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RESEARCH ARTICLE

Radiographic analysis of teeth calcification of breastfed rat cubs of mothers consuming excessive Arabica coffee

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ABSTRACT

Lack of nutrient intake, especially calcium, can inhibit tooth development because calcium is the major component of the inorganic material that forms enamel and dentin. Caffeine in coffee can decrease calcium levels in the body. Arabica coffee has a very high caffeine content compared to other types of coffee. Excessive caffeine consumption can cause the inhibition of PTH secretion. As a result, the nursing mother's body cannot absorb calcium optimally. This makes the baby lack calcium intake which is used for the dental calcification process. This study analyzes radiographic images of tooth calcification of rat cubs during lactation period from rat mothers which consumed excess doses of Arabica coffee. Forty-eight breastfeeding rat mothers (n = 48) were divided into a control group and a treatment group. The control group consisted of 24 rat mothers that were given aquadest. The treatment group consisted of 24 nursing rat mothers, which were given coffee orally at a dose of 2.7 grams/200 grams/day. After the rat mothers gave birth (H + 1 to H + 18), every 4 rat cubs were euthanized, then the mandibular bones were X-rayed. Dental calcification was viewed and analyzed based on radiographic images. The teeth of rat cubs whose mothers consumed excess doses of Arabica coffee experienced a delay in the calcification stage compared to the teeth of normal rat cubs. Consumption of Arabica coffee by nursing mothers can cause a delay in the stage of dental calcification in rat cubs.

Keywords: arabica coffee; dental calcification; dental eruption; development; growth; lactation period

INTRODUCTION

The growth and development of teeth begin early in the embryonic period. The process of dental development is divided into several stages: initiation (bud stage), proliferation (cap stage), histodifferentiation (bell stage), apposition, and calcification starting from the age of 14 weeks in the womb.[1] The process of tooth development does not stand alone but is influenced by several factors. Lack of nutrients can affect the development of teeth because the role of nutrients for oral tissues is no different from other tissues of the body organs. Lack of intake of carbohydrates, proteins, fats, iodine, calcium, magnesium, phosphorus, vitamin C, and vitamin D during the period of tooth growth and development can cause delayed tooth eruption.[2]

The prevalence of dental and oral growth disorders in Indonesia is increasing. Based on the data of the 2018 Basic Health Research, dental and oral problems affect 57.6% of Indonesians. Of this percentage, 93% affect children.[3] The cause of the disorder is the lack of intake of calcium-containing nutrients. Calcium deficiency during growth may cause growth inhibition. In addition to the low calcium intake, other factors can inhibit calcium absorption in the body. As a result, calcium is not distributed and metabolized properly. One of these factors is the consumption of foods or drinks that contain high caffeine. Caffeine is a substance that is widely contained in coffee, tea and c, and carbonated drinks.[4]

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Caffeine has been used for thousands of years, is one of the most extensively consumed food ingredients in the world, and has been shown to give energy.[5] It is found in common beverages and products such as coffee, tea, and soft drinks, along with cocoa and chocolate, and various pharmaceutical and dietary supplements.[6] In Indonesia, there are many types of Arabica coffee, especially in East Java. Arabica coffee has a higher caffeine content than Liberica type, but lower than Robusta type coffee.[7] An average coffee connoisseur drinks 1-3 cups (200 ml/cup) of coffee per day with a caffeine content of about 200 mg/day.[8] If caffeine is consumed more than 300 mg per day, it may affect health, especially during pregnancy and lactation. Caffeine can inhibit the secretion of parathyroid hormone (PTH) which is a calcium-sensing hormone in the body.[9]

Excessive caffeine consumption causes the inhibition of PTH secretion, resulting in nursing mother's body to be less sensitive to calcium. Consequently, the mother's body cannot absorb calcium optimally in the small intestine which has an impact on the waste of calcium in the urine.[9] This may cause the baby to suffer from calcium deficiency, which is supposed to be used for dental calcification process. Choirunnisa showed that consumption of Arabica coffee of 2.7 grams/200 gram body weight/day during lactation period affected the eruption of rat cub's teeth.[10] Other researchers also found that consuming Arabica coffee with an excess dose of 2.7 grams/200 grams body weight/day during lactation can reduce the density of the mandibular bones of Wistar rat cubs.[11] Considering these findings and limited references and research on radiographic images of calcified teeth of rat cubs, we seek to conduct an experimental study on the effect of Arabica coffee consumption during lactation on Wistar rats on radiographic images of calcified teeth of rat cubs.

MATERIALS AND METHODS

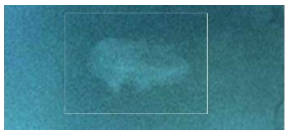
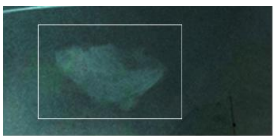
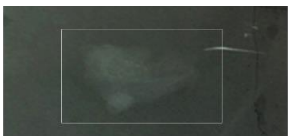
This research is experimental research in laboratory with a posttest-only group design. The sample was 48 breastfeeding Wistar rat mothers (*Rattus norvegicus*). The criteria of the experimental animals are as follow: (1) Wistar rats with body weight of 150-250 grams, (2) healthy, (3) the rats were never used in previous studies. Ethics approval was obtained from the Faculty of Dentistry Ethics Commission, University of Jember No. 1632/UN25.8/KEPK/DL/ 2022. The research group was divided into two: the control group and the treatment group. Grouping samples used a total sampling method. The study sample was divided into two groups, with each group observed at postnatal D+1 until D+18. The first is the control group (K) which consisted of 24 rats. It is the nursing group of mothers given aqua dest during lactation. Every day 4 rat cubs were taken and euthanatized (D+1 until D+18 postpartum). The second group is the treatment group (P) with 24 rats. It is a nursing group of mothers given a dose of coffee exceeding normal or equivalent to 2.7 grams/200 grams body weight/day. Every day 4 rat cubs were taken and euthanatized (D+1 until D+18 postpartum).

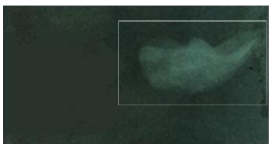
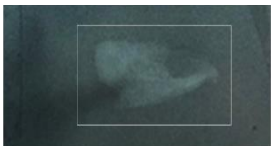
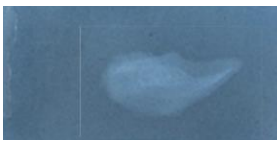
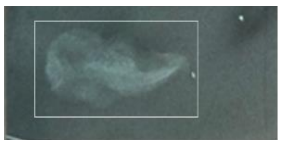
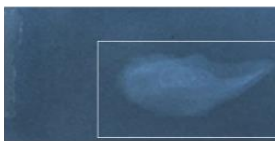
After the rat mothers gave birth (D+1 until D+18), every 4 rat cubs were euthanatized. Then surgery was performed to take a sample of the mandibular bone, and then a periapical X-ray of the mandibular bone was taken. The mandible of a rat cub was placed on a tray, then the cone of the X-ray was directed perpendicularly to it. Dental calcification was viewed and analyzed based on radiographic images. Dental calcification was seen and analyzed descriptively by 3 different observers. There are several stages of tooth calcification formation according to the Demirjian method. Stage A is the calcified occlusal point at the superior level of the crypt in the form of a cone or inverted cone, which is not accompanied by fusion from calcification in other parts. Stage B is fusion from the mineralization point; one or more cusps fuse and form an outline of the occlusal surface. Stage C is the formation of enamel which has been perfect on the occlusal surface. In this stage, expansion and confluence take place until the cervical region are visible. Dentin disposition

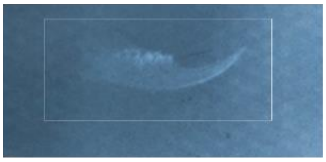
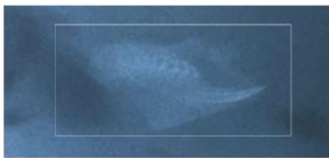
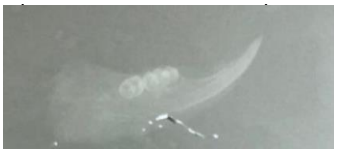
begins with an outline on the arch-shaped pulp chamber at the occlusal edge. In stage D the formation of the crown is complete until the cementoenamel junction. The superior line of the pulp chamber on the root tooth of one is curved, and the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums with spicula-shaped roots. In stage E, (molar) radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown. In stage F (molar) the bifurcation of the conundrum develops from the semi-lunar stage until the roots form a more definite and distinct outline and funnel-shaped tip. In stage G the formation of the roots is complete, but the apical foramen is still partially open (distal molar root). In stage H the apical foramen is completely covered (distal molar root). Periodontal ligaments surround the same width. Then we performed descriptive analysis of the data obtained to determine the differences in each group of researchers.

RESULTS

This study discusses the effect of Arabica coffee on Wistar rats during lactation and on the tooth

day	control group	treatment group
1	 Has not shown any calcification	 Has not shown any calcification
2	 Has not shown any calcification	 Has not shown any calcification
3	 Has not shown any calcification	 Has not shown any calcification
4	 Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	 Has not shown any calcification
5	 Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	 Has not shown any calcification

6			
	Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	Has not shown any calcification	
7			
	Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	Has not shown any calcification	
8			
	Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	Has not shown any calcification	
9			
	Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	Has not shown any calcification	
10			
	Stage B: Fusion of the mineralization point, one 1or more cusps fuse and form an outline of the occlusal surface	Stage A: The calcified occlusal point at the superior level of *the crypt* in the form of an inverted cone or cone, is not accompanied by fusion from calcification in other parts	
11			
	Stage C: The formation of enamel has been perfectly on the occlusal surface. Expansion and confluence until the cervical region is visible. The process of dentin disposition begins. Outline on the arch-shaped pulp chamber at the occlusal edge	Stage B: Fusion of the mineralization point, one or more cusps fuse and form an outline of the occlusal surface	

12		
	Stage C: The formation of enamel has been perfectly on the occlusal surface. Expansion and confluence until the cervical region is visible. The process of dentin disposition begins. Outline on the arch-shaped pulp chamber at the occlusal edge	Stage B: Fusion of the mineralization point, one or more cusps fuse and form an outline of the occlusal surface
13		
	Stage D: Crown formation is completed until CEJ. The superior line of the pulp chamber on the root tooth of one is curved, the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums. The roots look spicula-shaped	Stage C: The formation of enamel has been perfectly on the occlusal surface. Expansion and confluence until the cervical region is visible. The process of dentin disposition begins. Outline on the arch-shaped pulp chamber at the occlusal edge
14		
	Stage E: Radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown	Stage D: Crown formation is completed until CEJ. The superior line of the pulp chamber on the root tooth of one is curved, the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums. The roots look spicula-shaped
15		
	Stage E: Radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown	Stage D: Crown formation is completed until CEJ. The superior line of the pulp chamber on the root tooth of one is curved, the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums. The roots look spicula-shaped
16		
	Stage E: Radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown	Stage D: Crown formation is completed until CEJ. The superior line of the pulp chamber on the root tooth of one is curved, the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums. The roots look spicula-shaped

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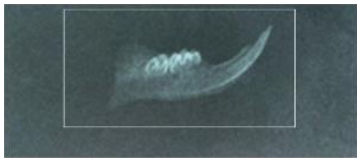
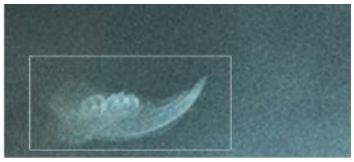
17		
	Stage E: Radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown	Stage D: Crown formation is completed until CEJ. The superior line of the pulp chamber on the root tooth of one is curved, the shape is concave in the cervical region. On the molar teeth, the pulp chambers are formed like trapeziums. The roots look spicula-shaped
18		
	Stage F: The bifurcation develops from the semi-lunar stage until the roots form a more definite and distinct *outline* and funnel-shaped tip	Stage E: Radicular bifurcation incisal formation is seen to form a semi-lunar or calcified point. The length of the root is still less than the length of the crown

Figure 1. Interpretation of radiograph images of molar mandibular calcification in rat cubs

calcification of rat cubs. Dental calcification of rat cubs in the control group (K) and the group of rat cubs whose mothers were given an excess dose of coffee (P) was observed through radiographic images. The results of radiographic observations between the two groups are presented in Figure 1.

Figure 1 illustrates the results of the observation and interpretation of radiographic images of the first molar mandibular of rat cubs. It shows that day 1 to day 3 of the post-partum period did not show calcification in both the control group and the treatment group. Until the 9th day, the rat cubs of the rat mothers given excess coffee still did not show any sign of calcification. The formation of tooth calcification in the control group appeared on day 4, while in the treatment group, it began on day 10. There was a delay in the calcification stage of rat cubs whose mothers were given excessive doses of coffee when compared to the control group. The calcification stage on day 18 of the control group was stage F, while the treatment group was one stage behind (E).

DISCUSSION

This study is an experimental laboratory study. The aim is to analyze the radiographic images of tooth calcification of rat cubs of rat mothers which were given excessive doses of Arabica coffee during breastfeeding. The results of the radiographic analysis of mandibular bone of the rat cubs showed that giving Arabica coffee during lactation could inhibit the tooth calcification process. The teeth of rat cubs of rat mothers which consumed excess doses of Arabica coffee indicated a delay in one stage of calcification than those of rat cubs whose mothers were not given coffee. Differences began to appear on D+4, where tooth calcification had not developed in rat cubs whose mothers were given excessive doses of coffee. In contrast, rat cubs whose mothers were given aquadest entered Stage A. Tooth calcification in rat cubs whose mothers were given excessive doses of coffee appeared to start to enter Stage A on D+10, six days apart with rats whose mothers were given aqua dest. On days 11 until 18, it appeared that the teeth calcification of the rat cubs whose mothers were given excessive doses of coffee

experienced a one-stage delay compared to the rat cubs whose mothers were given aquadest. The nutrients of metabolism needed by rat cubs in the control groups were provided by daily food intake without additional consumption of coffee which could cause calcification inhibition. Meanwhile, the group whose mothers were given excessive doses of Arabica coffee experienced a slower calcification process. This is due to high-dose coffee intake by the rat mothers. The caffeine level in coffee consumed by these breastfeeding rats may have caused increased calcium excretion in the urine. Consequently, calcium loss in their body may lead to the slow process of molar calcification of the rat cubs. This finding is consistent with that of Choirunnisa which states that caffeine consumption of more than 300 mg per day can inhibit the eruption process of rat cubs' teeth.[10] Caffeine consumption may cause diuresis, a condition where there is an increase in the volume of urine secreted by the body as well as the amount of loss of substances dissolved in water that fail to be reabsorbed in the renal tubules. One of these substances is calcium, an essential nutrient for the body.[9] Other studies have suggested that mother mice that consume excess doses of coffee may have lower average value of mandibular bone density in rat cubs.[11] About 99% of caffeine in coffee will be directly absorbed by the intestines and transported around the body within 30-45 minutes.[12] Increased coffee consumption can cause an imbalance in calcium levels in the body, thus resulting in low level of calcium.[13,5] Another study also revealed that high caffeine consumption is a risk factor for osteoporosis because it increases diuresis and urinary calcium loss, reduces intestinal absorption, and inhibits bone mineralization.[14]

The European Food Safety Authority (EFSA) and the UK National Health Service (NHS) advise that coffee consumption not exceed 200mg/day.[15] The maximum safe dose of caffeine during pregnancy is less than 300 mg/day (about three cups of coffee).[16,17] Studies in mice have shown that caffeine consumption during pregnancy and lactation could interfere with brain development, lead to long-term neurological and cognitive disorders in infants.[18] In humans, caffeine is catalyzed by the cytochrome P450 (CYP) enzyme CYP1A2 in the liver. The process begins with the removal of the methyl group to form primary metabolites namely paraxanthine, theobromine, and theophylline.[16] The half-life of caffeine in healthy adults is about 3-5 hours, and in pregnant women, it is slower. Caffeine is quickly distributed to all tissues and easily crosses the blood vessels to the brain to exert its effects.[16]

Parathyroid hormone (PTH), vitamin D, and calcitonin are the main regulators in calcium homeostasis which involves the kidneys, intestines, parathyroid glands, and skeleton.[9] When the body needs a large supply of calcium during the lactation period, calcium levels in the blood will decrease, triggering the parathyroid glands to secrete PTH which stimulates the production of calcitriol or vitamin D3. Calcitriol plays a role in calcium re-absorption in the kidneys. This calcitriol will then move toward the small intestine to increase calcium absorption in the mother and baby.[19] Lack of calcium during lactation could lead to inhibition of calcite growth, resulting in a decrease in calcium absorption in the intestines. This finding was also reported by Lu et al. who found that excess caffeine intake could inhibit the secretion of the hormone parathyroid by 10.4%. In addition, the activity of pKA (protein kinase A) and cAMP (*cyclic adenosine monophosphate)* may also decrease after caffeine administration. This suggests that cAMP and pKA are directly proportional to the secretion of PTH.[9] If PTH secretion is inhibited by caffeine, cAMP, pKA, and calcitriol become inhibited which may cause the body of mother rats to be less sensitive to the presence of calcium. As a result, calcium absorption in the small intestine is not optimal and could cause an increase in calcium excretion in the urine. Taken together, the decrease in the calcification process in rat cubs stems from calcium excretion in the urine. Thus, this study suggests a hypothesis: Consumption of excessive doses of Arabica coffee during lactation period can delay the process of tooth calcification of rat cubs.

However, we acknowledge that this study has a limitation because this study was not followed by histological confirmation of the results of the tooth calcification of the rat cubs. In the future, further research is needed to observe the histological image of the calcification process, with a longer observation time until the eruption process is complete.

CONCLUSION

The results of this study showed that the tooth calcification of the rat cubs breastfeeding from mothers which consumed excess doses of Arabica coffee experienced a one stage delay in calcification compared to the tooth calcification of rat cubs whose mothers did not consume coffee.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

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