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Table of Content: Volume 7 Number 4 April 2015

ARTICLES

Research Articles

- Three-year follow-up: Healing of a large periapical lesion related to a maxillary central incisor and,two canalled lateral incisor after a single visitroot canal treatment** 40
Ammar Abu Mostafa
- The prevalence of oro-dental anomalies among 14-17 years students in Panchkula District Haryana, India** 44
Preety Gupta, Nidhi Gupta, Rajesh Gupta, Vikram Arora and Nishant Mehta
- Is there hidden caries or is this a limitation of the conventional exams?** 48
Valdeci Elias dos Santos Junior, Arnaldo Vasconcelos Filho, Andreza Cristina de Lima Targino Massoni and Aronita Rosenblatt

Case Report

Three-year follow-up: Healing of a large periapical lesion related to a maxillary central incisor and two canalled lateral incisor after a single visit root canal treatment

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In this study, a patient with a large periapical lesion related to the maxillary left central incisor and two canalled lateral incisors was presented. A single visit non-surgical root canal treatment was performed for both teeth. A three-year follow-up radiograph revealed complete healing of the periapical lesion. This report confirms that the large size of a periapical lesion neither mandates surgical removal nor the placement of intra-canal dressing and that such a lesion can heal following a conservative single visit endodontic treatment. Furthermore, familiarity with the variations of root canal morphology is essential for successful endodontic treatment. Maxillary lateral incisors usually have a single canal; however, this case report describes endodontic treatment of maxillary lateral incisor with two buccopalatal root canals.

Key words: Healing of large periapical lesion, single visit root canal treatment, two canalled maxillary lateral incisor.

INTRODUCTION

Dental pulp gets infected mainly as a result of caries, operative dental procedures and trauma. Pulp infection usually involves a mixed, predominantly Gram-negative, anaerobic bacterial flora (Sundqvist, 1994). This infection leads to pulp necrosis, which subsequently stimulates an immune reaction in the periapical area that creates what is commonly known as a periapical lesion (Stashenko, 2002). Identifying whether the periapical lesion is a cyst or granuloma is notoriously difficult using radiography.

However, lesions exceeding 200 mm are most probably periapical cysts (Natkin et al., 1984). Additionally, it is also necessary for a lesion to present a straw-coloured fluid produced upon aspiration or on drainage, which contains cholesterol crystals as well as the relation of the lesion with a tooth with pulp necrosis to establish a provisional-“clinical” diagnosis of periapical cyst (Eversole, 1984).

Treatment of such large periapical lesions ranges from

non-surgical root canal treatments and/or apical surgery to extraction. However, the current treatment philosophy lies in the first place on non-surgical root canal treatment. If such treatment does not lead to resolution of the periapical lesion, additional treatment options should be considered (Caliskan, 2004). These treatment options might include non-surgical retreatment to rule out morphological abnormalities or treatment inadequacies. Surgical intervention might occasionally be required, in which curettage and apical resection are frequently performed. However, simpler approaches such as marsupialization or tube decompression may be alternatives for the treatment of large cystic lesions (Hoen et al., 1990).

Awareness of the complexity of root canal systems among dentists has increased, which has led to the development of newer techniques, instruments and materials. This awareness remarkably enhances the practitioner's ability in dealing with such cases (Regan and Guttmann, 2004). An awareness of root canal morphology in general, and possible complexity in particular, is essential for adequate endodontic therapy.

Although, Vertucci's (1984) study reported that 100% of the maxillary lateral incisors have one root and one canal; it seems that this generalization is not totally correct as case reports showed maxillary lateral incisors with more than one canal and/or root (Walvekar and Behbehani, 1997; Pereira et al., 2000). Most of these cases involve instances of germination, fusion, concrescence, or dens invaginatus, since maxillary lateral incisors are often located at the site of high embryological risk. This is attributed to the fact that the mesial nasal process and maxillary process fuse during the fourth and sixth weeks of human embryonic development medial to the lateral incisor or at the medial or middle one-third of the lateral incisor to form the maxilla (Mohan et al., 2012; Lim et al., 2012).

This report discusses root canal treatment of a maxillary central incisor and two canalled maxillary lateral incisors associated with a large periapical lesion.

CASE REPORT

A 21-year-old male patient visited the dental clinics of Riyadh Colleges in Riyadh, KSA, having no complaint and only for a check-up. Initial examination in the screening clinic and periapical radiographs demonstrated a large radiolucent lesion around the apices of the left maxillary central and lateral incisors with a well-defined margin, mainly related to the central incisor. The size of this lesion as digitally measured using Kodak-RVG software (USA) was 2.3 cm. Clinically, these two teeth were restored with proximal mesial composite restorations. Next, the patient was referred to the endodontic clinic. Upon investigating the history of these two teeth, the patient mentioned that he was exposed to trauma 3 years ago. Response to the cold vitality test for both the central and lateral incisors was negative. Response to percussion and palpation tests was also negative. Radiographically, a slight internal resorption defect was found in the apical third of the central incisor in addition to the previously described large periapical lesion. The two teeth

were diagnosed as having necrotic pulp associated with chronic apical periodontitis.

After local anesthesia and rubber dam isolation for the central and lateral incisors, root canal treatment was initiated for both teeth together. The two canals of the maxillary lateral incisor were detected depending on the tactile sensation of the operator, the furcation in the mid-root was felt and detected using K-file size 15. The working length was determined using an apex locator (Root ZX II Apex Locator, J.Morita, USA). For all the canals, preparation was completed using Easy Race instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland) with copious irrigation with sodium hypochlorite 2.5%. The master apical file was size 40 taper 6% in the central incisor and size 35 taper 6% in the two canals of the lateral incisor. Apical patency was maintained in all canals by pushing K-file size 15 beyond the apex. Ethylenediaminetetraacetic acid (EDTA) 17% solution (EDTA solution, Pulpdent, USA) was used as a final rinse to remove the smear layer.

No pus or exudate was found, and canals were properly dried using paper points (SybronEndo, Orange, CA, USA), then obturated in the same visit with Gutta percha (SybronEndo, Orange, CA, USA), and AH26 sealer (Dentsply, UK). The technique employed was continuous wave compaction by System-B and Obtura II (SybronEndo, Orange, CA, USA). The access cavities for both teeth were then temporarily restored by Cavit (3M ESPE, UK and Ireland). Figure 1 shows the teeth immediately after obturation. No postoperative pain was found, and then the teeth were restored by composite (3M ESPE, UK and Ireland) two days after obturation. A periapical radiograph taken three years later showed complete healing of the lesion (Figure 2). The patient could not come earlier than this due to unforeseen circumstances.

DISCUSSION

The consequences of dental trauma might include pulp necrosis, tooth resorption, calcific metamorphosis and tooth ankylosis (Haapasalo et al., 2007).

In this case, the trauma led to pulp necrosis in both teeth and little internal resorption in the apical third of the maxillary central incisor. Necrotic pulps provide a good nutritional supply for pathogenic bacteria, which must be present for the development of a periapical lesion. The main objective of root canal treatments for infected cases is based primarily on the removal of microbial infection from the root canal space, which is achieved by mechanical preparation of the canal and chemical disinfection (Seltzer, 1988). Root canal irrigant helps reduce the number of microorganisms inside the infected canals, and if a tissue-solvent solution is used, it can help to dissolve the necrotic tissue. Sodium hypochlorite was used in this case as it is an effective antimicrobial agent and has tissue-dissolving capabilities. Additionally, it has low viscosity, allowing easy introduction into the canal architecture (Spencer et al., 2007). In this case, neither pus nor inflammatory exudate was coming out of the canals; additionally, all canals were totally dried. Therefore, root canal treatment for both teeth was finished in one visit.

The antimicrobial effect of calcium hydroxide as an intra-canal medicament is well known, particularly in cases of infection associated with periapical lesion



Figure 1. Periapical radiographs with two different angulations taken immediately after obturation and temporization.



Figure 2. Periapical radiograph taken three years after obturation showing complete healing of the periapical lesion.

(Mohammadi and Dummer, 2011). However, studies have shown that one-visit root canal treatment can create favorable environmental conditions for periapical repair similar to the two-visit therapy when calcium hydroxide was used as antimicrobial dressing (Weiger et al., 2000; Figini et al., 2008). The findings of this report support this as total healing occurred after a single visit root canal treatment despite the presence of a large periapical lesion.

Maxillary incisors usually have single canals; however, in cases where a maxillary incisor has two canals, they are usually located mesiodistally (Thompson et al., 1985; Hosomi et al., 1989; Michanowicz et al., 1990; al-Nazhan, 1991; Lambruschini GM, Camps, 1993). In this case, the lateral incisor had a single root containing two buccopalatal root canals that started as a single canal in the coronal half, then separated to leave the root via two apical foramina, thereby having type V according to Vertucci's classification (Vertucci, 1984).

Continuous wave compaction was the obturation technique of choice for this case. For this technique, gutta percha is softened and vertically compacted. Using this technique helped fill the internal resorption defect in the central incisor three dimensionally.

Conclusion

This reported case proved that single visit non-surgical root canal treatment can successfully lead to the complete healing of a large periapical lesion. Additionally, and although rare, the clinician should expect a maxillary incisor with more than one canal.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Full Length Research Paper

The prevalence of oro-dental anomalies among 14-17 years students in Panchkula District Haryana, India

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The aim of this study was to find out the prevalence of oro-dental anomalies among 14 to 17 years students in Panchkula District Haryana, India. Oral examination among 3248 students (1608 males and 1640 females) aged 14 to 17 years in Panchkula District of Haryana was carried out in 18 secondary and intermediate schools. Oro-dental developmental anomalies seen in 946 students represents 29.1% of the studied sample, 42.1% males and 57.9% females; there was a significant statistical difference between them. Majority of the oro-dental developmental anomalies were presented in 16 years age group in the sample. The study reported that 29.8% of the participants were affected with atleast one dental anomaly. Enamel hypoplasia, followed by microdontia was the most common findings. Geographic tongue, commissural lip pits, fissured tongue and lingual varicosities showed a significant statistical difference among the two genders.

Key words: Oral anomalies, dental anomalies, prevalence.

INTRODUCTION

Developmental anomalies of teeth, oral soft and hard tissues are groups of conditions which arise due to disturbances in development and growth that involve these tissues. Some of them develop in utero and these are usually present at birth and persist throughout life. Others may not manifest themselves for many years (Mohanad and Wasan, 2009). Most of these anomalies are congenital (i.e., present at birth) and considerable number of these have genetic basis. The cause appears complex and multifactorial, involving the interaction of genetic and environmental factors (Makki, 2003).

Studies on the prevalence of dental anomalies show

divergent results (Ezoddini et al., 2007; Gupta et al., 2011; Thongudomporn and Freer, 1998; Uslu et al., 2009); while some investigations show the prevalence of tooth anomalies as low as 21% (Ooshima et al., 1996), other studies show the prevalence of almost 75% (Thongudomporn and Freer, 1998). Several investigations report a prevalence of tooth anomalies to be between 34 and 40% (Ezoddini et al., 2007; Gupta et al., 2011). The reasons for such discrepancies can be multifold. Ethnic differences can be one explanation, but the type of dental anomalies investigated and the use of different diagnostic criteria can also contribute to the

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divergent results.

Around 7% of children are born with some of the disturbances in the oro-facial region and most commonly are supernumerary teeth, missing teeth, fused teeth and peg lateral incisors (Clayton, 1956). Dental anomalies in comparison with more common oral disorders such as dental caries and periodontal diseases have low frequency, but their management procedure is more complicated, because they can result in esthetic problems, malocclusion, and lead to the other oral problems (Ghabanchi et al., 2010). In industrialized countries, there are about 10% of children with developmental disturbances, whereas in developing countries like India their percentage is higher, ranging between 15 and 20%. The identification of oral/dental and minor anomalies is of great importance for timely and accurate diagnosis of numerous genetic abnormalities of the craniofacial region (Patel and Kleinman, 2003). Hence, this study was done to know the prevalence of oro-dental anomalies among school going adolescents.

METHODOLOGY

This study was conducted among 3248 students (1608 males and 1640 females) aged 14 to 17 years in Panchkula District of Haryana, India during a four month period in 2014. Eighteen (18) secondary and intermediate schools from Panchkula district were randomly selected. The study population was taken with cluster sampling technique. The study sample was divided into 4 age groups (14 years = 812 students, 15 years = 812 students, 16 years = 814 students and 17 years = 810 students) with an equal male:female ratio in each age group. Before scheduling the survey, the official permission was obtained from the Institutional Ethical and Review Committee of Swami Devi Dyal Hospital and Dental College (dated: 2 January, 2014). Official permission was obtained from the heads of the institutes from the district. Informed oral consent was obtained prior to examination of each subject. A pilot survey was conducted in one of the school on 100 randomly selected subjects to know the prevalence of dental anomalies and feasibility of the survey. Children with any kind of medical history such as Down's syndrome, ectodermal dysplasia, cleft lip and cleft palate were excluded from the study. The clinical examination of the oral and dental anomalies was performed using sets of instruments consisting of plane mouth mirrors and sharp probes which are kept in a kidney tray containing 2.5% gluteraldehyde solution. Specially designed chart was used to record the personal data. The students were instructed to rub their teeth by piece of cotton supplied by investigator to get as much clean teeth as possible and to obtain clearly visualized field for examination. Clinical examination was done to know the prevalence of hard and soft tissues defects. All subjects were made to sit in a chair under natural light for examination (Type III). The recording clerk was made to sit near to the examiner so that the instructions could be effortlessly recorded.

Data analysis

The statistical software, namely, Statistical Package for Social Sciences (SPSS) version 16.0 was used for data analysis. Values were compared using Chi-square test. The p value of 0.05 or less was considered as statistically significant.

RESULTS

The total number of affected students was 946 students (398 males and 548 females) representing 29.1% of total sample. There was a statistical difference between males and females. Most of the oro-dental developmental anomalies were seen in 16 years age group, while the less affected age group was 14 years, as shown in Table 1. Also, there was a high statistical difference between the age groups (P -value<0.001).

The prevalence of different dental developmental anomalies examined in this study is as shown in Table 2 concerning the sex and statistical difference. Environmental diffuse opacity was the most common oro-dental developmental anomaly in this study (4.9%). Micro-dontia represented 4.01% which was considered the higher prevalence rate. Dens invaginatus had the lower prevalence in this study (0.03%). There was a highly significant statistical difference between males and females in enamel opacities of teeth (P -value=0.001).

Table 3 shows the prevalence rate of different oral soft and hard tissues anomalies; fissured tongue showed higher prevalence (4.2%) among other oral soft tissue anomalies and was considered the most common anomaly of the tongue in this study. Geographic tongue had a high significant statistical difference between sexes (P -value<0.001). Commissural lip pits, fissured tongue and lingual varicosities showed a significant statistical difference between males and females. Microglossia had lower prevalence (0.03%) among soft tissue anomalies. Torus palatinus was the only hard tissue anomaly seen in this study represented by 0.4%.

DISCUSSION

The presence of dental anomalies is commonly seen during routine dental check-up. In the present study, the prevalence of permanent tooth anomalies was assessed among 14 to 17 year old school children in rural and urban areas of Panchkula District, Haryana, India. Although there have been several studies reporting the prevalence of various dental anomalies, no reported study has been carried out in this region.

The results of this study indicate higher prevalence rates of dental anomalies among all the age groups in comparison to earlier studies. The reason for such difference could be different degrees of severity from the mildest developmental delay to the most severe tooth agenesis manifestation; dental anomalies may be expressed as microdontia, changes in dental morphology and ectopias and also varying definitions of dental anomalies (Garn et al., 1965). Earlier studies have also suggested genetic and hereditary background in the causation of dental anomalies affecting size, shape, number, position and timing of development (Vastardis, 2000; Markovic, 1982; Mossey, 1999; Baccetti, 1998).

Environmental diffuse and localized opacities were the

Table 1. Number and percentage of affected students of males and females by age groups.

Age (Years)	Total	Male	Female	%
14	160	54	106	16.9
15	267	148	119	28.2
16	298	112	186	31.5
17	221	84	137	23.4
Total	946	398	548	100

Table 2. Number, distribution, percentage and statistical difference of Dental anomalies.

Dental anomaly	Total	%	Male	Female	P-value
Enamel hypoplasia	82	2.5	26	56	0.001**
Environmental localized opacity	142	4.4	68	84	NS
Environmental diffuse opacity	158	4.9	59	99	NS
Attrition	13	0.4	8	5	NS
Retained deciduous teeth	101	3.4	45	56	0.007*
Supernumerary teeth	11	0.34	7	4	0.03*
Microdontia	129	4.01	61	68	NS
Macrodontia	44	1.4	19	25	NS
Talon cusp	7	0.22	4	3	0.02*
Rotation	66	2.03	31	35	0.05*
Dens invaginatus	1	.03	0	1	NS

NS: Non-significant; *Significant; **Highly significant.

Table 3. Number, distribution, percentage and statistical difference of oral soft and hard tissues.

Oral anomaly	Total	%	Male	Female	P-value
Cleft lip	4	0.12	2	2	NS
Commissural lip pits	3	0.09	3	0	0.047*
Fissure tongue	136	4.2	51	85	0.09*
Geographic tongue	30	0.92	10	20	<0.001**
Macroglossia	2	0.06	2	0	0.049*
Microglossia	1	0.03	0	1	NS
Lingual varicosities	3	0.09	3	0	0.047*
Torus palatinus	13	0.40	4	9	NS

NS: Non-significant; *Significant; **Highly significant.

most common oro-dental developmental anomalies in this study (4.9 and 4.4%, respectively). Studies conducted by Kathariya et al. (2013) and Ooshima et al. (1996) also reported similar frequency. Microdontia represented 4.01% of the cases, which is comparable to study done by Kathariya et al. (2013). This study separately recorded the prevalence of retained deciduous teeth (3.4%), rotation (2.03%), enamel hypoplasia (2.5%), attrition (0.4%), supernumerary teeth (0.34%), macrodontia (1.4%), talon cusp (0.22%) and dens invaginatus (0.03%). There was a highly significant

statistical difference between males and females in enamel opacities of teeth (p value < 0.001). The prevalence of supernumerary teeth is less when compared with study conducted by Gupta et al. (2011), where they recorded a prevalence of 2.40%, whereas occurrence of other anomalies can be compared with other studies done by Mahmood (2008).

On comparing prevalence among males and females, significant difference was observed only for the enamel opacities, which was also observed by Mohanad and Wasan (2009) in their study.

This study also compared prevalence rates of oral soft and hard tissues anomalies. Fissured tongue was seen in 4.2% of the study population which is less than the study conducted by Younis and Majeed (2002) and Muhammed and Qassim (2005) among higher age groups, in which they reported prevalence of 31.87 and 6.2%, respectively. This difference could be attributed to the fact that incidence of geographic tongue increase with age. Geographic tongue, commissural lip pits, fissured tongue and lingual varicosities showed a significant statistical difference between males and females. The reasons for such a difference should be explored and similar studies can be carried out to prove the gender predilection for several dental anomalies.

This clinical survey had a limitation that radiographs were not used in this study which could have underestimated the prevalence of dental anomalies which could otherwise be visible on a radiograph. However, this research does open new vistas for prevention and treatment planning programs for those who were suffering from these diseases or more prone to these diseases.

Conclusion

This study reported that 29.8% of the participants were affected with atleast one dental anomaly. Enamel hypoplasia followed by microdontia were the most common finding. Among hard tissues, there was a significant statistical difference between males and females in enamel opacities of teeth whereas among the soft tissues, geographic tongue, commissural lip pits, fissured tongue and lingual varicosities showed a significant statistical difference among the two genders. The high levels of these anomalies among the school children suggests to find the etiological factors involved in the occurrence of dental anomalies, as well as formulation of effective dental programs for the timely screening and treatment of such defects.

Conflict of interests

The authors declare that they have no conflict of interest.

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Full Length Research Paper

Is there hidden caries or is this a limitation of the conventional exams?

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The aim of this study was to verify the existence of hidden caries and compare the diagnosis agreement among different examiners by visual, dental radiography and computed microtomography images of hidden dental caries lesions on the occlusal surface of extracted molars. Two hundred and fifteen teeth were examined visually and extracted from cadavers of adolescents with a mean age of 12 years. Eleven teeth which showed hidden occlusal caries were included in the study. Occlusal sites were examined both visually and by dental radiograph by 3 different dental practitioners. Results were compared and validated independently by each examiner with computed microtomography; hence, the gold-standard in current investigation. The coefficient of agreement was calculated by the Cohen's Kappa test. The agreement among examiners for the diagnosis by standard microtomography was excellent ($K = 0.924$) and moderate for both the visual ($K = 0.515$) and the radiographic ($K = 0.583$) examinations. Computed microtomography-produced images which allowed the examiners to visualize radiolucent areas unseen in previous radiographic images and visual examinations showed anatomic communication between enamel and/or dentine to the external environment, not necessarily due to enamel collapse. Occlusal hidden caries was being over detected by clinicians because of the limitations of the conventional dental visual and radiographic examination, which indicate that the examinations are not accurate enough to detect the lesions.

Key words: Computed microtomography, dental diagnostics, dental caries.

INTRODUCTION

Since 1970, the pattern and prevalence of dental caries in permanent teeth have revealed a marked change (McDonald and Sheiham, 1992; Beltrán-Aguilar et al., 1999; Whelton, 2004; Bernabé, 2009; Bagramian et al., 2009). This fact indicates that dentine lesions under non-cavitated enamel actually represent about 50% of total

caries in many world communities (Bagramian et al., 2009). Although several labels for this type of lesion have been forwarded, namely, 'fluoride syndrome', 'fluoride bomb' and 'hidden caries' (Weerheijm, 1997; Zadik and Bechor, 2008; Hashizume et al., 2013), its aetiology is still undetermined.

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Dentists who routinely examine clinically caries-free children may be shocked to discover a large caries lesion on a radiograph that they may have missed in the visual examination (Bader et al., 2002; Mutsvari et al., 2012). The detection rate of such lesions depends upon the prevalence of caries in the population and on the frequency of bite-wing radiographic examinations. Information on the prevalence of these lesions in the world population is still unpractised, even though the available data shows that it ranges from 0.8% in Scotland (Creanor et al., 1990) to 50% in Germany (Weerheijm et al., 1992).

The visual examination is the method of choice to diagnose dental caries. However, clinically undetected occlusal caries are of difficult diagnosis (Bader et al., 2002) and routinely require additional examinations, such as conventional radiographs (Souza-Zaroni et al., 2006; Ricketts et al., 2007) for a more accurate one. However, the aforementioned technique's limitation results from overlapping images of the occlusal surface due to a two-dimension diagnosis (Neuhaus et al., 2009).

While the x-ray method projects the image on two planes, the CT-scan image reproduces the internal structures on three spatial planes. Thus, the structural relations are shown in depth, including the images of the segments and layers' internal structures, particularly the mineralized tissues, with excellent definition, which favor the division of three dimensional irregularities (Parks, 2000).

Thus, the aim of this study was to verify the existence of hidden caries and compare the diagnosis agreement among different examiners between the visual and radiographic examination and computed microtomography.

METHODOLOGY

In this *in vitro* descriptive study, 215 permanent molars were collected from donated cadavers of 12 to 15-year-old adolescents at the Forensic Medicine Institute of Pernambuco, Brazil and 11 (5.11%) of the 215 teeth were cleaned, dried and included in the study, the ones that exhibited caries images under microtomography examination (micro-CT scan).

The investigation was developed according to recommendations by the Committee in Ethics in Human Research of the University of Pernambuco, Brazil. Furthermore, parents or legal representatives agreed on the donation of the teeth and signed the informed consent.

Detection of caries without communication with the external environment

Due to their three-dimensional nature, CT scanning images were used as standard to detect the existence of lesions in dentine without any external communication. The teeth were then examined by three independent examiners who looked at the images and identified the absence (0) or presence (1) of the lesions. Examiner 1 was the gold standard on detecting caries in micro-CT scan and participated in all examinations.

Accuracy of diagnosis tests

Specimens were prepared by carefully cleaning the teeth with hand scale and rotating bristle brushes with water/pumice to remove deposits of calculus, plaque or debris. They were then stored in individually identified bottles with 10.0% formaldehyde solution.

The teeth were examined by three independent examiners employing visual radiographic and microtomographic methods. The three examiners were identified as Examiner 1, Examiner 2 and Examiner 3.

Visual examination (VE)

Visual examination was performed using a dental light unit. Three independent dentists, averaging 10 years clinical experience each, examined each tooth, looked at the occlusal surfaces and identified the absence (0) or presence (1) of cavitated caries lesions.

Radiographic examination (RE)

For the radiographic examination, the teeth were held with utility wax and radiographed with periapical film (Ektaspeed/Eastman Kodak Company®, Rochester, USA). The focus-film distance was 20 cm, with an exposure time of 0.5 s. Radiographs were taken using Gnatus X-ray equipment (10 mA and 70 kV) and the films were processed in a portable dark-box and in solutions (developer and fixer) for conventional Kodak Dental X-rays (Kodak®, São José dos Campos SP Brazil), at a temperature of 24°C, for 3 min. Defective radiographs were discarded and new radiographs taken. The teeth were then examined by three independent examiners who looked at the images and identified the absence (0) or presence (1) of the dentine lesions.

Computed microtomographic examination (MCTSE)

Teeth were imaged by micro-CT scan on a Siemens Inveon PET-CT (Siemens Molecular Imaging, Munich, Germany) for microtomographic examination. The x-ray source was set to voltage and current of 80 kVp and 500 µA, respectively. The x-ray source and CCD detector camera were positioned to each tooth so that the effective pixel size would be 41.80 µm isotropically. As many as 360 projections were retrieved for a 330-min exposure time. All projection data were reconstructed with Filtered Backprojection. The images were interpolated bilinearly, and filtered with a Shepp-Logan filter for higher resolution. Finally, conversion to DICOM format allowed the images to be viewed by DICOM medical imaging viewers.

The teeth were then examined by three independent examiners who looked at the images and identified the absence (0) or presence (1) of enamel and dentine lesions.

Statistical analysis

Because of its superior image quality over the other two methods, the Micro-CT was chosen as the gold-standard method to compare the accuracy of the micro-CT scan with the visual and radiographic examination of the occlusal lesions.

Moreover, the inter-examiners agreement for the tested methods (visual, radiographic and micro-CT scan) for occlusal lesion detection was also calculated and thus Cohen's Kappa statistic test expressed the coefficient of agreement for the three examiners.

The Kappa statistic test, formulated by Cohen in 1960, is an agreement coefficient that corrects the error due to chance by paired analysis. It compares the observed proportion of agreement

Table 1. Cross-tables showing the relationship between the different methods and Cohen's kappa values for Inter-observer for occlusal lesion. detection.

Variable	Examiners			Total	kappa IE
	1	2	3		
Visual					
(0) Absence	7	6	6	19	0.515
(1) Presence	4	5	5	14	
Total	11	11	11	33	
Radiography					
(0) Absence	3	3	4	10	0.583
(1) Presence	8	8	7	23	
Total	11	11	11	33	
Micro-CT scan					
(0) Absence	1	2	1	3	0.924
(1) Presence	10	9	10	6	
Total	11	11	11	33	

Table 2. Coincidence between visual examination and radiographic examination in relation to micro-CT scan examination for three Examiners.

Variable	Concordance		Total
	Yes	No	
Visual x Micro-CT scan			
Examiner A	3	8	11
Examiner B	6	5	11
Examiner C	6	5	11
Total	16	17	33
Radiography x Micro-CT scan			
Examiner A	2	9	11
Examiner B	3	8	11
Examiner C	2	9	11
Total	6	27	33

between the observed (P_o) and the proportion of agreement expected by chance. Rates vary between -1.0 (complete disagreement) and $+1.0$ (complete agreement); zero represents the agreement expected by chance.

RESULTS

Table 1 shows the results of the examination by the three examiners employing the visual, radiographic and micro-CT scan methods and the inter-examiner agreement for occlusal lesion detection calculated by Cohen's Kappa rates. Kappa rates were 0.515, 0.583 and 0.924 for the inter-observer agreement, respectively for visual, radiographic and microtomographic examinations (Table 1).

Table 2 presents the coincidence between visual examination and micro-CT scan examination, and between the radiographic examination and micro-CT scan examination for three examiners. So that visual inspection and examination by micro-CT scan could be compared, the coincidence for the presence or the absence of communication from the external to the lesion for both techniques was taken into account.

Figure 1a, b and c shows a new sequence of analysed images of tooth F. In this case, the examiners disagreed on visual examination and agreed on the radiographic and micro-CT examinations. One examiner stated that the clinical examination indicated a sound surface and the radiographic image was described as radiolucency in dentine, to which all examiners agreed (Figure 1a and 1b,

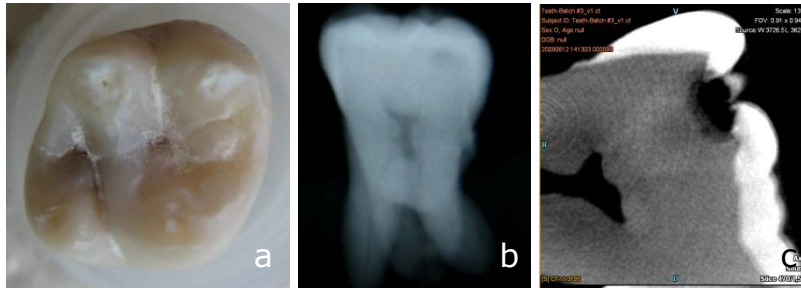


Figure 1. Tooth F: (a) photo of occlusal surface, (b) radiographic image, and (c) micro-CT scan image.

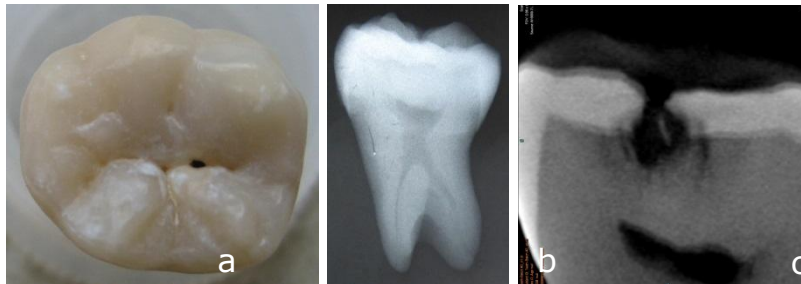


Figure 2. Tooth E: (a) photo of occlusal surface, (b) radiographic image, and (c) micro-CT scan image.

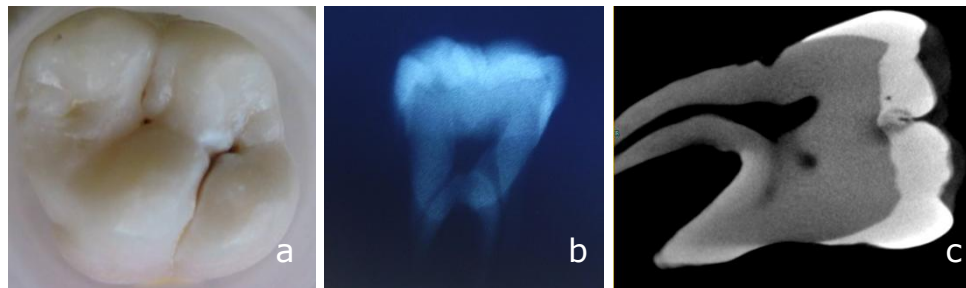


Figure 3. Tooth D: (a) photo of occlusal surface, (b) radiographic image, and (c) micro-CT scan image.

respectively). The micro-CT image revealed radiolucency in enamel and dentine, to which all the three examiners agreed (Figure 2c).

Tooth E is as shown in Figure 2a, b, and c. The three examiners agreed that there was a lesion in the occlusal surface when examined visually (Figure 2a). However, they failed to agree on the radiographic diagnosis (Figure 2b). All examiners detected the presence of radiolucency in enamel and dentine with micro-CT scan (Figure 2c).

With regard to tooth D, as shown in Figure 3a, b, and c, the examiners differed on the visual and radiographic examinations (Figure 3a and b, respectively). However, when the micro-CT scan examination was employed, the three examiners were able to identify radiolucency in enamel and dentine.

DISCUSSION

In the present research, occlusal lesions were determined by three methods of caries detection that enabled dental practitioners to decide on treatment options, of which two are used routinely in dental practice whilst the third method, micro-CT scan image, was chosen as the gold standard owing to its superior quality and accuracy. Results indicated that the visual and radiographic method exhibited only moderate correlation to micro-CT scan (Tables 1 and 2). The images observed by micro-CT scan revealed that the 11 teeth examined in current research showed communication between the external environment and the occlusal lesion. Communication with the external environment detected

by micro-CT scan invalidated the theory that described these injuries as hidden caries (Seow, 2000; Chong et al., 2003), since, by definition, this condition would definitely show dentine lesion under non-cavitated enamel. Interestingly enough, micro-CT scan showed broken enamel with dentine exposed to mouth environment. The low sensitivity and specificity of the conventional radiographs for the detection of lesions in the occlusal surface may be due to the nature of the two-dimensional, albeit low-quality, images (Poorterman et al., 2000).

When caries reached the dentin at the dentin-enamel junction, decay quickly spread laterally. Decay within the dentin followed a triangular pattern that pointed towards the tooth's pulp. In fact, decay pattern is typically described as two triangles (one triangle in enamel and the other in dentin) with their bases joined to each other at the dentin-enamel junction (DEJ). Actually, such base-to-base pattern is typical of pit and fissure caries (Thylstrup and Fejerkov, 1994). It should be emphasized that the radiograph-generated image failed to provide a clear view of this characteristic of occlusal caries, clearly seen on the micro-CT scan image.

Clinical diagnosis of occlusal caries is one of the most difficult issues in the field of dentistry, with uncertainties as to its outcome. Mouth mirror, good reflected light and a blunt or a sharp probe are the dentist's principal diagnostic tools, but they lack overall accuracy in terms of sensitivity and specificity (Poorterman et al., 2000). This difficulty has also been observed in current study on the diagnosis of the eleven examined teeth. For instance, when examiners diagnosed tooth F (Figure 1), they could only partially agree on the visual examination, whereas they totally agreed on the radiographic and micro-CT scan examination. In fact, Examiner 1 described the occlusal surface as sound, but Examiners 2 and 3 identified lesion on the same surface (Figure 1a). While the three examiners identified radiolucency in enamel and dentine by micro-CT scan (Figure 1c), the radiographic examination only allowed them to diagnose radiolucency in dentine (Figure 1b). Dental X-rays mostly fail on displaying enamel lesions and communication with the external environment. They were, however, very clear in the images produced by micro-CT scan (Table 2).

Although the three examiners were able to clinically identify lesion on the occlusal surface (Figure 2a), the dental radiographs were not helpful to show to Examiner 1 the radiolucency suggestive of lesion in dentine. Contrastingly, Examiners 2 and 3 identified radiolucency in dentine (Figure 2b) but micro-CT scan images failed to confirm any diagnosis by the radiographic examination (Table 2). In fact, it showed the presence of radiolucency in enamel and dentine (Figure 2c) and reflected the communication of the lesion with the external environment and thus confirmed only the findings of the visual diagnosis. Examination of tooth E confirmed the limitations of the dental radiographs previously mentioned

and stressed by current research. The aforementioned also indicated the importance of accurate clinical examination (Zafersoy-Akarslan et al., 2009) confirmed by agreement between clinical and micro-CT scan examinations for this tooth.

Visual and radiographic examinations were not accurate enough for inter-examiner agreement in the case of some teeth (Table 1). The diagnosis of tooth D is highly relevant to illustrate this fact (Figure 3a, b, and c). This observation reflected the difficulties of clinical examination and also showed the limitations of dental radiographs when compared with micro-CT scan images. In this context, the hidden caries lesions were not easily visualized by dental radiograph, because the visibility of caries was determined by the ratio of enamel to caries through which x-rays penetrate. Radiographic diagnosis of caries must always be supplemented with a careful clinical examination (Wenzel, 2014).

Visual examination, routinely used for detecting caries in dental clinics, has also been used in other studies (Souza-Zaroni et al., 2006; Zafersoy-Akarslan et al., 2009). In current research, the occlusal surfaces were graded according to each examiner's clinical experience to demonstrate the lack of consensus diagnosis among the different dentists. This hypothesis became clear when the Kappa for inter-examiner rate ($K=0.515$) was employed (Table 1). It actually established only moderated agreement and became a reflection of the difficulty mentioned in several studies to perform more accurate clinical caries diagnoses on occlusal surface (Barder, 2002). Further, the observers hailed from different training and routine, and thus with different degrees of experience in detecting caries. Low inter-observer agreement could be related to these factors and variation in caries detection among dentists is a common phenomenon (Zafersoy-Akarslan et al., 2009). There is no guarantee that the examiners will agree on diagnoses, since their decisions are based on previously acquired knowledge and experience. Since examiners' experience obviously influences the diagnostic, it is important to highlight that the comparison of results involves subjective aspects, such as knowledge and clinical experience (Souza-Zaroni et al., 2006).

The reported specificity of visual examination for detecting occlusal lesion was described as high by various authors. Although in current research, all the teeth were considered sound by visual examination, CT scan images showed radiolucency without any doubt (Table 2). This fact also indicated that the various theories labelling this type of lesion as 'fluoride syndrome', 'fluoride bomb' and 'hidden caries' (McDonald and Sheiham, 1992; Beltrán-Aguilar et al., 1999; Whelton, 2004; Bernabé, 2009; Bagramian et al., 2009) may be nothing more than a misdiagnosis for hidden occlusal caries merely relying on visual and dental radiographic examination. Moderate inter-examiner agreement in current investigation occurred for radiographic examination ($K=0.583$) (Table 1).

Since the examinations were performed by the aforementioned technique, the diagnoses with an identified lesion only on dentin were predominant (Tables 1). Consequently, the lesion which extended from the enamel, as displayed by micro-computed tomography examination, was not perceived.

On the other hand, micro-CT scans identified lesions in dentin and enamel on occlusal surfaces more clearly than the traditional radiographic survey. This might be perceived when Kappa inter-examiners rates ($K=0.924$) were provided (Table 1). However, our findings are not in support of the routinely recommendation for the use of micro-CT scan in daily clinical use due to the large amount of ionizing radiation emitted by this examination. This *in vitro* study aimed to verify the limitations of conventional tests for the detection of hidden caries.

Conclusion

Results of this work indicate that there is strong limitation for accurate diagnosis of hidden caries due to conventional examinations limitations, visual and dental radiography. New imaging and other more advanced technologies are needed to improve those procedures.

Conflict of interests

Authors declare that they have no conflict of interest, nor any interference with the results of this article.

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