Green Pigmentation in Human Teeth. A Stereomicroscopic Study

Rakauskaite A*/ Juodzbalys G**/ Pauza DH***/ Cicciù M****

Green pigmentation in teeth is an uncommon condition associated with bilirubin deposits in hard dental tissues. Its occurrence can cause anxiety to both the child and parents and is not diagnosed easily by clinicians. The aim of this study is to analyze the current knowledge about the etiology, the intraoral alterations, and the macroscopic and microscopic features of green teeth pigmentation related to a high bilirubin levels. A primary tooth was extracted and manually sliced into 600 microns thin sections. The slenderized slices were examined with a light microscope AxioImager M1 to evaluate the microscopic teeth structure.
The clinical characteristics of teeth may help in the diagnosis of current or past systemic diseases. Pediatricians should be able to quickly note the signs in order to perform the proper diagnosis. This study may help clinicians gain more knowledge about the current status of this uncommon pathology.

Key Words*: green teeth, bilirubin, steromicroscope*

INTRODUCTION

Green teeth are an extremely rare form of teeth pigmentation caused by bilirubin deposition in hard dental tissue [1-7].If there is an increase in levels of bilirubin in the organism, defined as hyperbilirubinemia, it may also be distributed in different tissues in the entire body. After normalization of bilirubin levels, the soft tissue pigmentation immediately disappears due to rapid cell metabolism. Permanent deposition of bilirubin in the organism's hard tissues and in teeth can suggest loss of metabolic activity after maturation [1, 3, 8]. Green staining of the teeth is greater if increased level of bilirubin concentration is present during the dental development period. Primary teeth start to develop during the fourth to sixth month of foetal life and ends at 11 months after birth while deposition of hard tissues of the permanent teeth begins at birth and usually ends by 8 years of age [1, 5, 6, 8]. Dental tissues formed after the normalization of bilirubin concentration or remission of the disease become normal both in color as well as structure [4, 8]. Langmead and Thursfield first proposed that green teeth could be associated with jaundice and foetal or neonatal hyperbilirubinemia. Congenital biliary atresia is characterized as one of the most frequent causes of hyperbilirubinemia in this particular period of life. The clinical characteristics of this disease include pronounced jaundice, hepatosplenomegaly, pruritus, ascitis, xanthemas, growth retardation, portal hypertension, bleeding, osteomalacia, ascites and most importantly – liver failure [6, 9, 10]. The most frequent deposition of bilirubin as a green color occurs in the primary dentition, nevertheless there are cases reported where the permanent teeth were also affected with a particular green area in the crown. If a patient has a history of congenital biliary atresia and was cured by liver transplantation, it is considered that permanent dentition will be affected [3, 7, 8]. The extent and localization of pigmentation is definitely associated with the period of dental tissue development and depends on the duration and moreover, on the severity of hyperbilirubinemia [4, 11].

This paper presents a case of a patient with an atypical green pigmentation of the permanent teeth from biliary atresia. Microscopic analysis of affected primary teeth is analyzed.

Case Report

On November 6, 2012 a nine years old boy with his mother was referred to a private dental practice for general dental care. During the intraoral examination, an atypical green pigmentation of the permanent teeth was noticed. The medical history of this patient included liver transplantation due to biliary atresia in 2005, when he was 2 years old. The intraoral examination revealed green pigmentation of two thirds of the incisal part and normal colouration of the cervical third of the already erupted permanent teeth (Figure 1). An obvious margin separating the pigmented zone from the non-pigmented one was observed (Figure 2). The brightest

***Asta Rakauskaite DDS,** Department of Maxillofacial Surgery, Lithuanian University of Health Sciences, Kaunas, Lithuania.
** **Gintaras Juodzbalys DDS, PhD,** Department of Maxillofacial Surgery,Lithuanian University of Health Sciences, Kaunas, Lithuania.
***Dainius H Pauza, MD, PhD,** Institute of Anatomy, Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania.
****Marco Cicciù, DDS, PhD, MSc,** Assistant Professor, Human Pathology Department, University of Messina School of Dentistry,Italy.

Send all correspondence:
Prof Marco Cicciù Department of Human Pathology, Dental School, Messina University Via Consolare Valeria 98100, Messina, Italy.

Tel.: 0039-090 221 6909

E-mail: acromarco@yahoo.it

Figure 1. Figure showing permanent maxillary and mandibular central and lateral incisors: A green pigmentation of two thirds of incisal part and normal colour of cervical third of teeth. Anatomy and structure is normal. B - Clear demarcation line separating pigmented and non-pigmented tooth zone is visible especially precisely in mandibular incisors.

Figure 2. Green discoloration of extracted first primary molar roots are observed, coronal part differently is normal at their colour. Roots are affected by physiological resorption – 1/3 of apical part of the root is resorbed.

colour was observed on the maxillary central incisors, the mandibular central and the lateral incisors: the demarcation lines in these teeth were clearly seen as well. The green tone of the other permanent teeth was lighter and the demarcation line was not so evident (Figure 2). The colour of the crowns of primary teeth was normal.

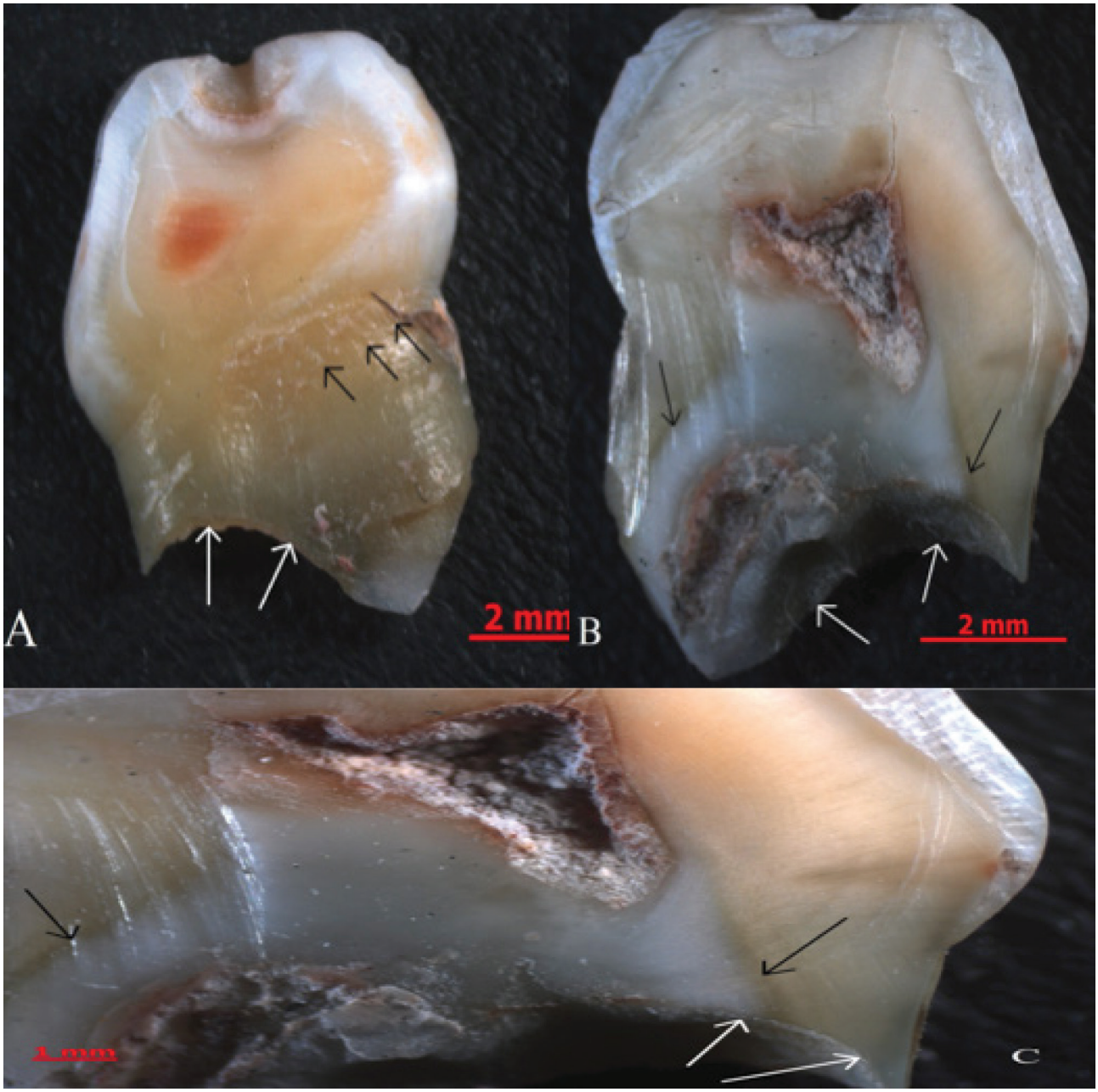
Treatment was decided during the first visit. On November 8 and November 13, 2012 two primary left and right first molars were extracted due to periodontitis. The roots of the extracted teeth were not fully affected by physiological resorption – only 1/3 of the apical part of the root was resorbed. Green discoloration of the roots was observed, while the coronal parts of these extracted teeth differently were normal (Figure 3). The patient's mother confirmed that the colour of the primary teeth had been normal, however as they started to be replaced by permanent teeth, it was noticed that the roots of primary teeth were stained green.

One of the extracted primary teeth was obtained for anatomical investigation and analysis. Further intraoral examination revealed: caries of the mandibular right and left permanent first molars and of all the primary second molars. The structure of the enamel was normal; no enamel hypoplasia was evident and the condition of the gums was also normal and healthy. Oral hygiene was satisfactory.

From the patient birth in 2003 till one and the half-year's ago (after liver transplantation at 2006), regular blood test results were monitored and tabled. The most important blood test parameters reflecting the hepatic system and depending on patient's age, before and after the liver transplantation were extracted and analysed. The following blood test parameters were analysed: variation of total and direct bilirubin; variation of enzymes indicating liver function, i.e. liver transaminases (AST, ALT); enzymes linked to the biliary tract (gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP)). The possible relationship between teeth pigmentation and the above-mentioned blood parameters was evaluated, depending on already established age of the human, when the hard tissues of the particular teeth are developing.

The first primary left mandibular molar was examined. The extracted tooth was washed with water vapour and desiccated. Following 10 days, the tooth was longitudinally sliced into sections of 2mm using a straight handpiece Chirana 110D (Chirana Medical, Stará Turá, Slovak Republic) and a diamond polishing disc, applying an average speed of 30,000 rpm, with 35 m/Nm torque. The tooth slices had an approximate thickness of 2mm and were observed under a Stemi 2000–CS stereo-microscope (Carl Zeiss, Gottingen, Germany). The tooth slices were then manually thinned to 600 microns with the aid of fine sandpaper and polished manually on a natural leather surface. The slenderized slices were examined under an AxioImager M1 light microscope (Carl Zeiss, Gottingen, Germany).

Figure 3. Stereomicroscopy of 2 mm width section. Pulp (B, C) and signs of physiological root resorption (A, B, C) indicated by white arrow. A – The start of bilirubin pigmentation 1mm below the cemento-enamel junction is indicated by black arrow. B, C – The accumulation of bilirubin in the dentin of the root is observed. B, C – More vivid color of bilirubin localized in the surface of dentin and cement junction, indicated by black arrow.



RESULTS

Three additional follow-up control visits were made on November 19, 28, and December 13, 2012. The following procedures were performed: treatment of caries (16, 46, and 36 teeth), one tooth per visit. The diagnosis was caries media. Caries lesion cavities were lined with light curing ready-for-use glass ionomer composite cement, Ionoseal (Voco, Cuxhaven, Germany) and restored with composite restorative material. The main difficulty of this procedure was achieving a good esthetic result due to incompatible and uncommon colour tone and opacity of the teeth. Composite material of Charisma Opal D3 (Heraeus Kulzer, Hanau, Germany) was used, as it was decided to be the most aesthetically and compatible in this situation. All concentrations of direct and total bilirubin registered before liver transplantation was recorded. An obvious difference was observed between two periods – before (from birth till 24 months) and after liver transplantation (from 24 months to the last registered blood test result at 39 months) – both parameters significantly decrease. All concentrations of direct and total bilirubin registered before liver transplantation exceeded the normal range. The highest concentration of total bilirubin (401 μmol/l) was marked at 21 months after birth; the highest rate of direct bilirubin (211 μmol/l) was marked at s12 – 13 month after birth. The mean concentration of total and

direct bilirubin before liver transplantation was 251.28 µmol/l and 117.99 µmol/l, after liver transplantation – 18.8 µmol/l and 7.2 µmol/l, respectively.

The main change of all monitored enzymes concentrations after liver transplantation was a significant decreased. The highest concentrations before liver transplantation were as follows: 580 u/l (AST) and 430 u/l (ALT) at 6 months, 482 u/l (GGT) at 7 and 2380 u/l (ALP) at 11 months. The mean concentrations before liver transplantation were: 304.1 u/l (AST), 202.94 u/l (ALT), 181.75 u/l (GGT), 1309.1 u/l (ALP) and all of them significantly exceed the normal range. The mean concentrations after liver transplantation were normal: 36.54 u/l (AST), 30.17 u/l (ALT), 39.6 u/l (GGT) and 613.36 u/l (ALP). The period, when the highest concentration of direct bilirubin was recorded, coincides with completed root development of the primary mandibular and maxillary central incisor, and the mandibular lateral incisor. Moreover, this period also coincides with the beginning of calcification of the permanent maxillary lateral incisor and first premolars of both jaws. All the primary and permanent teeth, involved from the birth to 24 months developing during hyperbilirubinemia when the blood presented excessive concentration of bilirubin t.

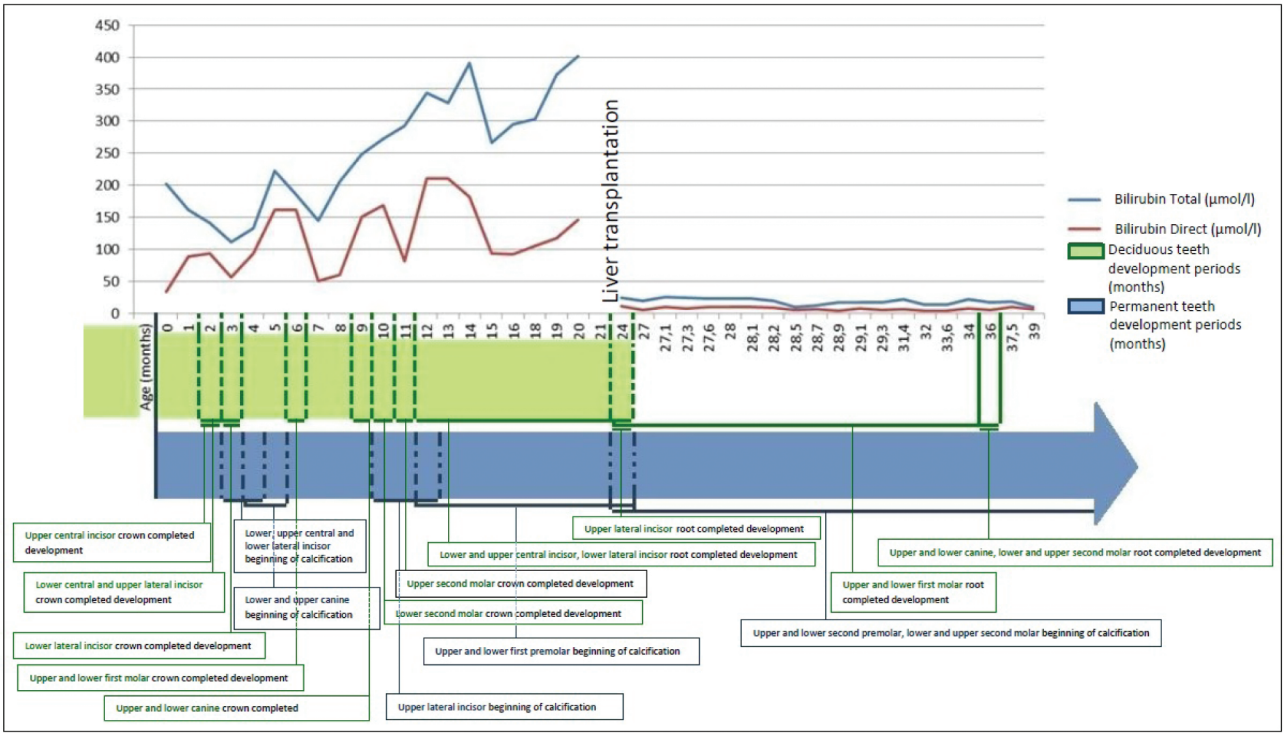
The period of 6, 7 and 11 months after birth, when the highest concentrations of AST, ALT, GGT, ALP were recorded, coincides with completed crown development of the primary maxillary and mandibular first molars and canines, and second molars. Furthermore, the calcification of the permanent maxillary and mandibular central and lateral incisors, and the maxillary and mandibular canines had already begun. All of the teeth, from both the primary and permanent dentition that developed from the birth to 24 month after birth, developed during the exceeded concentration of all parameters. (Graph 1, 2).

Microscopic examination

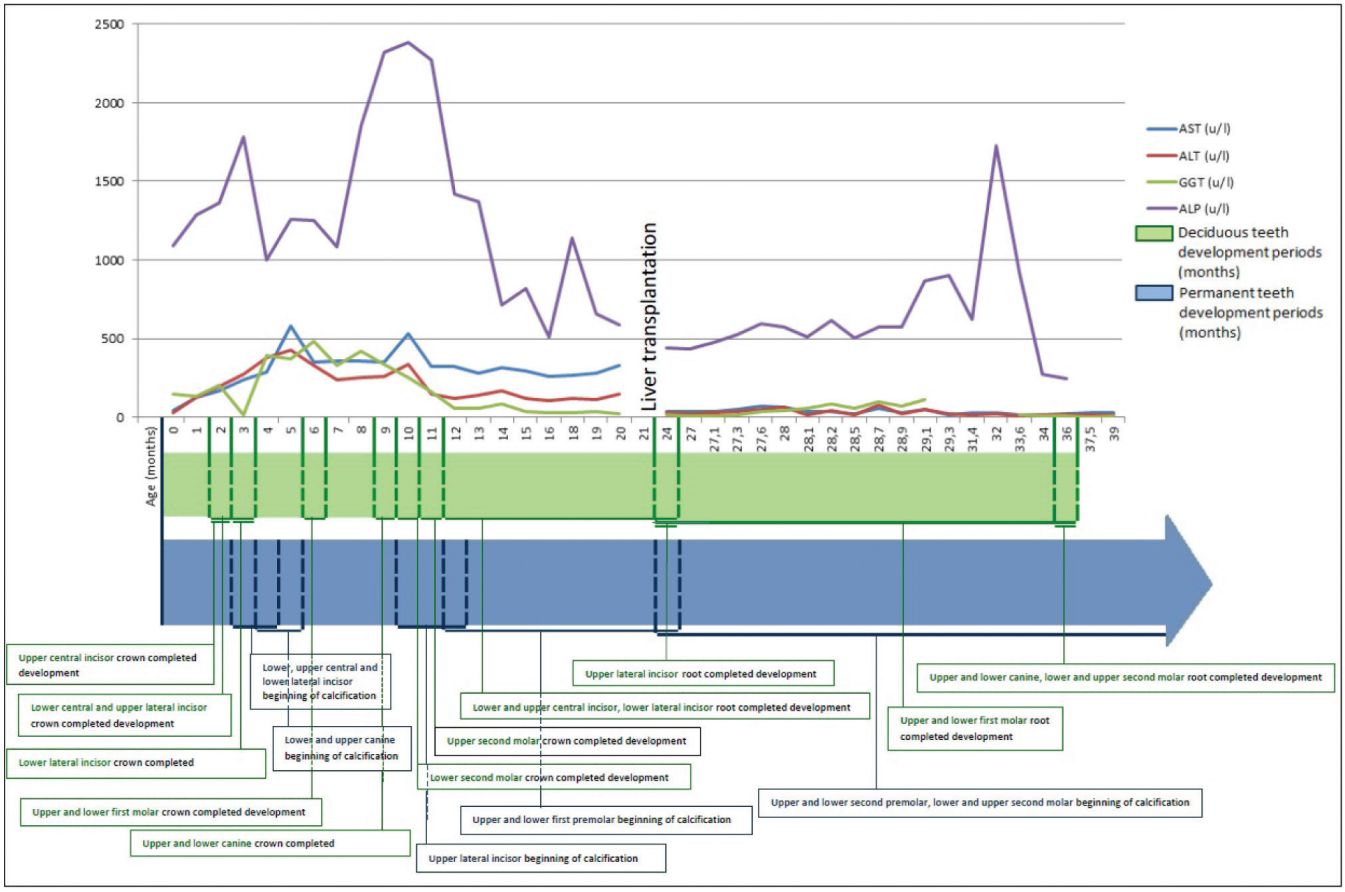
Analysis of the initial 2mm width tooth section under stereomicroscope (Figure 3A, 3B, 3C): An irregular shape of the apical part of the section showed signs of physiological root resorption. The pigmented part of the tooth root gradually started about 1mm below the cemento–enamel junction (Figure 3A). Green pigmentation accumulated in the dentin: no sign of enamel and cement pigmentation was observed. More vivid colour pigmentation was localized in the surface of the dentin and cement junction – in Figure 3B and 3C it is seen as a green line. In the internal surface of the section (Figure 3B, 3C) the coronal pulp camera and root canal pulp with the remains of pulp tissue was observed.

Analysis of the 600µm width slices of the affected tooth under light microscope revealed the following results (Figure 4A, 4B and 4C): the clear zone of the bilirubin accumulation in the partially resorbed root is indicated by black point with more vivid colour or higher concentration in the part of dentin and cement junction (Figure 4A). As in stereoscopic view, only the pigmentation of the dentin was observed, neither the cement nor enamel was affected (Figure 4A and 4B). The, color of the enamel, its thickness and direction of the prisms of the tooth was normal. However the direction of the dentin tubules was different between the pigmented and no pigmented part: in the former, the diameter of the tubules was observed, while in the latter part, the tubules changed direction and the longitudinal sections were observed (Figure 4C).

Graph 1. A – Graph, which indicates the variation of total and direct bilirubin depending on patient's age and stage of disease i.e. before and after liver transplantation. B – Variation of the enzymes depending on patient's age and stage of disease i.e. before and after liver transplantation.



Graph 2. A – Correlation between bilirubin concentration and teeth development periods. B - Correlation between AST, ALT, GGT, ALP concentrations and teeth development periods.



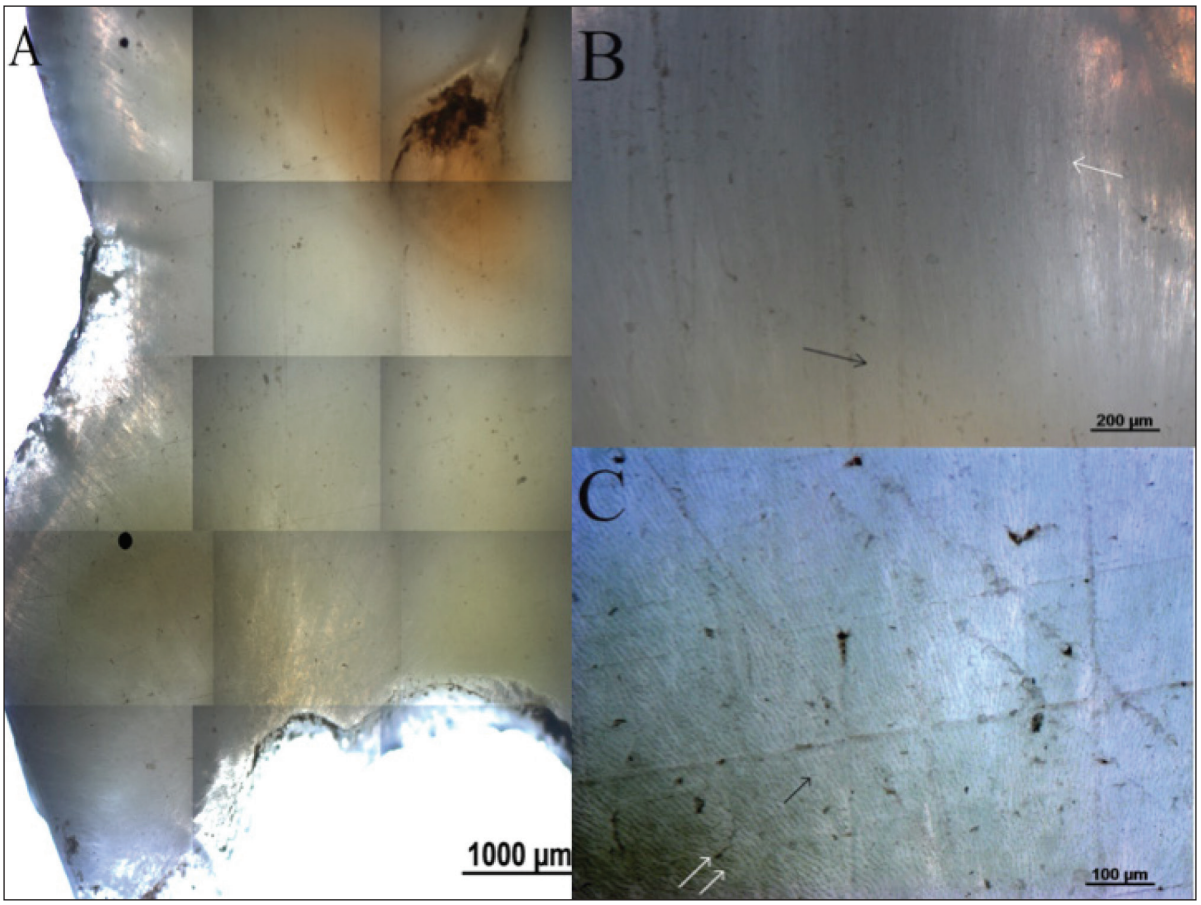
DISCUSSION

There are several reasons mentioned in the literature for the cause of hyperbilirubinemia and subsequently bilirubin deposition in the teeth (either primary, permanent or both dentition). The most frequent etiologic factor, which causes hyperbilirubinemia and eruption of teeth with green pigmentation, was biliary atresia [1- 8, 12, 13]. Telation between biliary atresia and frequency of green teeth might be associated with an extremely high concentration of direct bilirubin, which is >100 μmol/L and in this particular disease, exceeds the normal range by more than five times[11]. Other etiologic factors include hemolytic disease [1, 5, 13, 14, 15], biliary hypoplasia, acute liver failure, and occlusion of bile duct or its absence [3, 8]. Literature also shows cases in which the pigmentation of teeth occurred because of hyperbilirubinemia was caused by intrahepatic cholestasis, associated with sepsis by *K oxytoca* and *Candida, Staphylococcus aureus* species or cytomegalovirus infection [2, 3, 13, 15]. There was one case of green teeth in a 3 years old patient whose medical anamnesis included diagnosis of cystic fibrosis shortly after birth and hepatic function deterioration ensued, accompanied with sustained hyperbilirubinemia [16-18].

The present study results and the anatomical investigation analysis revealed the same facts as previous studies [1, 8, 16]. Light microscopy confirmed two earlier studies but opposes another regarding the direction change of the tubules of pigmented and no pigmented dentin: in no pigmented parts of the dentin, the diameter of the tubules can be observed while in the pigmented parts the tubules change their direction and longitudinal section (Figure 4C) [1, 8, 16, 19]. The methodology of our anatomical investigation revealed that it was not necessary to perform additional histological preparations of analysed teeth, which had been performed in previous analysis, to achieve qualitative results. Moreover, the risk of possible degradation and colour alteration of bilirubin was avoided. In this present study, the localization of the green colour is clearer compared to previous studies [16, 21-26].

In this reported case, dentitions were involved in the pigmentation but differences between the localization were recorded. The ability to notice deposition of bilirubin and green staining of the teeth was only possible at the beginning of mixed dentition when the roots of primary teeth were exposed after extraction because the crowns of the primary teeth had no sign of green colour. Conversely, erupted permanent teeth are affected on the crowns and therefore were noticed just after eruption (Figures 1 and 2).

Figure 4. A – Zone of bilirubin accumulation in the partially resorbed root with more vivid colour or higher concentration in the part of dentin and cement junction is indicated by black point. A, B – Pigmentation of dentin is observed, other structures are not affected. B – Normal cement indicated by white arrow, affected – by black. C – The direction of tubules of the dentin is different between pigmented and not pigmented part: in not pigmented part of the dentin the diameter of the tubules can be observed, while in the pigmented part tubules change their direction and the longitudinal sections is observed; direction of tubule in sound zone is indicated by black arrow, in affected – white.



CONCLUSIONS

The literature review revealed that green pigmentation of the teeth caused by bilirubin is an extremely rare form of teeth pigmentation. However, this intraoral manifestation has a sufficiently narrow field of causes related to severe liver diseases coinciding the period of teeth development and therefore, can be differentiated from other pigmentations. Despite this fact, it is rare that the doctors treating the causal disease and dentists would take into consideration this kind of teeth pigmentation and not only predict, but also inform the patient's parents about possible forthcoming dental problems. After maturation and eruption of all permanent teeth, treatment of this condition by aesthetic restorations should also be performed. Naturally, before any treatment plan can be carried the medical history about the past liver disease, its severity and bilirubin concentration must be carefully analysed.

REFERENCES

1. Watanabe K, Shibata T, Kurosawa T, Morisaki I, Kinehara M, Igarashi S, Arisue M. Bilirubin pigmentation of human teeth caused by hyperbilirubinemia. J Oral Pathol Med; 28:128-30. 1999.
2. Fernandes KS, Magalhães M, Ortega KL. Green teeth. J Pediatr;158:510. 2011.
3. Guimaraes LP, Silva TA. Green teeth associated with cholestasis caused by sepsis: a case report and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 95:446–51. 2003
4. Lin YT, Lin YT, Chen CL. A survey of the oral status of children undergoing liver transplantation. Chang Gung Med J; 26:184-8. 2003.
5. Rosenthal P, Ramos A, Mungo R. Management of children with hyperbilirubinemia and green teeth. J Pediatr; 108:103-5. 1986.
6. Rangé H, Camy S, Cohen J, Colon P, Bouchard P. Dental treatment of an adult patient with a history of biliary atresia. Quintessence Int; 43:337-41. 2012.
7. Sommer S, Magagnin K, Kramer PF, Tovo MF, Bervian J. Greenteeth associated with neonatal hyperbilirubinemia caused by biliary atresia: review and case report. J Clin Pediatr Dent; 35:199-202. 2010.
8. Amaral TH, Guerra Cde S, Bombonato-Prado KF, Garcia de Paula E Silva FW, de Queiroz AM. Tooth pigmentation caused by bilirubin: a case report and histological evaluation. Spec Care Dentist; 28:254-7. 2008.
9. Langmead F. Anomalous jaundice with enlargement of liver and spleen, and bile stained teeth. Proc R Soc Med;5:148. 1912.
10. Thursfield H. Green teeth subsequent to a prolonged jaundice in the first weeks of life. Proc R Soc Med;5:147-8.1912.
11. W E Tjon A Ten, Houwen RH. Green teeth. Arch Dis Child; 92(3): 250. 2007.
12. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol; 62:1006-12. 2009.
13. Swann O, Powls A. Green Teeth in Neonatal Sepsis. N Engl J Med; 9: 367:8. 2012.
14. Hartley JL, Davenport M, Kelly DA. Biliary atresia. Lancet; 14; 374(9702):1704-13. 2009.
15. Battineni S, Clarke P. Green teeth are a late complication of prolonged conjugated hyperbilirubinemia in extremely low birth weight infants. Pediatr Dent; 34:103-6. 2012.
16. Bimstein E, Magliocca K, Cohen D, Morelli G, Katz J. Hyperbilirubinemic stain: location and extent in dental tissues. J Clin Pediatr Dent; 36:75-8. 2011.
17. Sommer S, Kramer PF, Magagnin K, Tovo MF. Unusual case of green pigmentation in human teeth resulting from neonatal hyperbilirubinemia. Gen Dent; 56:21-3. 2008.
18. Naudi AB, Ammari AB, Fung DE. A report of 2 cases of green pigmentation in the primary dentition associated with cholestasis caused by sepsis. J Dent Child (Chic); 75:91-4. 2008.
19. Guadagni MG, Cocchi S, Tagariello T, Piana G. Case report: Alagille syndrome. Minerva Stomatol;54: 593-600. 2005.
20. Alto LA, Pomarico L, Souza IP, Janini ME. Green pigmentation of deciduous teeth: report of two cases. J Dent Child (Chic); 71:179-82. 2004.
21. Morisaki I, Abe K, Tong LS, Kato K, Sobue S. Dental findings of children with biliary atresia: report of seven cases. ASDC J Dent Child; 57:220-3. 1990.
22. Herbert FL, Delcambre TJ. Unusual case of green teeth resulting from neonatal hyperbilirubinemia. ASDC J Dent Child; 54:54-6. 1987.
23. Zaia AA, Graner E, de Almeida OP, Scully C. Oral changes associated with biliary atresia and liver transplantation. J Clin Pediatr Dent; 18:38-42. 1993.
24. Seow WK, Shepherd RW, Ong TH. Oral changes associated with end-stage liver disease and liver transplantation: implications for dental management. ASDC J Dent Child; 58:474-80. 1991.
25. Shibata T, Watanabe K, Maki F, Arisue M, Kurosawa T, Tohma M, Hosokawa M, Okada F, Takeichi N. The LEC (Long-Evans Cinnamon) rat as an animal model for bilirubin-induced tooth pigmentation. Anticancer Res; 17:2141-5. 1997.
26. Shibata T, Watanabe K, Oda H, Arisue M, Kurosawa T, Tohma M, Hosokawa M, Takeichi N. Experimental bilirubin pigmentation of rat dentine and its detection by a qualitative analytical method. Arch Oral Biol; 41:509-11. 1996.