ISSN 2305-9664

Journal of Contemporary Dental Sciences

Volume : 9, No : 01 January, 2021

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An Official Publication of Sapporo Dental College, Dhaka, Bangladesh
Recognized by Bangladesh Medical & Dental Council (BM&DC)

ISSN 2305-9664

Journal of Contemporary Dental Sciences (JCDS)

Recognized by Bangladesh Medical and Dental Council (BM&DC)

Vol.9, No. 1, January 2021

An Official Publication of Sapporo Dental College Uttara, Dhaka

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Graphics Idea : Maruf Hussain, SDCH

Printed at
IDEA Printers, Katabon, Dhaka. 02-9662116
E-mail : ideaprinters@yahoo.com

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Editorial

It has been observed that obesity is often associated with anemia and low-grade inflammation. BMI is used to measure adults' nutritional condition and obesity, whether Hemoglobin concentration is used to screen for anemia. The article by Rafique T et al. identified that there was a significant negative correlation between blood hemoglobin concentration and BMI which ultimately outline that an increased BMI could be an indicator of lower Hb levels.

Another study by Khan AAY et al. on rodent incisors explored that origin of cementum matrix was from cementoblast and epithelial root sheath contribute very little in cementogenesis. However, acellular cementogenesis is very similar in both incisors and molars.

Ahmed Z et al. in their article identified that Cisplatin, a chemotherapy medication used to treat a number of cancers, is capable to induce apoptosis without activating caspase 3/7.

The rest of the issue's article covered rare case reports. One of the article by Aman J et al. covered 3 case reports of rare double mesiodens while another study by Tafhim S et al. reported 2 cases of intraosseous mucoepidermoid carcinoma which is very rare in terms of bone origin.

In conclusion, in one end this issue highlights some important topics like importance of BMI indicators, cementogenesis in teeth and role of cisplatin and cellular apoptosis. On the other hand, this issue covers some interesting rare cases like double mesiodens and intraosseous mucoepidermoid carcinoma.

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Original Article

Caspase 3/7 not activated during cisplatin induced apoptosis in sensitive HSC-3 cells and resistant BHY cells

Z Ahmed [1,2], AAY Khan[3], Y Deyama[1], Y Yoshimura[1], K Suzuki[1]

Abstract

Purpose: We recently established HSC-3 cell and BHY cell as cisplatin-sensitive and cisplatin-resistance respectively and cisplatin capable to induce apoptosis in both the cells. The purpose of this study is to find out the role of effector caspases in apoptotic cell death in both the sensitive and resistant cells. ***Methods:*** After treatment with different concentration of cisplatin in both the cell apoptosis was detected by agarose gel electrophoresis. Necrosis was determined by measuring the LDH release using CytoTox-ONE™ Homogeneous Membrane Integrity Assay. To determine the role of effector caspases in apoptotic cell intracellular caspase-3/7 activity was measured using Caspase-Glo® 3/7 Assay Kit by luminometer. ***Results:*** Cisplatin induces delayed apoptosis in resistant BHY cells. There are no significant differences in necrosis and caspase 3/7 are not activated during the treatment regimen in both the cells. ***Conclusion:*** Cisplatin capable to induces apoptosis without activating caspase 3/7.

Key Words: Oral squamous carcinoma cell; Cisplatin; Apoptosis; Caspase-3 ***(J Cont Dent Sci 2021;9(1): 1-6)***

Introduction:

Oral carcinomas become an increasingly global disease, more than half of these carcinomas are found late, resulting permanently altering a patient's ability to chew, swallow, talk, as well as their appearance[1-3]. Therefore, it is important to treat oral cancers at the early stages. Usually the treatment requires an efficient combination of surgery, radiotherapy, and chemotherapy [4,5].

Cisplatin, a platinum-based drug, used to treat oral squamous cell carcinoma is one of the standard first-line chemotherapeutic agents[6]. However, several factors can lead to the failure of chemotherapy treatments, including acquired resistance to the chemotherapeutic agents[7,8]. Its efficacy is limited due to acquired resistance and dose-limiting side effects, mainly nephrotoxicity[9]. It was demonstrated that tumor cell exposure to cisplatin ultimately results in apoptosis[10,11]. Unfortunately, the mechanism or mechanisms by which nuclear cisplatin/DNA adducts generate the cytoplasmic cascade of events leading to apoptosis have not been defined.

Apoptosis is a feature of programmed cell death which occurs via activation of intracellular death proteins, is accompanied by biochemical and morphological changes that involve chromatin condensation and margination at the nuclear periphery, extensive double-stranded DNA fragmentation, cellular shrinkage and blebbing[12]. The common signaling pathway in cells undergoing apoptosis is the activation of caspases. Caspase-3-like proteases activated by proteolytic cleavage are considered to play a critical role in the induction of apoptosis[13,14]. Additionally, apoptotic cells are phagocytosed by macrophages immediately, so the release of intracellular molecules which causes an inflammation or shock is limited to a low level compared with necrosis[15].

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Article Received: 05-10-2020; Article Acepted: 29-11-2020;

Thus, the induction of apoptosis in cancer cells by anticancer drugs is an appropriate aim in the therapy of malignant tumors[16].

Caspases belong to a family of cysteine proteases that specifically cleave their substrates after aspartic acid[17]. To date at least 14 different mammalian caspases have been identified and implicated in different aspects of programmed cell death[18]. Their activation signals a point of no return in the apoptotic pathway. However, the mechanisms by which anticancer drugs induce cell death are not clearly defined.

We have already established HSC-3 and BHY cells as cisplatin sensitive and cisplatin resistant respectively and intercellular drug accumulation is one of the major causes of cisplatin resistance. Moreover, we have also reported the ability of cisplatin to induce apoptosis, DNA platinization, DNA adducts repair in sensitive and resistant variant of oral cancer cells. Based on the unique ability of cisplatin to induce apoptosis in different cancer cells, we were interested in investigating the potentiality of cisplatin to induce apoptosis in cisplatin 'sensitive HSC-3 and cisplatin' resistant BHY cells. We also examined the signaling pathways of cisplatin by studying the induction of necrosis and activation of caspase-3/7 in the sensitive and resistant variant of oral squamous carcinoma cells. Moreover, we wished that the studies might further help us to understand the molecular mechanism by which cisplatin induce apoptosis in oral squamous carcinoma cells.

Materials and methods

Drugs and reagents

Cisplatin (Sigma, St Louis, MO); Trypsin-EDTA (GibcoBRL®, Life Technologies, Canada); Dulbecco's modified eagle's medium (D-MEM) (ICN Biomedicals Inc., Aurora, Ohio); Fetal bovine serum (FBS) (HyClone, Perbio, Canada); Kanamycin sulfate (Meiji, Japan); All other drugs and chemicals were obtained from Wako Pure (Osaka, Japan).

Cells and culture conditions

Two different types of human oral squamous carcinoma cells were used in this study and were provided by the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan). The cells were cisplatin-sensitive HSC-3 cell (squamous cell carcinoma of tongue, low metastatic type) and cisplatin-resistant BHY (epithelial like squamous cell carcinoma of mouth, non- metastatic but highly invasive to muscle and bone).

Cells were grown in a humidified incubator gassed with 5% CO_2 - 95% air at 37^{O}C. The media used for the cells were D-MEM supplemented with 1 mM L-glutamine, 4.5 g/l glucose, 3.7 g/l $NaHCO_3$, 66.5 mg/l kanamycin sulfate and 10% FBS. The cells were maintained in 100 x 20 mm tissue culture dish (Falcon®, Becton Dickinson Labware, Franklin Lakes, NJ) and media were renewed every second day. Subcultures were obtained by trypsin-EDTA treatment. Experiments were performed when the dishes became at least 80% confluent.

Detection of apoptosis

Apoptosis was detected by the analysis of DNA fragmentation. Cells were treated with 1 mM cisplatin for 0, 12, 18, 24 and 48 hours. Then DNA was separated as described earlier and added 0.2 ml of 8 mM NaOH and 1M HEPES (23µl / 1 ml 8 mM NaOH) and mixed by tapping finger. Thus 10 ml of obtained DNA samples and 2 ml of loading dye were subjected to 1% agarose gel electrophoresis at 100 V using 1 x TBE as the running buffer. The electrophoresed gel was stained with (3 ml /50 ml) ethidium bromide and visualized under ultraviolet light and documented by photography.

Detection of necrosis

Cytotoxicity assay was performed by determination of lactate dehydrogenase (LDH) level of the cells. Leakage of LDH was measured as an index of lethal membrane injury (necrosis) using CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega, USA). Briefly, 2 x 10^5 cells were seeded in 96-well plates and at the end of the incubation of cells with 1 mM cisplatin for different time frames, released LDH was measured and percent cytotoxicity was calculated according to the procedure recommended by the manufacturer.

Assessment of caspase-3/7 activity

To determine intracellular caspase 3/7 activity in cisplatin treated HSC-3 cell and BHY cells, cells were incubated in 96-well plates with or without 1 mM cisplatin for 0, 3, 6, 12 and 24 hours and caspase-3/7 activity was measured using Caspase-Glo® 3/7 Assay Kit (Promega, USA) according to the manufacturer's instruction by luminometer.

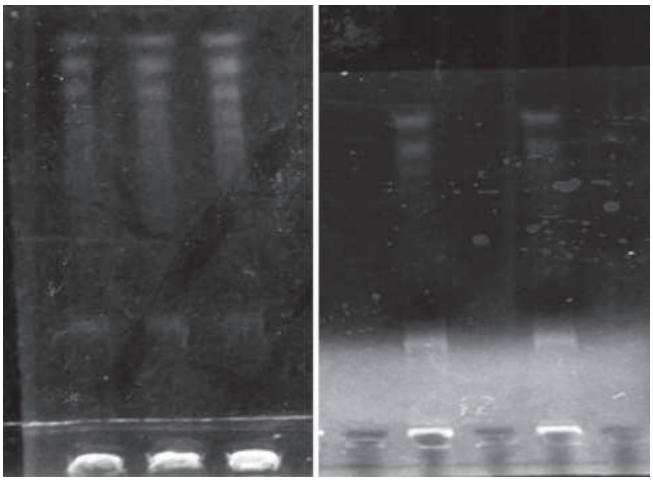
Results

Apoptosis

Analysis of DNA from apoptotic cells by agarose electrophoresis produces a characteristic DNA ladder that is widely regarded as a biochemical hallmark of apoptosis. Therefore, DNA was extracted from 1mM cisplatin treated cells for different time frames and analyzed for DNA fragmentation by electrophoresis.

The results presented in Figure 1 clearly illustrates that cisplatin was capable of inducing apoptosis in both the cells. In case of HSC-3 cells DNA fragmentation was observed after 24 hours of 1 mM cisplatin exposure, while for BHY cells it was visible after 48 hours of 1 mM cisplatin exposure. In addition, in HSC-3 cells as no DNA fragmentation was observed following 12 hours incubation, suggesting that a period greater than 12 hours and possibly as long as 24 hours is required to produce this effects. On the other hand, in case of BHY cells a period greater than 24 hours and possibly as long as 48 hours is required to produce apoptotic effect.

Figure 1: Agarose Gel Electrophoresis Assay LDH Assay after 1mM cisplatin application.



HSC-3 Cells *BHY Cells*

Figure 1: Apoptosis was detected by the analysis of DNA fragmentation. BHY cells and HSC-3 cells were treated with 1 mM cisplatin for 0, 12, 18, 24 and 48 hours. Then DNA were separated and samples with loading dye were subjected to agarose gel electrophoresis. The electrophoresed gel was stained with ethidium bromide and visualized under ultraviolet light and documented by photography.

Necrosis

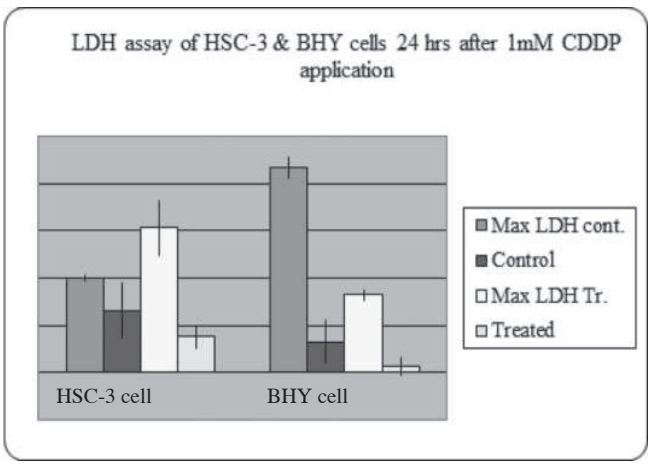


Figure 2: After exposure to the 1 mM dose of cisplatin, released LDH was measured and percent cytotoxicity was calculated. A time dependent study of the percentage of LDH release was conducted using CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega, USA).

After exposure to the 1 mM dose of cisplatin, a time dependent study of the percentage of LDH release was conducted. As depicted in Figure 2 in HSC-3 cells, the percentage of released LDH remains stable within the first 18 hours of 1 mM cisplatin treatment. However, LDH release (%) was significantly increased in 24 hours. On the other hand, in BHY cells LDH remains stable throughout this time frame.

Caspase

Based on the results obtained above, our next aim was to examine the involvement of specific caspase, namely caspase-3/7 which is known to be one of the main executioner / effector caspases. The results presented in Figure 3 clearly indicated that cells treated with 1 mM cisplatin did not increase in caspase 3 /7 activity.

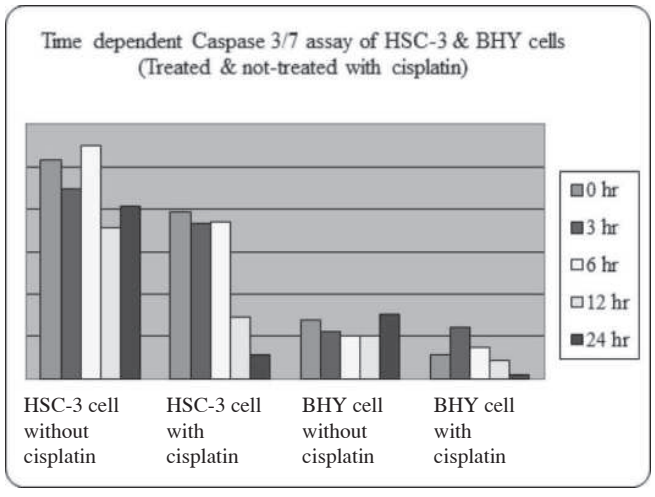


Figure 3: To determine intracellular caspase 3/7 activity in cisplatin treated HSC-3 cell and BHY cells, HSC-3 cells and BHY cells were incubated in 96-well plates with or without 1 mM cisplatin for 0, 3, 6, 12 and 24 hours and caspase-3/7 activity was measured using Caspase-Glo® 3/7 Assay Kit (Promega, USA) by luminometer.

Discussion

Cell death is usually classified into two broad categories: apoptosis and necrosis. Necrosis, which is always pathological, is a passive, catabolic process, that represents a cell's response to extreme accidental or toxic insults. On the other hand, apoptosis occurs under normal physiological conditions and is an active process requiring energy. However, apoptosis can also be elicited in a pathological way by toxic injury or during disease processes[19]. Therefore, it is important to determine between apoptosis and necrosis in pathological conditions, as therapeutic intervention could be considered in apoptotic cell death with putative new pharmacological agents aimed at interfering with the key molecular events involved.

DNA ladder that is widely regarded as a biochemical hallmark of apoptosis. Therefore, DNA was extracted from 1mM cisplatin treated cells for different time frames and analyzed for DNA fragmentation by electrophoresis. As shown in the figure 1 DNA ladder was visible after 24 hours of cisplatin treatment in sensitive HSC-3 cells where as in resistant BHY cells it is visible after 48 hours of cisplatin treatment figure 1. Our data strongly suggests that cisplatin is able to induce apoptosis in both the sensitive and resistant variant of the cells. But it induces delayed apoptosis in cisplatin-resistant cell in comparison with the cisplatin sensitive variant. In this study, leakage of LDH was measured as an index of lethal membrane injury 'necrosis'. As shown in figure 2 released LDH was stable throughout the treatment period. So we can conclude as cisplatin did not induce necrosis in this study.

Apoptosis is a well described sort of cell death induced by a variety of substances. The processes in an apoptotic cell are well characterized and several reports describe that mitochondria play a crucial role [20, 21]. Release of cytochrome C and other proteins from mitochondria[22,23] often induces a series of events which finally leads to activation of caspase - 3, followed by DNA ladder formation and cell death.

The caspase family is broadly divided into two groups, namely the initiator caspases (caspases 2, 8 and 9) and the effector caspases (caspases 3, 6, 7 and 14). The initiator caspases undergo activation in response to an apoptotic stimuli and in turn activate the effector caspases that are responsible for the cleavage of a wide variety of physiologic substrates[24]. Among the ten or more caspases, caspase-3-like proteases activated by proteolytic cleavage are considered to play a critical role in the induction of apoptosis[13,14]. Caspase-3 has been specifically implicated as the effector caspase responsible for cleavage of the human DNA fragmentation factor that subsequently activates the DNA endonuclease required for formation of apoptotic DNA ladders. Cisplatin may cause mitochondrial release of cytochrome c and caspase-3 activation[25]. In addition, in human osteosarcoma cells, cisplatin induces apoptosis through a sequential activation of caspase-8, caspase-3 and caspase-6[26]. However, it has been also reported that cisplatin-induced apoptosis in A2780 ovarian tumor cells may proceed via a caspase-3 independent pathway[27]. The data presented in this article in figure 3 shows that caspase 3/7 activity did not increase in cisplatin treated cells in comparison with not treated cells in both the cisplatin-sensitive and cisplatin-resistant variant oral squamous carcinoma cells. The role of caspases 3/7 inhibitor which was not investigated in this study highlighted major limitation of the study.

This data and the reports describe earlier clearly indicates that the role of effector caspases in apoptotic cell death is still controversial. Further investigation is necessary to identify the way in which caspases are activated and the role of caspases in apoptotic cell death in cancer cells treated with cisplatin.

Based on results described in this article and our recently published report [28-30] we can come to a conclusion that cisplatin is capable to induce apoptosis in both cisplatin-sensitive and cisplatin-resistant cancer cells. Intracellular cisplatin accumulation plays a key role in cisplatin resistance. In cisplatin-resistant cells reduced intracellular cisplatin accumulation leads to reduced DNA platination followed by delayed apoptosis. Caspase 3/7 was not activated in the cisplatin induced apoptotic time frame.

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Correlation of Hemoglobin Concentration to Body Mass Index (BMI) among Undergraduate Dental Students: A Cross-sectional Study

T Rafique[1], MM Liza[2], F Yeasmin [3], A Ahamed[4]

Abstract

Background: *The most common type of anemia is iron deficiency anemia. Along with other tests, hemoglobin (Hb) concentration is used to identify iron deficiency anemia. It is also used to screen for anemia. Obesity, which BMI can assess, is linked to low-grade inflammation, is frequently associated with anemia, and predisposes to long-term problems in old age. The outcome of the relationship between anemia and body mass index (BMI), a measure of adults' nutritional condition, has proven inconclusive.* ***Aims:*** *The study aimed to determine the correlation of hemoglobin level with BMI in undergraduate dental students.* ***Materials & Methods:*** *A cross-sectional study was performed on 270 undergraduate dental students. Weight and height were measured to calculate BMI. Hb was measured with a hematology analyzer (Sysmex) from venous blood. The correlation between hemoglobin concentration and BMI was assessed by the Pearson correlation coefficient. A P <0.05 was considered statistically significant.* ***Results:*** *In our study, there is a significant negative correlation (r = -0.724) between BMI and Hb levels, (p = 0.000); there is also a significant negative correlation (r = -0.56) between Hb level and weight, (p = 0.000).* ***Conclusion:*** *There was a significant negative correlation between blood hemoglobin concentration and BMI. Therefore, increased BMI can be an indicator of lower Hb levels.*

Key Words: Body Mass Index (BMI), Hemoglobin (Hb). ***(J Cont Dent Sci 2021;9(1): 7-11)***

Article Acepted: 09-11-2020;

Article Received: 07-09-2020;

Introduction:

Hemoglobin (Hb) is the protein that contains iron in red blood cells[1]. The concentration of which indicates the state of anemia in the population is less than the established cutoff value, impairing the capacity of blood to carry oxygen in the body [2]. Anemia is a common blood disorder in which the number of red blood cells or the Hb concentration falls below the established cutoff value, impeding the blood's ability to transport oxygen throughout the body[1].

Anemia is a severe public health and nutrition issue affecting developing and developed nations, with serious consequences for human health and economic and social growth[3].

Adolescents are susceptible to the condition known as anemia due to increased iron demands associated with their rapid growth. In the population of adolescent females, the occurrence of menstruation is associated with an elevated likelihood of developing iron-deficiency anemia during adolescence and reproductive age [4].

Most people with iron deficiency anemia are young adolescents and college-going students. Low Hb concentration and abnormal body mass index (BMI) adversely affect people's health, which promotes morbidity and mortality rates[5]. Low Hb concentration due to iron deficiency causes fatigue, reduces the ability to perform work, and impaired cognition, leading to poor academic performance among students[6]. Even mild anemia is known to lower immunological competence and has an adverse effect on productivity[3]. On the other hand, obesity is distinguished by the presence of chronic, persistent systemic inflammation, which is linked to anemia of chronic disease, increased levels of serum ferritin, decreased levels of serum iron, and reduced levels of Hb[7]. Being overweight or obese is considered a significant risk factor for the early onset of chronic diseases among adults. Also, it hampers the Students' academic performance to a notable extent [8].

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Dental students are part of the medical fraternity and comprise a well-educated and knowledgeable segment of society. Dental school students are expected to have an in-depth knowledge of the advantages of adopting a healthy lifestyle and diet. However, they become susceptible to nutritional disorders, such as obesity and anemia, as a result of the stress of the curriculum, significant changes in lifestyle and behavior, increased consumption of readily available and cheap fast foods, inadequate and inappropriate dietary habits, and a rigid work profile [9].

Several studies have examined the correlation between Hb levels and BMI in undergraduate medical students and adolescents. The correlation between anemia and BMI, an adult nutritional status measurement, has produced inconsistent results. However, there is no study on the correlation between hemoglobin level and BMI in undergraduate dental students. Thus, the present study was conducted to determine the correlation of Hb level with BMI in undergraduate dental students of Bangladesh.

Materials and Methods

270 dental students of both sexes between the ages of 18 to 21 years who are enrolled at Sapporo Dental College, Uttara, Dhaka, volunteered for the study. From December 2018 to June 2019, this cross-sectional study was conducted at the Department of Physiology and Biochemistry. Following permission from the college's ethical committee, participants provided written informed consent.

A convenient sampling technique was followed. Participants, both male, and female, who had a medical history of chronic conditions such as diabetes, infections, and treatment with iron, folate, or vitamin B-12 within the previous year, as well as those who had donated blood within the last six months and among female students who had a history of menorrhagia, pregnancy, and lactation, were excluded from the study.

Weight and height were precisely assessed to determine BMI for age. The scale for weight was digital. Spring-loaded bathroom scales were avoided. Rather than carpet, the scale was put on hard ground (such as tile or wood). Participants took off their shoes and heavy clothing. They stood at the center of the scale with both feet. Weight was reported to be the closest decimal fraction (55.5 pounds or 25.1 kilograms, for example)[10].

Participants were told to stand upright, keep their arms at their sides, keep their shoulders level, and look straight ahead and parallel to the floor. Participants stood with heads, shoulders, buttocks, and heels on the wall for measurement. A flat headpiece forms a straight angle with the wall and is lowered until it hits the crown. The measurer's eyes were level with the headpiece. Lightly mark where the headpiece bottom touches the wall. The height was measured from the floor base to the wall mark with metal tape. Record height to the nearest 1/8th inch or 0.1 centimeter. Then, a centimeter is converted into a meter[10].

The body mass index (BMI), formerly known as the Quetelet index, is used to assess a person's status of nutrition. Body mass index (BMI) is calculated by dividing a person's weight in kilograms by the square of their height in meters[11]. For adults, normal (BMI = 18.5~24.99 kg/m2), overweight (BMI = 25~29.99 kg/m2), and obese (BMI = 30~34.99 kg/m2), respectively, according to the latest WHO criteria[11].

Blood samples were obtained in EDTA-containing tubes for hemoglobin measurement with a hematology analyzer (Sysmex)[12].

The hemoglobin levels were compared to the standards for grading anemia according to WHO guidelines, with hemoglobin levels of <12 g% being anemic[13].

Standard deviation and mean were used to illustrate the data. The Pearson correlation coefficient was used to determine that Hb levels were related to BMI and Weight. STATA MP Version 16 was used for statistical analysis. A two-tailed P-value of less than 0.05 was considered significant.

Result

A total of 270 undergraduate BDS students were participated in the study. Among the participants, 61.48% (166) were female and 38.52% (104) were male (Figure 1). Age, anthropometric parameters and hemoglobin in study participants were presented in Table 1 as background information. Correlation between BMI & Hb concentration and Weight and Hb concentration were presented in Table 2 and Table 3 respectively.

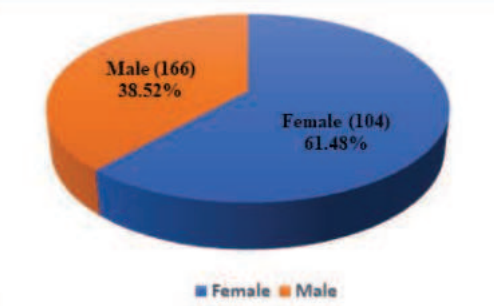

Figure 1: Gender distribution of study participants.

Table 1: Background information of study participants (n=270)

Variables	Mean±SD
Age (years)	19.2±.86
Height (meter)	1.59±.11
Weight (kg)	80.15±12.20
BMI (kg/m2)	25.23±3.86
Hb (g/dL)	11.90±1.17

***BMI:** Body mass index, Hb: Hemoglobin, SD: Standard deviation

Table 1 shows the mean (±SD) age of the participants was 19.2 (±.86) years. The Mean (±SD) Height (meter), Weight (kg), BMI (kg/m2), Hb (g/dL) were 1.59±.11, 80.15±12.20, 25.23±3.86, 11.90±1.17, respectively.

Table 2: Correlation between BMI and Hb concentration.

Variables	Correlation coefficient (r)	P value
BMI	-0.724	*0.000
Hb		

***BMI:** Body mass index, Hb: Hemoglobin

Table 2 shows correlation between BMI and Hb level. There was a very strong significant, negative correlation (±0.724) between BMI and Hb level (p = 0.000).

Table 3: Correlation between Weight and Hb concentration.

Variables	Correlation coefficient (r)	P value
Weight	-0.56	*0.000
Hb		

***Hb:** Hemoglobin

Table 3 shows a correlation between Weight and Hb level. There was a very strong significant, negative correlation (-0.56) between Weight and Hb level (p = 0.000).

Discussion

In our study we got significant, negative correlation between BMI and Hb level. Similar to our studies, the Himalayan Institute of Medical Science study discovered a negative correlation between BMI and Hb among medical students[14]. MKCG Medical College in Berhampur, Odisha, found a negative correlation between BMI and Hb, like our study[15]. While a study done among Amritsar medical students revealed a positive correlation of Hb in both males and girls with BMI grades[3]. In our study we also got significant, negative correlation between weight and Hb level.

In our study we also got significant, negative correlation between weight and Hb level. But in a study done in Karnataka, India, there was no significant relationship between weight and Hb level among adolescents, but they got significant positive correlation between height and Hb level [7]. We did not get any significant correlation between height and Hb level. BMI and hemoglobin were shown to have a non-significant positive connection among medical students at CMH Kharian Medical College in Pakistan[16]. Obesity or being overweight, on the other hand, is a risk factor for many diseases, including type 2 diabetes, hypertension, heart disease, stroke, sleep apnea, and malignancies; additionally, studies have shown that increasing BMI harms iron status, with the effect being more profound in females[17]. The reason for iron deficiency anemia in people with a high BMI is unknown. Iron deficiency in obese persons may be caused by low iron intake due to an imbalanced diet, which appears to be the most logical explanation in our study, as most dental students were hostelers and consumed a substantial amount of junk and premade nutritionally deficient food[18]. Other hypothesized reasons for iron insufficiency in persons with high BMI include decreased iron absorption in the small intestine, increased iron needs due to increased blood volume, and obesity-related to chronic low-grade inflammation[19]. Peter et al. found an identical relationship between high BMI and hemoglobin concentration in overweight and obese females[20]. Bulliya et al. also proved a negative relationship between obesity and hemoglobin levels[21].

Limitations

The study used a convenient sample from a single dental college. A significant number of participants with diet and lifestyle data in the study could provide more generalized results.

Conclusion

According to the findings of this study, being overweight or obese increases the risk of anemia. There is a negative correlation between hemoglobin concentration and BMI. Therefore, increased BMI can be an indicator of lower Hb levels. Further study with a broad and diversified sample size is necessary to determine the relationship between Hb and BMI. As low hemoglobin concentration and abnormal BMI harm an individual's performance, adequate screening and attention are required, especially for undergraduate dental students in Bangladesh.

Acknowledgement

We are grateful to the Chairman of The Foundation for Health Education, Service and Research, the Principal of Sapporo Dental College, and members of the ethics committee of Sapporo Dental College for granting permission to perform this study. We also thank all the participants and laboratory technicians of Sapporo Dental College for their full cooperation.

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Acellular cementogenesis in rat incisors: An immunohistochemical, lectin cytochemical and enzyme histochemical study

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Abstract

Background and objective: *There have been only a few studies examining the acellular cementum of rodent incisors. Therefore, this study was designed to detect the acellular cementogenesis in rat incisors by light microscopy. The aim of this study is to observe the acellular cementogenesis in rat incisors and to discuss the similarities of acellular cementogenesis between rat molars and continuously growing incisors.* ***Material and method:*** *Maxillary incisors of 21 days old six Wister rats were used. Routine histological staining, immunohistochemical staining, digestion tests were conducted. The role of proteoglycans (PGs), osteopontin (OPN) and bone sailoprotein (BSP) was also investigated by an immunoperoxidase method. To characterize PGs antibody against 3 species of glycosoaminoglycans (GAGs), [chondroitin ′ 4 ′ sulphate (C4s), chondroitin ′ 6 ′ sulphate (C6s) and keratin sulphate (KS)] were used.* ***Result:*** *At the onset of dentin mineralization acellular cementum appeared as a thin hematoxylin stained layer on the continuously developing root of the maxillary incisors. Acellular cementum and cementodentinal junction (CDJ) of both incisors and molars showed a very similar histological and immunohistochemical features which were intensely hematoxylin – stainable, deficient in collagen fibrils and rich in BSP and OPN.* ***Conclusion:*** *The findings mentioned above suggest that: The origin of cementum matrix is from cementoblast, the epithelial root sheath contribute very little in cementogenesis, acellular cementogenesis is very similar in both incisors and molars only the width of the CDJ in incisors are thin than the molars.*

Key Words: Cementogenesis; Rodent incisors; Cementodentinal junction (CDJ); Non collagenous glycoprotein

(J Cont Dent Sci 2021;9(1):12-18)

Article Received: 25-09-2020; Article Accepted: 12-11-2020;

Introduction:

Rodent incisors are continuously growing teeth. The continuously growing teeth are represented not only by rodent incisor but also molars in certain other species, including rabbits, guineapigs and field voles. The rat's upper incisors are about 4 mm long and 1.5 mm wide, the lower ones are about 7 mm long and 1.2 mm wide[1].

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A tooth consists of three layers of mineralized tissues: a hard external layer of enamel, a hard layer of cementum covers the root. Both the enamel and cementum surround dentin, which makes up the bulk of the tooth.

Cementum is composed of mineralized collagen fibers and interfibrillar matrices. Bone Sialo Protein (BSP) and Osteopontin (OPN) belong to the non-collagenous glycoprotein group and are the major constituents of the interfibrillar matrices[2-5].

BSP and OPN are phosphorylated sialoproteins with Arg-Glycin-Asp domains. They are closely associated with mineralization, cell differentiation, matrix-matrix attachment and cell- cell/matrix attachment in collagen-based hard tissues [6,7].

The Proteoglycans (PGs) are macromolecules composed of core protein with one or more glycosaminoglycans (GAGs) attached by covalent bonds through a specific sequence of trisaccharides (galatose-galactose-xylose).

The GAGs are linear, highly anionic polysaccharide units of uronic acid and hexosamine. There are a number of GAGs types: chondroitin-4-sulphate (C4S), Chondroitin-6-sulphate (C6S), unsulphated chondroitin (COS), dermatan sulphate (DS), keratin sulphate, heparin sulphate, heparin and hyaluronon. The PGs exist in several forms depending on their size, amino acid sequence of the core protein and the nature and the number of GAGs chains etc. Small leucine-rich PGs (SLRPs; decorin, biglycan, lumican and fibromodulin) have been suggested to be implicated in biomineralization in cooperation with other extracellular matrix molecules [8-12].

This study is designed to detect acellular cementogenesis in rat incisors. Moreover, this study figures out the similarities of the acellular cementogenesis procedure and attachment mechanism through non collagenous proteins as well as glycosaminoglycans between continuously growing root of rat maxillary incisors and rat molars. The objective is to immunodetect PGs, BSP and OPN on the acellular cementum during cementogenesis and common attachment mechanism in mineralized tissue.

Materials and methods

This study used six, 21 days old male Wistar rats weighing about 50 gm. The animals and tissue specimens were treated in accordance with the Guidelines of the Experimental Animal Committee, Hokkaido University Graduate School of Dental Medicine.

Animals and tissue preparation

After anesthesia by an intraperitoneal injection of sodium pentobarbital, the animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 minutes at room temperature. Maxillary incisors, together with the surrounding alveolar bone and covering tissues, were dissected out and immersed in the same fixative over-night. Tissues were then demineralized in 100% EDTA alcohol for 10 weeks in room temperature with multiple changes. After complete demineralization was ensured by radiography the specimens were dehydrated in graded series of ethanol and before immerse in 80% alcohol the jaw had been cut into two halves and embedded in paraffin. Next, 7µm thick serial longitudinal sections of the maxillary incisor were cut in the bucco-lingual plane of the tooth for different staining.

Routine Histological Staining

To demonstrate morphology and collagen fiber, sections were stained with hematoxylin and eosin and silver impregnation. For proteoglycans demonstration Periodic Acid Schiff (PAS) and Toluidine Blue (pH 6.0) were employed.

Immunohistochemistry

Immunodetection of GAGs

All primary antibodies and enzymes were purchased from Seikagaku (Tokyo, Japan). The staining techniques for these antibodies have been established and applied to rat molars[13], rat salivary glands[14], rabbit alveolar bone [15] and human tooth germs[16].

Deparaffinized sections were immersed in methanol containing 0.3% hydrogen peroxide to inhibit endogenous peroxidase and pretreated with one of the three chondroitinases. Corresponding chondroitinase were used to generate neoepitopes for the 2-B-6, 3-B-3 and 1-B-5 antibodies and to unmask native epitopes for the 5-D-4 antibody. The sections were incubated successively with the primary antibodies, biotinylated anti-mouse rabbit polyclonal antibody (DAKO, Japan) and rinsed with phosphate-buffered saline after every incubation.

Immunodetection of BSP and OPN

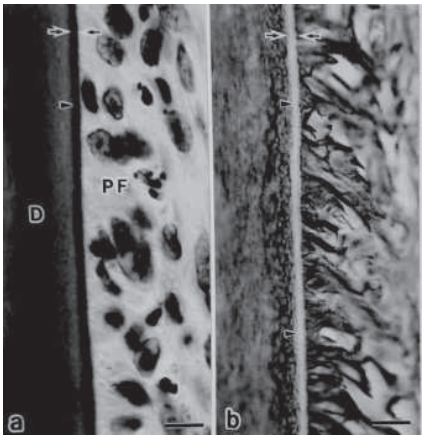
For BSP immunodetection, two kinds of antibodies were used as primary antibodies. The primary antibodies for BSP detection are (i) anti-mouse BSP rabbit polyclonal antibody (LSL, Tokyo, Japan) and (ii) anti-human BSP rabbit polyclonal antibodies (Chemicon, Temecula, Canada; Alexis, Lausen, Switzerland). For OPN immunodetection, one kind of antibodies were used as primary antibody; anti-mouse OPN rabit polyclonal antibody (LSL, Tokyo, Japan). In accordance with the supplier`s instructions, these antibodies react to rat antigens in paraffin sections.

After deparaffinization sections were pretreated with 2.5% testicular hyaluronidase (Sigma, St Louis, Mo., USA). They were then incubated with the primary antibodies, biotinylated anti-mouse rabbit polyclonal antibody (DAKO), anti-rabbit swine polyclonal antibody (DAKO) and streptavidin-boitin-horse-radish peroxidase complex (DAKO). Immunostaining was visualized as described above.

Results

General Histology

The acellular cementum appears as a hematoxylin stained thin layer, which is about (2-3 micro meter) thick. The cementodentinal junction took the form of the layer (about 1 micro meter thick) that was more intensely hematoxylin stained than the rest of the cementum (Figure 1a). Silver impregnated sections showed that the cementodentinal junction was deficient in collagen fibrils (Figure 1b). Principal fibers were arranged at right angles to the cementum surface. The cementum contained the ends of principal fibers as extrinsic fibers.



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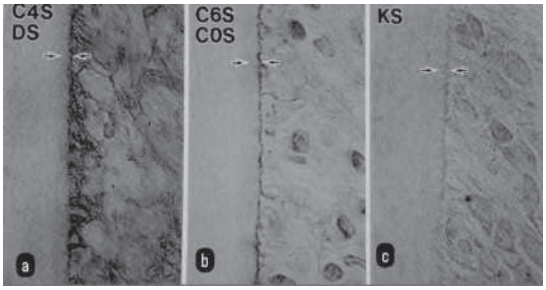

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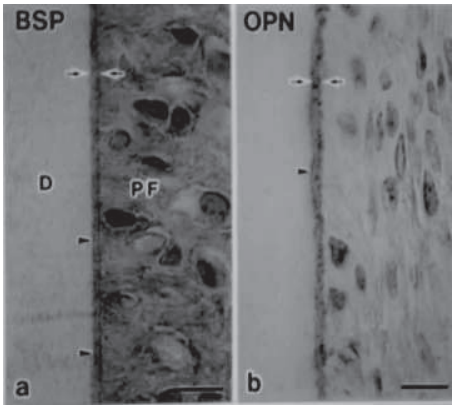

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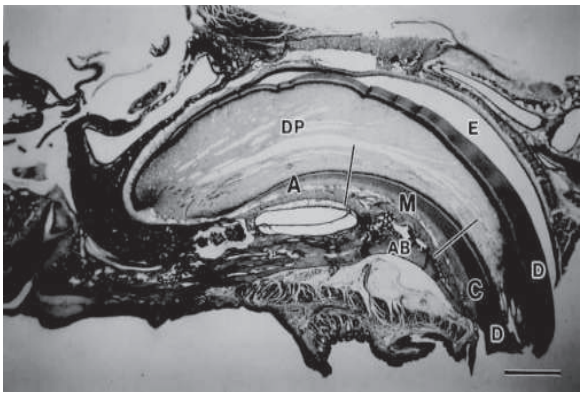

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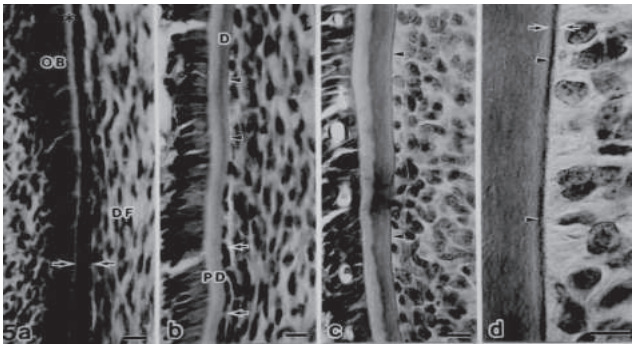

Fig 05

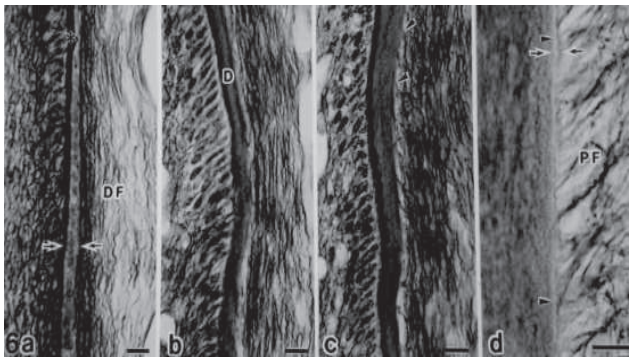

Fig 06

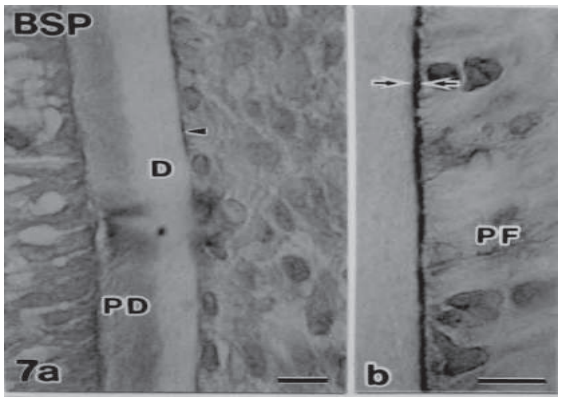

Fig 07

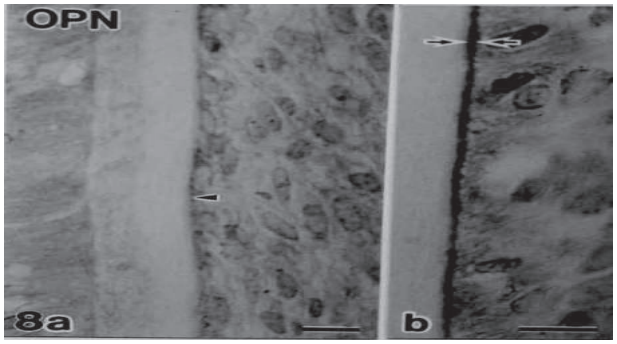

Fig 08

Immunohistochemistry

GAGs

Neither the cementum nor the cementodentinal junction showed significant immunoreactivity against any of the antibodies C4S, C6S and KS (Figure 2a, 2b, 2c). The cementodentinal junction was totally immuno negative against these antibodies. But the principal fibers moderately stained by these antibodies.

BSP and OPN

Fundamentally, all types of anti-BSP antibodies provided a similar labeling pattern for BSP. Therefore, sections stained with antibodies purchased from Chemicon are shown on this study. Sections stained with anti-OPN used in this study were purchased from LSL, Tokyo, Japan. Tadatomo et al. [17] has reported the authenticity of these antibodies in a study on root resorption of rat molars. Anti-BSP antibody stained the cementum moderately and the cementodentinal junction intensely (Figure 3a). Anti-OPN antibody stained the cementodentinal junction (Figure 3b). The principal fibers showed moderate to intense immunoreactivity against the two antibodies.

Developing acellular cementum

General histology

The continuously growing root of maxillary incisors was examined in 21 days old rat. For convenience of description, the root was divided into cervical, mid and apical regions (Figure 4).

Apical region

At the apical end, Hertwig`s epithelial root sheath consisted of an outer and an inner cell layer. In the cervical direction, dental papilla cells facing the epithelial sheath differentiated into columnar

odontoblasts and initiate formation of the initial predentin (Figure 5a, 5b, 5c, 5d). Where the epithelial sheath disintegrated and the epithelial cells became smaller and flattened. With the onset of dentin mineralization, hematoxylin-stained initial acellular cementum appeared on the mineralized dentin and large cementoblasts appeared on the root surface (Figure 5d).

In silver impregnated sections (Figure 6) the boundary between mineralized dentin and predentin is unclear and a clear non-silver impregnated layer appeared between the attached principal fibers and the root dentin.

Mid region

The acellular cementum increased in thickness. The cementodentinal junction, which was intensely stained by hematoxylin and the unstained clear layer in silver was also observed. The principal fibers were well organized, form bundle and enter the acellular cementum as extrinsic fibers (Figure 1a, 1b).

Cervical region

The acellular cementum developed to a maximum thickness of 3 µm and the principal fibers became thicker. The cementodentinal junction was more discernible than in the mid region (Figure 5d).

Immunohistochemistry

BSP and OPN

Sections stained with anti-BSP antibodies purchased from Chemicon and anti-OPN antibodies purchased from LSL, Tokyo, Japan are shown

Apical region

At the apical end both the anti-BSP and anti-OPN antibody stained the odontoblast, predentin, principal fibers and epithelial sheath (Figure 7a, 7b). Both the antibody intensely stained the initial cementum (Figure 8a, 8b).

Mid and cervical region

Anti-BSP antibody stained the cementum and cementodentinal junction clearly. The anti-OPN antibody stained the cementodentinal junction and the cementum matrix clearly. Both the antibody stained the developing principal fibers moderately.

Discussion

Previously immunolocalization of proteoglycans and bone related noncollagenous glycoproteins in developing acellular cementum of rat molar had been investigated by Yamamoto 2004[18]. This study determined acellular cementogenesis in rat incisor on the basic of the formation of process of the cementodentinal junction. In this study an intensely haematoxylin stained, fibril poor initial cementum layer appeared with the onset of dentin minerelization and as the acellular cementum develop the layer became the cementodentinal junction.

With the onset of dentin mineralization, the initial cementum appeared on the dentin surface as a haematoxylin-stained fibril poor layer subsiquently primitive principal fibers attached to the initial cementum. As the acellular cementum containing extrinsic fibers covered the initial cementum, the initial cementum formed the cementodentinal junction[18]. Structure and formation process of the cementodentinal junction were very similar for both rat molar and incisors. Using human molar and rat molar we have found that the cementodentinal junction contains a small amount of intermingled fibrils and much accumulated proteoglycans and mucopolysaccharide.

Fibril intermingling is not a consistent feature throughout the cementodentinal junction[19-23]. The presence of proteoglycans and mucopolysaccharides in cementodentinal junction of rat incisor prove that the cementodentinal

junction of incisor is a fibril poor proteoglycan rich layer.

Using molars, we have proposed that the cementodentinal attachment is created mainly by the adhesion of proteoglycans and strengthened by mineralization. The fibril intermingling between dentin and cementum is a secondary or accessory factor for the attachment[24]. In this study by using incisor we have observe the presence of proteoglycans in the junction which acts as an adhesive and adheres two different mineralized tissue (dentin and cementum) together and forms a strong cementodentinal junction.

According to the classical theory of cementogenesis ectomesenchymal cells migrate from the dental follicle toward the root surface, where they insinuate between Hertwig's epithelial root sheath cells and begin to deposit cementum matrix constituents [25, 26]. In this study we found after the breakdown of the Hertwig's epithelial root sheath the cementoblast cells appeared and laid down over the root as a cementum matrix. Hertwig`s epithelial root sheath consisted of two or three layer of squamous cells and was surrounded by a basal lamina at the apical end of the sheath. In the cervical direction, the sheath thickness decreased into two cell layers: inner layer with cuboidal cells and outer layer consist of squamous epithelial cells[20]. After the acellular cementum is established, the initial cementum layer or the cementodentinal junction functions as a binding device for cementum and dentin[27]. The adhesive functions of the cementodentinal junction are closely associated with noncollagenous proteins like BSP, OPN.

The origin of cementum is a matter of controversy, though the data found in this study suggest that the cementum origin is cementoblast. These finding suggested that the first cementoblasts that appear during root development are mesenchymal cells of the dental follicle and confirms previous studies on the mesenchymal origin of cementum forming cell[25,28].

The classical theory suggested that mesenchymal cells of the dental follicle become cementoblast and secret cementum after having transmitted the barrier of the Hertwig's epithelial root sheath[29-33]. In the study according to the position we can differentiate cementum from fibroblasts. We found large cementum on the site of cementogenesis at the onset of dentin mineralization and breakdown of Hertwig;s root sheath.

Conclusion

Acellular cementum and cementodentinal junction (CDJ) of both incisors and molars showed a very similar histological and immunohistochemical features which were intensely hematoxylin stainable, deficient in collagen fibrils and rich in BSP and OPN. Only the width of CDJ in incisors are thin than the molars. The origin of cementum matrix is from cementoblast cells. This cementoblast cells plays the major role during the development of cementum and the Hertwig's epithelial root sheath contributes very little during cementogenesis.

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Double Mesiodens: A Series of 3 Case Reports

J Aman,[1] MG Mahmud,[2] N Anber,[3] M R Rahi,[4] S Parvin,[5] MAI Khan[6]

Abstract

A Mesiodens is a supernumerary tooth which occupies a central position between the central incisors, most commonly in the maxilla. This situation is more prevalent in permanent dentition as compared to primary dentition, occurring predominantly in the male population. Mesiodens may be single, paired, erupted or unerupted or may remain impacted. Mesiodens cause variable complications such as median diastema, displacement, rotation, disturbances in eruption, unpleasing aesthetics, occlusal disharmony, inflammation of the soft tissues, cystic transformation, among others. Although occurrence of a single Mesiodens are reported in the majority of available literature, cases of double mesiodens (mesiodentes) have also been described. This case report presents 3 cases of double mesiodentes treated and documented at Pediatric OPD of Sapporo Dental College and Hospital, Dhaka.

Keywords: Supernumerary Teeth, Double Mesiodens, Mixed Dentition. ***(J Cont Dent Sci 2021;9(1):19-23)***

Introduction:

Supernumerary teeth are developmental abnormalities of odontostomatologic origin characterized by one or more extra tooth/teeth present in the dentition in relation to the normal dental formula[1]. A mesiodens is a supernumerary tooth lodged between the central incisors of either Maxilla or Mandible[1-3]. The occurence of a mesiodens is more common in permanent dentition in comparison to primary dentition[3]. The prevalence of mesiodens in permanent teeth has been reported between 0.15% to 3.8%, while in primary dentition it was reported at 0.2-0.8%[4,5]. Such teeth show maxillary predilection (5:1) with a higher incidence in male compared to female (3.1:1)[5]. The shape of a mesiodens may vary but a conical or peg shape is the most recognizable shape[4-6]. A mesiodens may erupt or may remain unerupted causing disturbances in the eruption process of the corresponding permanent/primary teeth. It has been described that an unerupted extra tooth may prevent eruption of a tooth, create a gap between teeth, or may give rise to a cyst of odontogenic origin such as a dentigerous cyst[6]. An erupted mesiodens is aesthetically unacceptable and may cause displacement or rotation of the corresponding teeth, occlusal disharmony and inflammation of the soft tissues, among other complications[6-8]. In most instances supernumerary teeth are recognized by their unusual size, shape and numbers. Some unerupted extra teeth may be discovered accidentally during a routine dental checkup, in particular through a dental radiograph[6]. Presence of a mesiodens often would become a cause of concern for both the parent and the patient, who may not have any knowledge about an extra tooth. Cases of mesiodens show genetic history, have been associated with syndromes like cleft lip and palate, Cleiodecranial dysplasia and Gardener's syndrome, or may appear as sporadic cases with variable environmental factors[5,6,7]. According to the available literature, though a single mesiodens is a much more common anomaly, cases of double mesiodens or mesiodentes, have been reported [6-10].

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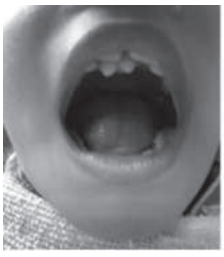
Article Received: 01-09-2020; Article Acepted: 01-11-2020;

We have described three (3) cases of double maxillary mesiodentes in this series of case report. These patients visited Pediatric Out-patient Department (POPD) of Sapporo Dental College and Hospital (SDCH) over a period of two years between 2019 and 2020. Verbal permission to report the cases was obtained from the parent/s of the children at the time of diagnosis and treatment planning.

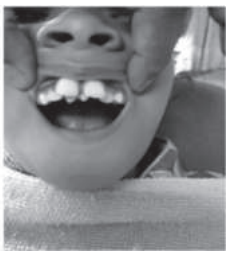
Case description

Case 1

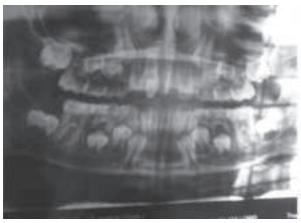
Yasin Arafat (not his real name), a boy of 11 years, visited the Pediatric OPD of SDCH with his parents on July 2019. The parents expressed their worry as them and the child had noticed two whitish, pointed peg-like structures growing out from the anterior part of upper jaw, palatal to the upper central incisors for the last 3 months. The structures, according to their description, were slowly coming out, painlessly. Though they noticed the permanent incisors being pushed out labially and slightly distally creating a noticeable gap between them, they had no clue about the newly erupting structures. The two conical, tooth like structures were causing injury to the tongue. The parents stated their concern by saying that they never heard of such happening and now have come to SDCH for proper diagnosis and treatment. The child, healthy and jovial in nature, stated that he noticed hardening of the anterior palatal area 3 months back, and those two structures, initially pointed and very sharp, came out of the anterior part of palate penetrating through the mucosa causing very little pain. The mucosa surrounding them would often bleed during brushing.



Case 1, Fig 1



Case 1 Fig 2



Case 1, Fig 3

Case 1, Fig 4

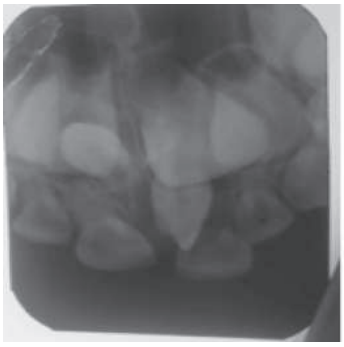
A through history was obtained before clinical examination. There was no positive family history of such occurrence. On clinical examination it was found that they were peg or conical shaped teeth. An OPG and IOPA radiograph revealed no further abnormal structures except these two. The surrounding palatal area, the lips and the tip of the tongue were inflamed. Presence of plaque was also noted. This was diagnosed as a case of 'double mesiodentes'. The parents were informed of the diagnosis, reassured and the treatment plan, which was to extract both the teeth, were discussed.

Treatment: With the parents giving consent to extraction, both the supernumeraries were removed by intra-alveolar or forcep extraction under local anesthesia. The extraction sockets were packed with gauge pack and post extraction advice were given to parents and the patient. The parents were advised for follow-up after 7 days. On the following visit palatal mucosa showed signs of healing and the maxillary incisors had already started to move palatally and towards midline reducing the gap.

Case 2

An eight-year old boy was brought to pediatric OPD of SDCH for consultation. The minor's upper left primary maxillary incisor was slightly mobile and the mother of the patient complained of a sharp wedge-shaped structure protruding from the palatal aspect of the same tooth. The patient had noticed it a month ago and now it was causing a traumatic bite and the gingiva showed redness.

The right-sided primary central incisor was present and showed no mobility. On examination the child had some plaque deposits, oral hygiene was average and all his permanent first molars were present and had reached occlusal level. The lower permanent central and lateral incisors had erupted. No caries was detected. An IOPA radiograph showed the presence of a peg shaped tooth wedged between primary left central incisor and the presence of an underlying permanent incisor. More than half of the root of the same tooth was resorbed. Another tooth shadow was noticed on the right side of the midline. It was an inverted peg shaped supernumerary tooth that was probably obstructing the eruption path of right permanent incisor. The primary right central incisor had no radiographic signs of root resorption. Considering the related findings it was concluded as a case of peg-shaped double mesiodens, one inverted and the other one erupting palatally, both preventing eruption of permanent incisors. The treatment plan included extraction of both primary central incisors and the peg shaped supernumerary teeth. For the left side the plan was to remove left primary central incisor and the extra tooth by forcep extraction (Intra alveolar extraction). For the right side surgical removal (transalveolar extraction) of the inverted supernumerary tooth after forcep extraction of right central incisor and follow-up for emergence of right permanent right central incisor was planned. It was assumed that removal of the extra teeth would create an unimpeded pathway for eruption of permanent central incisors and both would erupt without orthodontic intervention. Orthodontic intervention was also considered in case of failure of eruption. The diagnosis, treatment plan and the expected outcome were explained to the child's parent in clear and simple words. The mother looked worried stating she had some superstition regarding removal of extra tooth and said she would like to discuss with the father and would want to come back on a later date. Unfortunately the family did not show up for the procedure and we failed to contact them.



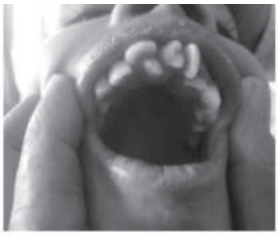
Case 2, Fig-1,

Case 2, Fig-2

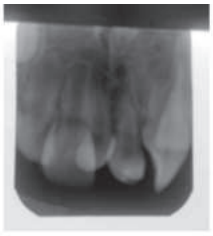
Case 3

The third patient was a boy of nine years of age, hailing from outside of Dhaka. His problem, according to his parents was 'funny teeth' protruding from upper jaw. The boy was shy, would not communicate much and would put his hands in front of his mouth while talking, clearly to hide his teeth. Our pediatric OPD team, after much effort, was able to perform a clinical examination, which was followed by IOPA and OPG x-rays. His maxillary left permanent central incisor was displaced and rotated distally by an erupted tubercle shaped tooth. This tooth had occupied the natural space for the permanent central incisor. The right permanent central incisor was somewhat pushed labially by a cone shaped supernumerary tooth. The upper left lateral incisor was also pushed palatally, while the right central incisor remained unmoved at its natural occlusal position. The maxillary malocclusion along with the atypical shape of the front teeth and presence of the supernumeraries were the reason behind his unusual 'toothy' appearance. We concluded that this was also another case of double mesiodens affecting the aesthetic and causing occlusal disharmony in the mixed dentition period. The treatment plan was to remove the extra teeth under local anesthesia and correct the alignment of the anterior teeth by removable or fixed orthodontics if no natural alignment were noticed on the follow up visits.

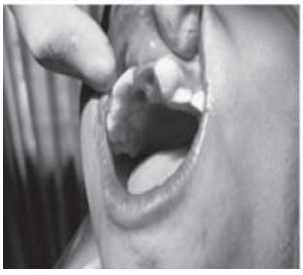
The extraction procedure was carried out with success and patient follow up was scheduled accordingly.



Case 3, Fig 1,



Case 3, Fig 2



Case 3, Fig 3,

Case 3, Fig 4

Discussion

The three cases of double mesiodentes presented in this case report were aged between 8-11 years representing the mixed dentition period. The maxillary anterior region about the central incisors was the common site for these supernumeraries. All three were male children. The patients did not have any positive family history. The worried parents of these children patients had come to the OPD of SDCH with similar complaints of non-aesthetic presentation of the teeth, malocclusion and occlusal disharmony, traumatic bite, plaque accumulation and frequent injuries to gingivae, lips and tongue. Intra oral periapicals and OPG radiographs were performed to confirm the diagnosis. Reviewing the literature, these clinical presentation of the 3 cases had similar findings reported in available case reports.[1-3,6-8,13]

Supernumerary teeth are developmental anomalies widely considered to be arising from hyperactivity of dental lamina, appearing in the bud stage of tooth development or by splitting of the tooth bud resulting in supernumerary teeth (dichotomy theory)[1,7,9]. With detailed history, thorough extra and intraoral clinical and radiographic exam, we diagnosed all the three cases as paired supernumerary teeth occurring in the maxillary central incisor region described in the literature as 'double mesiodens' or 'mesiodentes' [1-5,7,9,10].

According to their shape and size, mesiodens are classified as two morphological types, supplemental or rudimentary. The supplemental types represent their corresponding tooth (eumorphic), while the rudimentary (dysmorphic) ones appear in different sizes or shapes such as – tubercle, odontome like, conical, round or any other atypical shape[10,11]. Case 1 and 2 had conical or peg shaped supernumeraries, typically representing an extra tooth, while case three had one conical and one tubercle shaped Mesiodens.

Most common complications of mesiodentes have been described as- delay or prevention of eruption (26-52%) and displacement/rotation (28-60%) of maxillary permanent incisors[11]. Other less common complications include diastema formation, crowding, occlusal disharmony, dilacerations of permanent teeth, cyst formation and eruption into the nasal cavity.[11,12] The first patient had his permanent incisors labially displaced by the paired supernumeraries. An inverted Mesiodens was preventing the eruption of right central incisor of the second case presented here. The third patient had his left central incisor displaced and rotated with right anterior teeth showing labial proclination. The occlusal disharmony and premature contact of incisors were causing traumatic bite, plaque accumulation, ginigival inflammation and laceration of tongue in all three patients.

Removal of the mesiodentes followed by spontaneous correction of the occlusion or by intervention was the management plan for all three patients. It has been suggested that the extra tooth/teeth should be extracted as early as possible to improve alignment, aesthetics and to prevent complication arising from retaining the teeth for longer period[10-12].

The possible etiology of eruption of extra teeth, the complications related to retaining such teeth in the mouth and the treatment procedure and predictable outcome after extraction were explained to the parents of the children in detail. In all the cases, the parents and the patients were concerned about their oro-facial appearances and the injury to the oral tissues caused by the peg shape of the extra teeth. Extraction of the supernumeraries was the treatment of choice for all three cases and the patients' parents were advised to bring back their children for follow up after 7 days following extraction, which they did. Subsequent follow up visits were also advised in order to monitor prognosis. In the matter of case no 2, the failure of the patient to show up was probably the fact that the parents-worries got the better of them and they considered the extraction procedure complicated; whatever happened with them we would never know.

Conclusion

Mesiodens, either singular or multiple, have both academic and clinical significance. Proper diagnosis, treatment and periodic patient recall are all part of a comprehensive management plan for such cases. Intervention, as soon as a diagnosis is made, can prevent related problems and minimize future expensive treatment costs. Parents and patients counseling are also important for breaking misconceptions about supernumerary teeth. Though majority of cases of supernumerary teeth come into notice when reported by concerned parents or are accidental finds in oral radiographs [13], a screening for supernumerary teeth during recording patients-history may be helpful for early diagnosis of such developmental aberration. Proper documentation may also help in finding the prevalence or incidence of mesiodens/ double mesiodens in Bangladeshi population.

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Management of a Discoloured Incisor Tooth with Large Periapical Lesion- A Case Report

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Abstract

Periapical lesions commonly occur in incisors resulting from inflammatory responses to microorganisms around the teeth and root canals, followed by trauma. In such cases, microbial elimination or minimization from the pulp system using efficient chemomechanical preparation nonsurgically can lead to a successful outcome. In addition, discolouration of these teeth can often be corrected successfully with intracoronal bleaching. In the reported case, nonsurgical endodontic therapy successfully resolved a large periapical lesion of tooth # 32, which suffered from trauma. Intracoronal bleaching followed by endodontic treatment also corrected the discolouration. After 1 year, the incisor tooth showed clinical and radiographic signs of success.

Key Words: Discolouration, Periapical lesion, Bleaching, Root canal treatment. ***(J Cont Dent Sci 2021;9(1):24-28)***

Introduction:

Periapical lesions result from inflammatory responses to microorganisms around the teeth and root canals. Trauma, caries, or tooth wear commonly initiate periapical radiolucencies[1]. Treatment approaches for periapical lesions range from nonsurgical endodontic therapy with or without endodontic surgery to tooth extraction. Microbial elimination or minimization from the pulp system using efficient chemomechanical preparation can lead to a successful outcome[2].

Tooth discolouration varies in aetiology, appearance, location, and severity and could be classified as intrinsic, extrinsic, or both[3,4]. Extrinsic discolouration is caused by chromogens derived from habitual intake of dietary sources such as wine, coffee, tea, carrots, oranges, chocolate, tobacco, mouth rinses, or plaque on the tooth surface[5]. In contrast, intrinsic discolouration typically results from systemic or local causes. Systemic causes include drug-related (tetracycline), metabolic, fluorosis, and genetic (hyperbilirubinemia, amelogenesis imperfecta, and dentinogenesis imperfecta). Local causes include pulp necrosis, intrapulpal haemorrhage, pulp tissue remnants after endodontic therapy, coronal filling materials, root resorption, and ageing[6].

Different options are available for managing discoloured teeth. These include full veneers, laminates, crowns, and non-invasive techniques like bleaching. Even though the outcomes of laminate veneers or full porcelain crowns are more acceptable and predictable, the procedures require tooth preparation, resulting in substantial natural tooth structure loss that cannot be reversed.

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Article Received: 18-09-2020; Article Acepted: 20-11-2020;

In addition, both approaches may mask the discolouration and fail due to fracture, debonding, and marginal leakage[7].

Local causes of discolouration of teeth can often be corrected successfully with intracoronal bleaching. Over the years, many bleaching agents such as oxalic acid, calcium hypochlorite, hydrogen peroxide, carbamide peroxide, and sodium perborate have been used with varying results[8]. However, the most commonly used agents for bleaching endodontically treated teeth (intra-coronal bleaching) are 30%–35% hydrogen peroxide and sodium perborate in combination or separately[9]. Intra-coronal bleaching procedure is well documented within the literature[10]. It's an efficient and safe procedure, although it's been related to cervical root resorption (CRR). Previous studies indicate that previous traumatic injury, the patient's increasing age, and a high concentration of hydrogen peroxide and heating are risk factors that promote cervical root resorption[11,12]. The present article reports the management of a discoloured incisor tooth with a large periapical lesion that resulted from trauma.

Case report

A 35-year-old male patient complained of pain, and aesthetically unacceptable lower left lateral incisor (tooth # 32). The patient had a history of trauma 10 years back. No relevant medical history and history of allergy was noted. Clinical and radiographic examinations were carried out. The intraoral examination revealed that tooth # 32 was tender on percussion. Radiographic examination revealed a large periapical radiolucency (6.5 x 7 mm) associated with the tooth (Fig. 2-A). The tooth had become nonvital and infected following the injury. The case was diagnosed as an acute exacerbation of chronic periapical periodontitis on tooth # 32.

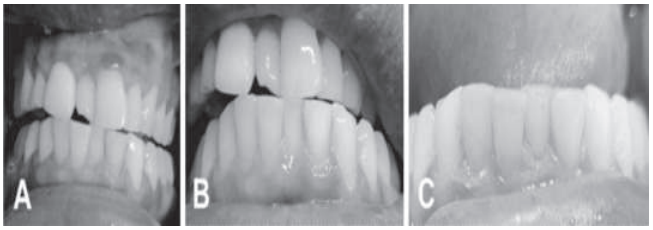

Fig. 1 A - C. Treatment of discoloured tooth # 32 by bleaching. A, Before bleaching; B, After bleaching; C, 1-year-follow-up

The complete treatment plan and procedures were discussed with the patient, and informed consent was obtained before initiating the treatment procedures. At this initial appointment, access to the root canal was established, and a purulent discharge from the canal was noticed. Cotton rolls and high-volume evacuation were used for isolation. The canal was gently irrigated with normal saline (Normal/The ACME Laboratories Ltd, Dhaka, Bangladesh). A cotton pellet was placed within the pulp chamber, and the cavity was kept open. The patient was discharged with the advice of warm saline gurgling over the next 48 hours. A 3rd generation of Cephalosporin (Cefixime 400 mg, 12 hourly for 7 days) was also prescribed to aid in periradicular microbial control. The materials used for root canal treatment are shown in Table 1.

Table 1. Materials used for Root canal treatment

Material	Name/Manufacturer
Normal Saline	Normal/The ACME Laboratories Ltd, Dhaka, Bangladesh
2% Sodium Hypochlorite Solution	Irrisol/HAI Laboratories, Dhaka, Bangladesh
Paper Point	Absorbent Paper Points/DiaDent, Korea
Pure Calcium Hydroxide Powder	Calcium Hydroxide/Deepti Dental Products, Ratnagiri, India
Zinc Oxide Cement	e-Temp/DiaDent, Korea
Calcium Hydroxide Sealer	Sealapex/SybronEndo, Glendora, USA
Gutta Percha Points	Gutta -percha Points/DiaDent, Korea

After 48 hours on the next appointment, the working length was determined. First, necrosed material from the canal was removed. Then, mechanical preparation of the canal was done to an apical size of 30 and irrigated with 2% sodium hypochlorite solution (Irrisol/HAI Laboratories, Dhaka, Bangladesh) and normal saline alternately. After drying with the sterile paper points (Absorbent Paper Points, DiaDent, Korea), the canal was medicated with pure calcium hydroxide (Calcium Hydroxide, Deepti Dental Products, Ratnagiri, India) mixed into a paste form with normal saline. Finally, the access cavity was filled with zinc oxide cement (e-Temp/DiaDent, Korea).

The patient was recalled after 7 days, and the treated tooth was found to be asymptomatic. The access cavity was reopened, copious irrigation was done with 2% NaOCl solution using a side vented needle and finally irrigated with normal saline. The root canal was dried with sterile paper points. Then, the root canal was obturated with gutta-percha (Gutta-percha Points, DiaDent, Korea) as filler and calcium hydroxide as a sealer (Sealapex, SybronEndo, Glendora, USA) by lateral condensation technique.

On the next appointment, after 7 days, the tooth was found asymptomatic. Therefore, bleaching treatment was initiated according to the patient's aesthetic requirement. First, a preoperative photograph was taken (Fig. 1-A) for reference. Then, the Vita classic porcelain shade guide (Vita Zahafabrik) was used under normal daylight to record the shade of teeth # 42 and # 32 before bleaching. The shade of tooth # 42 was A2. Next, approximately 2 mm of gutta-percha (GP) was removed from the pulp chamber (Fig. 2-B).

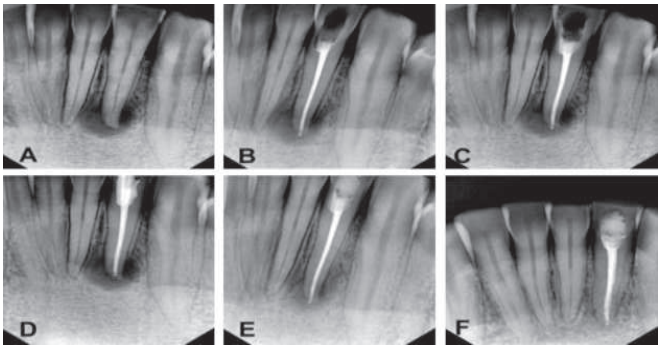

Fig. 2 A - F. Root canal treatment followed by intra-coronal bleaching and follow-up of tooth # 32. A, Preoperative intraoral periapical view showing large circular radiolucency around the apex of tooth # 32; B, Removal of approximately 2 mm Gutta Percha obturating material from the pulp chamber after root canal treatment; C, Placement of approximately 2 mm thick Glass Ionomer cement barrier; D, Placement of bleaching agent and restoration by zinc oxide cement; E, 3-months-follow-up showing bone regeneration and gradual disappearance of the periapical radiolucency; F, 1-year-follow-up revealing complete periapical healing by bone regeneration.

Then, to maintain a mechanical seal between the obturated canal and bleaching gel, 2 mm thick glass ionomer cement (GlasIonomer FX ULTRA/Shofu Dental Corporation, Japan) was placed over the GP (Fig. 2-C). Next, water-soluble cream (Vaseline) was applied to protect soft tissues. Next, the bleaching process was undertaken using 35% hydrogen peroxide gel (Opalescence Endo/Ultradent Products Inc., USA). Next, bleaching gel was placed into the pulp chamber with the help of a spatula and properly condensed with a wet cotton pellet. Finally, the access cavity was sealed with zinc oxide cement (Fig. 2-D). The materials used for bleaching are shown in Table 2.

The bleaching gel was changed and replaced after every week until desired shade was obtained. 3 rounds of bleaching treatment produced satisfactory results (Fig. 1-B). Then the pulp chamber was washed, dried, and neutralized with pure calcium hydroxide mixed into a paste form with normal saline. After 1 week, the tooth was restored with a bonded (CLEARFIL S3 BOND Universal/Kuraray Noritake Dental Inc., Japan.) composite resin (CLEARFIL AP-X/Kuraray Noritake Dental Inc., Japan) restoration.

In the follow-up visit after 3 months, the tooth was found asymptomatic and aesthetically acceptable.

Clinical evaluations showed no palpation or percussion sensitivity, and the radiograph showed a reduction of periapical radiolucency by bone regeneration (Fig. 2-E).

Table 2. Materials used for Intra-coronal bleaching

Material	Name/Manufacturer
Glass Ionomer Cement	GlasIonomer FX ULTRA/Shofu Dental Corporation, Japan
35% Hydrogen Peroxide	Opalescence Endo/Ultradent Products Inc., USA
Adhesive	CLEARFIL S^3 BOND Universal/Kuraray Noritake Dental Inc., Japan
Light Cure Composite Resin	CLEARFIL AP-X/Kuraray Noritake Dental Inc., Japan

Follow-up after 12 months revealed similar clinical findings (Fig. 1-C). In addition, the radiograph showed complete periapical healing by bone regeneration (Fig. 2-F). During follow-up visits, the patient was counselled about the importance of oral hygiene maintenance.

Discussion

Up to 94.4% of periapical lesions show partial or complete healing when managed with a conservative approach using nonsurgical endodontic therapy[13,14]. The nonsurgical endodontic treatment proved effective for the reported case as well.

The presented case exhibited a large periapical lesion (Fig. 2-A). Inflammatory periapical lesions are considered large when their diameter is more than 5 mm[15]. Shaping and cleaning the root canals aided with calcium hydroxide as the intracanal medication is recommended to resolve such lesions. An antibacterial calcium hydroxide-based paste dressing was placed in the canal and kept for 7 days in the reported case. Studies have shown calcium hydroxide dressing promotes periapical healing, notably in young adults[16,17]. Similarly, in the presented case, complete periapical healing occurred within 1 year of nonsurgical endodontic therapy. Radiographic examination demonstrated bone regeneration with increasing density, trabecular reconstruction, and lamina dura formation (Fig. 2-F).

Approximately a 2 mm glass ionomer cement barrier was placed over the root filling material (Fig. 2-C) to ensure a mechanical barrier between the sealed root canal and the bleaching gel, which according to previous studies, prevents leakage, chemical injury, periodontitis and CRR[18-20].

Residual H_2O_2 from bleaching treatment may adversely affect the bonding strength of composites. Therefore, waiting at least 7 days after bleaching before restoring the tooth with resin composites has been recommended. In addition, catalase treatment at the final visit may enhance the removal of residual peroxides from the access cavity. Packing calcium hydroxide paste in the pulp chamber for a few weeks before the placement of a final restoration to counteract acidity caused by bleaching agents and to prevent root resorption has also been suggested. In the reported case, after reaching the desired shade, the pulp chamber was filled with calcium hydroxide for seven days before placing the final material. This step eliminated residual oxygen, which interferes with the polymerization of the filling material. In addition, it neutralized the medium, reducing the risk of cervical resorption[20,21].

It is well documented in some previous studies that the success rate of endodontically treated anterior teeth with or without crowns shows no significant difference[22]. Thus, supporting our view that endodontically treated discoloured anterior teeth can be treated without crowns[23].

Conclusion

In the presented case, nonsurgical endodontic treatment with calcium hydroxide medication has proven its ability in apical healing. Intracoronal bleaching with 35% hydrogen peroxide also successfully corrected discoloration. Clinical and radiographic follow-up after 1-year showed impressive outcomes.

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Published by :

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