density from centre periapical lesion into the boundaries of alveolar bone lesions. Within voxel intensity value measurement that showed numerical information contained in each CBCT voxel image.

In a digital analysis, there is a difference in pixel values in areas that initially do not absorb photons with absorbing photons. Saeed stated that variations in grayscale values when correlated with histological differences had a direct correlation between grayscale values and lesion content.¹⁷ X-ray photons released passing through soft and hard tissue, will be absorbed and penetrate the tissue. The absorption of x-ray photons will ionize the atoms of the tissue material example atomic compact bone (Z = 13.8) greater than soft tissue (Z = 7.4) then intensity is reduced. Various tissue and object provide radiographic contrast. Contrast resolution is the ability of an image to reveal subtle differences in image radio-density. The differential photoelectric absorption of x-ray photons in various tissue as different degrees of Image intensity is a result of attenuation of x-rays by tissues that vary in density, atomic number, or thickness.¹⁸ We evaluate our study using intensity of voxel value that's a precise density of the images. This is recommended analysis methods to reveal the density changes in the trabecular bone associated with a periapical lesion.

T-test was used for statistical analysis, our results of the paired t-test this study showed that, on average, a significant difference was found in voxel intensity of surrounding bone and centre of the lesion that assumes surrounding bone was denser than the periapical lesion. According to Figure 3 that showed Image intensity changes from radiopaque to radiolucent or decreased density, indicating a loss of bone density and loss of trabecular amount. If the trabeculae decalcified, the trabeculae will not appear on the radiograph. This is related to the composition and nature of the image and is used to represent density.

There are several methods for assessed periapical lesion and bone structure using radiography. Estrela et al. reported measurement CBCT and periapical Index to score the radiolucent area. Gungor et al. assessed the accuracy and reliability of linear distances and voxel density measurements of cone-beam computed tomography (CBCT) at different voxel sizes. Voxel density (VD) is used to transform the radiodensity of the relevant region into numerical values. Values. Parsa et al. performed VD measurements in spongeous bone using CBCT, MSCT, and micro-CT (considered a gold standard reference) and found a correlation of 0.82 between CBCT and micro-CT.

This research generally has results that are consistent with research Geiger et al. which states that the periapical abscess has a difference with surrounding bone tissue which can represent the value of relative density of the lesion and surrounding bone tissue.23 The study used a quantitative description by measuring the mean grey value of periapical lesion and surrounding bone lesion.²³ Sogur et al. has similar analyses with our methods that assessed periapical lesion intensity for the diagnosis however they used 2D radiograph while our study using CBCT 3D radiograph.²⁴ CBCT 3D assessment of volume periapical lesion evaluation in Schloss et al. study more precise than periapical radiograph 2D25, also given indication CBCT 3D imaging is recommended as a valuable tool for the diagnostic periapical lesion.25

There are varies characteristic colony that can found in this research, showed in Table 4. Characteristics of colonies can be used to identify bacterial species. Characteristics that can be used are colour, shape, and surface texture. Some bacterial species cannot form circular colonies because the cells are able to move and gather on the agar surface, especially if the surface is moist. The growth phase of various types of bacteria looks different in colony growth in a dense medium.

The varies of diameter colonies as we can see on Table 4, showed that the cells will divide exponentially and form small colonies which are derivatives of the original cells. Colonies will grow rapidly on the edge of the colony, while cells near the centre will divide more slowly or even die from nutrition or be exposed to toxic bacterial products. Where one living bacterium is on the agar plate, the bacteria will divide to form a colony.²⁷

From microscopic examination in Table 4, there were two different types of bacterial shape, Gram staining, and formation which are Gram-positive coccus in staph and strep formation this Gram-positive coccus bacteria can be Staphylococcus aureus or Streptococcus viridans which are the predominant bacteria in periapical abscesses as Mahalle study stated that Staphylococcus aureus was isolated in 13 instances (43.3%), Streptococcus viridians in 4 instances (13.3%) followed by anaerobic Streptococci in 4 instances (13.3%).28 With the acquisition of 13 different types of bacterial colonies, it shows that periapical abscess is a disease caused by multiple bacteria. Different types of bacteria show proper medicinal selection for each treatment of cases of periapical abscesses. The success and effectiveness of treatment can be supported well through the identification of bacteria from lesions. Within the limits of the present study, it can be concluded that abscesses periapical chronic has isolates of mixed nature. Further research is needed to identify bacteria with such characteristics.

The weakness of this preliminary study is the short research period which caused the minimum sample number. From the radiographic aspect, the minimum sample number caused standard voxel intensity value for chronic periapical abscess

undetermined. In microbiology examination, this study only using microscopic and macroscopic examination, the chronic periapical abscess undetermined. Based on findings radiographic aspect, related to voxel Intensity of periapical lesion would inspire further studies with a larger sample to estimated standard voxel intensity value for chronic periapical abscess. Further research from the microbiological aspect is needed to identify species bacteria using biochemical examination.

CONCLUSION

Demineralisation of periapical bone can be determined by voxel intensity value in CBCT 3D radiograph using semi-auto segmentation ITK SNAP, could be recommended as a quantitative interpretation for detection periapical lesion and chronic periapical abscesses is a poly-microbial disease.

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