Demarcated Opacities as Predictors of Progression of the Molar Incisor Hypomineralisation: a Pilot Study

Introduction

The question of an appropriate therapeutic approach to molar incisor teeth hypomineralisation (MIH) arose simultaneously with the research of their etiology and diagnostic criteria. As early as 2003, the studies of durability and adequacy of use of specific dental materials in the therapeutic treatment of affected teeth were published (1). The primary concern was clearly to identify the best therapeutic options for severely affected first permanent molars. The key question was if it is better to extract severely hypomineralised First Permanent Molars (FPM) early on or to attempt to preserve them.

Over the following years, more attention was given to treatment options for teeth affected by a mild form of MIH and esthetically focused treatment of MIH-affected incisors. General treatment protocol was proposed (2, 3, 4) which placed great importance on remineralization of initial lesions and desensitization of teeth, as well as prevention of dental caries and post eruptive enamel breakdown (PEB) (5).

One of the first studies that discovered the link between

Uvod

Usporedo s istraživanjem etiologije i kriterija za dijagnostiku molarno-incizivnih hipomineralizacija (MIH) postavilo se pitanje odgovarajućega terapijskog pristupa. Već 2003. godine objavljena su istraživanja o dugotrajnosti i adekvatnosti primjene pojedinih stomatoloških materijala u terapiji zahvaćenih zuba (1). Naravno, najprije se nastojalo utvrditi najbolje opcije za tretman teže zahvaćenih prvih stanih molara. Osnovni problem u pristupu pacijentima s teško hipomineraliziranim PSM-im bio je ustanoviti je li takve zube bolje pravodobno ekstrahirati ili ih pokušati sačuvati.

U godinama koje su slijedile više se pozornosti posvećivalo opcijama za tretman zuba s blagim oblicima hipomineralizacije te tretmanu inciziva iz estetskih razloga. Predložen je opći protokol (2, 3, 4) u kojemu je istaknuto značenje remineralizacije inicijalnih lezija, desenzibilizacije zuba te prevencije karijesa i posteruptivnog puknuća cakline (PLC) (5).

Prvo istraživanje koje je pokazalo povezanost veličine i lokalizacije demarkiranih opaciteta s težinom kliničke slike da-
the size and location of demarcated opacities (DO) and the severity of MIH in different age groups was conducted in 1987, but the primary focus of the study was to identify etiological factors involved in the onset of the defect (6).

Location of demarcated opacities was analyzed to establish the difference between the progressions of the defect on molars as opposed to its progression on incisors. Demarcated opacities on incisors were mostly located on vestibular surfaces. Low incidence of PEB occurrence in incisors was commonly attributed to limited exposure of incisors to masticatory forces. Demarcated opacities on incisors were thus primarily an esthetic concern for patients (7). There were documented cases, although very rare, of demarcated opacities located on the incisal edge where they were exposed to masticatory forces and thus progressed to PEB occurrence. On the other hand, the DO on molars could be located on surfaces which were not exposed to physical forces of mastication. A recent study that investigated hypomineralised FPM did not confirm that location of DO in the areas exposed to masticatory forces had any influence in aggravation to its breakdown (8).

Apart from its location, two other clinical characteristics of demarcated opacity - its color and size - are also important. Clinical studies documented the direct correlation between the color of demarcated opacities and the porousness and susceptibility of affected enamel to the development and progression of dental caries. Clinicians discovered in practice that darker opacities tended to be more severe which prompted histological studies to establish why that was the case. Yellow and yellow-brown-colored opacities were of full enamel thickness and were more prone to PEB development. With regards to histological evaluation, they were more porous and more susceptible to development and rapid progression of caries. On the other hand, creamy-yellow or white-yellow-colored opacities were inner hypomineralisations covered by a thin layer of fully mineralized enamel (9). It was necessary to study if the likelihood of preventing PEB was lower in cases where demarcated opacities were darker-colored. It was considered that the color of the opacity was an important predictor for the PEB occurrence (8).

MIH studies have not given proper attention to the size of demarcated opacities. The size was mostly used as an exclusion criterion for the opacities of less than 2 mm in diameter, as recommended by the Weerheijm criteria for the evaluation of MIH presence (10). Having assumed that the size of demarcated opacity was a good indicator of the severity of the defect as well as of the duration and severity of influence of the etiological factors, it was important to study this parameter.

In addition to the abovementioned three parameters related to the appearance of the affected tooth, the number of affected teeth, which was varying from one first permanent molar to, although it was very rare, all index teeth (4 permanent molars and 8 permanent incisors) being affected, was also of great clinical significance. It has been documented that the progressed stages of hypomineralisation (PEB occurrence, atypical fillings and extraction due to MIH progression) were more likely found in patients with a greater number of affected teeth, as well as that the likelihood of defects on incisors tira iz 1987. godine, ali primarni fokus toga istraživanja bila je etiološka pozadina nastanka poremećaja (6).

Lokacija demarkiranog opaciteta analizirana je, ali više zbog distinkcija između progresije bolesti na molarima i inci- zivima. Na incizivima se demarkirani opaciteti najčešće nalaze na labijalnoj površini. Smatra se da upravo zbog toga što na tu plohu ne djeluju mastikacijske sile, na njoj se rijetko doga- đa posteruptivno puknuće, ali ta promjena uglavnom smatra pacijentima (7).

Dokumentirano je, iako rijetko, da demarkirani opacitet može biti na incizalnom rubu/plohi, izložen je pritom silama mastikacije i kao takav napreduje u PLC. S druge strane, DO na molarima može biti na plohamo koje nisu izložene fizio- loškim silama mastikacije. U novijim studijama istraživači nisu potvrdili da lociranost DO-a u područjima izvrnutima silama utječe na mogućnost progresije u PLC-u (8).

Osim lokalizacije demarkiranoga opaciteta značajne su još dvije njeoge kliničke karakteristike - boja i veličina.

U kliničkim opservacijama dokumentirano je da je boja demarkiranih opaciteta u izravnoj vezi sa stupnjem poro- znosti cakline i njezine suspektnosti za razvitak i brzinu progresije karijesa.

Na osnovi histoloških studija objašnjenje je ono što je odavno uočeno u kliničkoj praksi – da su tanniji defekti kli- nički zahtjevniji. Pri promjeni žute ili žutosmeđe boje hipo- mineralizacija zahvaća cijelu debljinu cakline, a promjene su sklonjeno posteruptivnom puknuću. Histološki se odlikuju ve- ćom porožnosti cakline i suspektnosti su kad je riječ o nasta- ku i brzini progresije karijesa. S druge strane, promjene u slu- čaju kremastu žutu ili bijeložute boje histološki su uočene kao subpovršinske hipomineralizacije iznad kojih se nalazi tanki, dobro mineralizirani sloj cakline (9). Smatrali smo potreb- nim ispitati umanjuje li tannija boja demarkiranog opacite- ta mogućnost da se preverita posteruptivno puknuće cakline. Smatra se da je boja opaciteta značajan prediktor PLC-a (8).

Veličina demarkiranog opaciteta je u ispitivanjima MIH-a znatno zanemarena, uglavnom je spomenuta kao kriterij za isključivanje zuba iz analize ako je veličina demarkiranog opaciteta manja od 2 mm, što je i preporučeno u Weerheij- jevim dijagnostičkim kriterijima MIH-a (10). Bazirano na pretpostavki da je veličina demarkiranog opaciteta dobar po- kazatelj stupnja poremećaja, te u vezi s dužinom i jakošću djelovanja etiološkog čimbenika, smatrali smo bitnim ispitati i taj parametar.

Uz ta tri parametra koji opisuju izgled zahvaćenog zuba, veliko kliničko značenje ima broj zahvaćenih zuba koji može varirati od jednoga prvog stalnog molara do, što je iznimno rijetko, svih indeksnih zuba (4 prva stalna molar i 8 stalnih inciziva). Dokumentirano je da kod pacijenata s većim brojem zahvaćenih zuba češće uočavamo ozbiljnije defekte (po- steruptivno puknuće, netipični ispuni i ekstrahirani zubi kao posljedica MIH-a), te da mogućnost defekata na incizivima raste s povećanjem broja zahvaćenih molara (11).

Smatra se da, što su etiološki čimbenik i/ili čimbenici ja- či i/ili dulje djeluju, to je broj zahvaćenih zuba veći i teže je stupanj hipomineralizacije. Istraživanja su pokazala da posto- jki korelacija između broja aficiranih zuba i oblika hipomineralizacije te razdoblja djelovanja etiološkog čimbenika. Broj
increased in parallel with the number of affected molars (11). The severity of hypomineralisation was believed to increase with the strength and durability of etiological factor(s) influence. The number of affected teeth depended on the stage of enamel formation (perinatal, perinatal or postnatal) in which the noxious factor occurred — latter occurrence resulted in more affected teeth (12). It was worth studying the correlation between the progression of demarcated opacity of one tooth and the total number of affected teeth of a patient.

Clinical forms of MIH presence were demarcated opacity (DO), post eruptive enamel breakdown (PEB), atypical filling (AF) and extracted tooth due to MIH progression (EX). Recently it was recommended to register atypical caries as an additional category (13). The distribution of clinical forms of MIH (DO; PEB; AF; EX) varied in different population groups. It was very important to establish the prevalence of teeth with DO presence which were the least severe form of MIH occurrence. DO occurrence was registered on 45.8% (Finland), 74.0% (Greece) and 84.5% (Germany) index teeth in studies in which the subjects were older than 9 years (11, 14, 15). A study conducted in Bosnia and Herzegovina in 2004 established that MIH occurrence affected 12.3% of 12-year-olds, and 47% of examine index teeth (light/dark), location (tooth surface exposed/not exposed to masticatory forces), size of demarcated opacity and number of teeth affected. The photographs served to calculate the size of tooth surface affected by demarcated opacities. The MATLAB computer program was used to create an application to analysis of tooth surface affected by demarcated opacities. The MATLAB® computer program was used to create an application to

Material and methods

Primary sample included

446 participants aged from 6 to 9 years were examined in Sarajevo in 2009. The prevalence of MIH presence in this sample was 11.5% (n=51). Only the subjects with MIH presence, where parental consent for further participation in one year period was obtained (n=25), were included in this study.

The research method was a longitudinal, analytical study and the sample was constituted from first permanent molars and incisors with demarcated opacities of the above mentioned study participants, where their baseline examination was performed in 2009. Each tooth was photographed with intra-oral camera (SONY DCC F717 Cyber-shot). The features of DOs for each tooth were registered as follows: color (light/dark), location (tooth surface exposed/not exposed to masticatory forces), size of demarcated opacity and number of teeth affected. The photographs served to calculate the size of tooth surface affected by demarcated opacities. The MATLAB® computer program was used to create an application to

Uzorak i postupci

Primarni uzorak

Primarni uzorak činilo je 446 ispitanika u dobi od 6 do 9 godina, a pregledani su u Sarajevu 2009. godine. Utvrđena je prevalencija MIH-a od 11,5 %, (n = 51). Daljnjim istraživanjem obuhvaćeni su ispitanici s potvrđenom dijagnozom MIH-a čiji su se roditelji složili s njihovim jednogodišnjim sudjelovanjem u studiji (n = 25).

Uzorci longitudinalne analitičke studije bili su prvi stalni moli i stalni incizivi na kojima su uočeni demarkirani opaciteti u navedenoj ispitivanoj skupini. Prvi pregled obavljen je 2009. godine. Zubi su fotografirani intraoralnom kamerom (SONY DCC F717 Cyber-shot).

Za svaki demarkirani opacitet korišteni su sljedeći parametri: boja (svijetla/tamna); lokacija (ne izložen/izložen slična zvakanja), veličina demarkiranog opaciteta i broj zuba zahvaćenih hipomineralizacijama.

Fotografije su korištene za izračun površine zuba zahvaćenih demarkiranim opacitetom. U kompjutorskom programu...

12. Zanimljivo je izpitati vezu progresije demarkiranog opaciteta na jednom zubu s ukupnim brojem zahvaćenih zuba kod istog pacijenta.

Klinički oblici MIH-a su demarkirani opacitet (DO), post eruptivno puknuće cakline (PLC), netipični ispuni (AI) i zubi ekstrahirani zbog progresije (EX). Nedavno je predložena i dodatna kategorija — netipični karijes (13).

Zanimljivo je pratiti distribuciju kliničkih pojavljivih oblika MIH-a (DO; PLC; AI; EX) u raznim istraživanim populacijama.

Registracija zuba s DO om kao najblažim oblikom MIH-a vrlo je važna. Blagi oblik uočen je kod 45,8 % (Finska), 74 % (Grčka) i 84,5 % (Njemačka) indeksnih zuba u populacijama pregledanih ispitanika starijih od 9 godina (11, 14, 15). U studiji iz Bosne i Hercegovine (2004. god) u kojoj je utvrđena prevalencija MIH-a od 12,3 % kod dvanaestogodišnjaka, istaknuto je da je kod ispitanika 47 % indeksnih zuba bilo zahvaćeno promjenama. DO je zabilježen na 25 % indeksnih zuba, PLC na 8,6%, AI na 4,6 % a, 8,8 % indeksnih zuba ekstrahirano je zbog posljedica progresije MIH-a (15). Distribucija pojavljivih oblika hipomineralizacija upućuje na zaključak da od svih zuba s demarkiranim opacitetom, polovina ne prelazi u teže kliničke oblike (PLC, AI, EX), odnosno ne mijenja se do 6 godina poslije nicanja (16).

Namatnulo se pitanje što je to odgovorno za post eruptivno puknuće cakline, odnosno zašto ga na pojedinim zubima nema. U ovom istraživanju nastojalo se odgovoriti i na pitanje u kojoj su mjeri boja, veličina i lokacija DO-a odgovorne progresiji bolesti te istražiti povezanost napredovanja bolesti s ukupnim brojem zahvaćenih zuba kod istog pacijenta.

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calculate the tooth surface affected by demarcated opacities expressed as a percentage of the total tooth surface (vestibular/oral or occlusal surface) (Image 1.). The teeth with decay, filling or developmental defects of other etiology (n=3) were excluded from the study sample.

The follow-up examination was conducted in 2010. Each tooth was photographed and the presence of progression from demarcated opacity to post-eruptive enamel breakdown, an atypical filling or teeth extracted due to MIH were noted.

Further methods of statistical analysis were used: description, inferential analysis, distribution, correlation, and binar logistic regression analysis. The Statistical Package for Social Science, version 15.0 (SPSS Inc., Chicago, IL, SAD) was used for the purpose of these statistical testings.

Results

The research sample consisted of 43 teeth with demarcated opacities in total, with 29 permanent incisors and 14 first permanent molars included. The most prevalent teeth in the sample were upper first permanent incisors (n=22), followed by lower first permanent molars (n=8), upper first permanent molars (n=6), and upper second permanent incisors (n=5). Also, one mandibular first permanent incisor and one second permanent incisor were included.

The number of teeth with light-colored opacities (n=35) was higher than the number of teeth with dark-colored opacities (n=8). Opacities were more numerous on surfaces which were not related to those exposed to masticatory pressure (n=32 compared to n=11). Out of 32 surfaces which were not exposed to masticatory pressure, 5 were located on first permanent molars and 27 on incisors. Demarcated opacities located on surfaces exposed to masticatory pressure were registered on 9 first permanent molars and 2 permanent incisors.

The size of tooth surface affected by demarcated opacities, expressed as percentage of total tooth surface, ranged from 1.33% to 56.56%. The number of affected teeth ranged from two to six. The observed parameters of demarcated opacity and its progression after one-year period are presented in Table 1.

The number of dark opacities was small: only 5, out of which the progression was noted in 3 cases. A smaller number of dark opacities was noted in comparison with light-colored opacities. The progression of dark opacities was noted in only 3 cases.

MATLAB® otvorena je aplikacija za izračunavanje postotka vrijednosti površine zahvaćene demarkiranim opacitetom u odnosu prema ukupnoj površini vestibularne/oralne ali okluzalne/incizalne plohe (slika 1.). Zubi s karijesom, ispunom ili razvojnim defektom druge etiologije (n=3) bili su isključeni.

Završni pregled bio je 2010 godine. Zubi su ponovno fotografirani te su evidentirani progresija demarkiranog opaciteta u posteruptivno puknuće cakline, netipični ispun ili ekstrakcija zuba.

Korištene su metode statističke analize – deskripcija, inferencijalna analiza, ispitivanje normalnosti distribucije podataka, korelaciju i binarna logistička regresijska analiza.

Za obradu podataka i statističku analizu korišten je program SPSS 15.0 (SPSS,Inc. Chicago, II, SAD).

Rezultati

Uzorci su bila 43 zuba s demarkiranim opacitetima – 29 stalnih inciziva i 14 prvih stalnih molara. Najzastupljeniji zubi bili su prvi stalni incizivi u gornjoj čeljusti (n = 22), prvi stalni molar u donjoj čeljusti (n = 8), prvi stalni molar u gornjoj čeljusti (n = 6), zatim drugi stalni incizivi u gornjoj čeljusti (n = 5) te po jedan prvi i drugi stalni inciziv u donjoj čeljusti.

Broj zuba sa svijetlim demarkiranim opacitetima (n = 35) bio je znatno veći negoli s tamnima (n = 8). Brojniji su bili demarkirani opaciteti locirani na glatkim plohamo kojima ne podnose pritisak žvačnih sila negoli oni na površinama izloženim pritisku (32 prema 11). Od 32 površine s DO-om koje nisu bile izložene žvačnom pritisku, 5 je bilo na molarima, a 27 na incizivima. Demarkirani opaciteti na plohamo izvrnutim silama uočeni su na 9 prvih stalnih molara i 2 stalnim incizivima.

U postotku se zahvaćenost zubnih površina demarkiranim opacitetom kretala se od 1.33 % do 56.56 %. Broj zahvaćenih zuba kod promatranih ispitanika kretao se od 1 do 6.

Distribucija zuba prema parametrima demarkanog opaciteta u odnosu prema progresiji nakon jedne godine prikazana je u tablici 1.

Od malog broja zuba s tamnim demarkiranim opacitetom, (n = 5), na njih tri je zabilježena progresija. Veći broj
Number of teeth investigated were those with demarcated opacity located on surfaces which were exposed to masticatory pressure (occlusal/incisal), and the progression was observed only on those teeth.

A binary logistic regression analysis was used for prediction of disease progression from DO to PEB, AF or EX. The progression of demarcated opacity was observed as a dependent variable. Five potential predictive variables were considered: color of demarcated opacity, location of demarcated opacity, size of affected surface, number of MIH-affected teeth and number of MIH-affected teeth.

### Table 1: Distribution of observed parameters in affected teeth in the sample in accordance with progression after one year.

| Initial appearance of demarcated opacities | Progression after one year | Total teeth affected (N) * |
|-------------------------------------------|----------------------------|---------------------------|
|                                           | **Yes** | **No** |                         |
| Light color of demarcated opacity         | 2       | 33     | 35                       |
| Dark color of demarcated opacity          | 5       | 3      | 8                        |
| Number of teeth                           | 7       | 36     | 43                       |
| Location of demarcated opacity            |         |        |                          |
| Vestibular/oral                            | 0       | 32     | 32                       |
| Occlusal/Incisal                          | 7       | 4      | 11                       |
| Number of teeth                           | 7       | 36     | 43                       |
| Size of affected surface                   |         |        |                          |
| 1-5%                                      | 1       | 10     | 11                       |
| 5-10%                                     | 1       | 11     | 12                       |
| 10-15%                                    | 2       | 5      | 7                        |
| 15-20%                                    | 1       | 6      | 7                        |
| >20%                                      | 2       | 4      | 6                        |
| Number of teeth                           | 7       | 36     | 43                       |
| Number of MIH-affected teeth              |         |        |                          |
| 2                                          | 1       | 0      | 1                        |
| 3                                          | 2       | 7      | 9                        |
| 4                                          | 4       | 6      | 10                       |
| 5                                          | 0       | 17     | 17                       |
| 6                                          | 0       | 6      | 6                        |
| Number of teeth                           | 7       | 36     | 43                       |

### Table 2: Correlations for predictive regression model

| Correlations of observed parameters | Disease progression | Light/ dark color of opacity | Oclusal- incisal/ Vestibulo- oral localization of opacity | % of tooth surface affected with opacity |
|-------------------------------------|---------------------|------------------------------|-----------------------------------------------------------|-----------------------------------------|
| Permanent first molar/ incisor      | Pearson correlation | -0.366*                     | -0.305*                                                   | -0.616**                                 |
|                                     | Significance        | 0.016                       | 0.046                                                     | 0.000                                   |
| Light/ dark color of opacity        | Pearson correlation | 0.599**                     | 1                                                         | 0.405**                                 |
|                                     | Significance        | 0.000                       | 0.007                                                     | 0.002                                   |
| Oclusal- incisal/ Vestibulo- oral localization of opacity | Pearson correlation | 0.752**                     | 0.405**                                                   | 1                                       |
|                                     | Significance        | 0.000                       | 0.007                                                     | 0.002                                   |
| Number of teeth affected            | Pearson correlation | -0.420**                    | -0.078                                                    | -0.339*                                 |
|                                     | Significance        | 0.005                       | 0.621                                                     | 0.026                                   |

* *significance at p< 0.05
** significance at p< 0.01

| Variables u modelu binarne regresije | B   | S.E. | Walid  | 95,0% C.I. for EXP(B) |
|--------------------------------------|-----|------|--------|------------------------|
| Dark color of opacity                | -5.104 | 2.007 | 6.469 | 0.000 - 0.310          |
| Number of affected teeth             | -2.159 | 1.065 | 4.111 | 0.014 - 0.931          |
| Constant                             | 10.290 | 5.002 | 4.232 | 29444.279              |
sidered: tooth (first permanent molar or permanent incisor), color of demarcated opacity (dark or light), the location of demarcated opacity (vestibular/oral or occlusal/incisal), the size of demarcated opacity (expressed as a percentage of the total tooth surface) and the number of teeth affected.

To identify the relationship between predictors and dependent variable, a correlation test was used in order to investigate the overall possibility of their use as predictors for post-eruptive enamel breakdown (Table 2).

Moderate to severe correlation within the predictors was noted between independent variables such as: the type of tooth (first permanent molar/permanent incisor), the color of the opacity (light/dark), the location and the number of affected teeth; and one dependent variable such as the progression of disease. The correlation between the progression of the disease and the percentage of tooth surfaces affected, as independent variable, was not found.

An Enter model of Binary Logistic Regression was used for the regression analysis to confirm the adequate use of the model: Omnibus test of model coefficients had a significant value (Chi-square test was 21.967, \(p<0.001\), with 2 degrees of freedom). The Hosmer and Lemesh test showed a non-significant value of \(p>0.05\). The Cox & Snell R Square test and the Nagelkerke R Square test showed that this model had explained 40-67% of variance of the results in the sample.

According to the Enter Logistic Regression model two variables provided an adequate explanation of the prediction of demarcated opacity progression. Those were dark color of DO and a total number of teeth affected in study participants. (Table 2)

The location of demarcated opacity was the independent variable that did not meet criteria. Disease progression appeared only on teeth with DO, and was localized on surfaces exposed to masticatory forces, and therefore this parameter was not analyzed by means of logistic regression.

Discussion

The first study focused on clinical characteristics of demarcated opacities was the study conducted by Koch et al. in 1987 (6). This was also the first study conducted on several age cohorts within the same population, on representative sample of participants. The methodology of this study was based on division of tooth surface in 4 areas. The occlusal area was one, while remaining three areas were incisal, middle and gingival area of vestibular surface. The following two clinical parameters of hypomineralised teeth were observed: color (white, yellow, brown) and appearance of enamel surface (rough, abraded, disintegrated, covered with atypical filling). Teeth were registered as hypomineralised if the changes (color or surface) were present on more than 1/3 of a tooth area.

Histomorphological and biochemical characteristics of hypomineralised enamel were investigated on a sample of 73 hypomineralised teeth (9). It was discovered that dark-colored opacity was a result of hypomineralisation in the entire matrano je pet potencijalnih predikcijskih varijabli: vrsta promatranog zuba (prvi stalni molar ili stalni inziciv), boja demarkiranog opaciteta (svijetla ili tama), lokacija demarkiranog opaciteta (vestibular/oralna ili okluzalna/incizalna), veličina, tj. postotak površine zuba s demarkiranim opacitetom i ukupan broj dipomineraliziranih zuba.

Povezanost prediktora i zavisne varijable analizirala se testiranjem povezanosti kako bi se ispitala mogućnost njihove upotrebe kao prediktora posteruptivnog puknuća cakline (tablica 2.).

Uočena je umjerena do snažna interkorelacija prediktora: prvi stalni molar/stalni inziciv, boja tamna/svijetla, lokacija, broj zahvaćenih zuba i zavisne varijable: progresija bolesti. Nije ustanovljena interkorelacija između progresije bolesti i varijable: postotak zahvaćene površine.

Za regresijsku analizu korišten je model binarne logističke regresije analize sa obilježjima koja objašnjavaju adekvatnost modela: omnibus test (Hi-kvadrat test 21,967, \(p<0.001\), 2 stupnja slobode) Hosmer-Lemeshov test sa nesignifikantnom vrijednošću od \(p>0.05\), a vrijednosti Cox i Snell-R. Squaroa izvornoj temelj zahvaćana broj zubova iz inicijalnog oblika (demarkiranog opaciteta) ili broj zuba boleznih (tamna) i ukupan broj zuba s inicijalnim oblikom MIH-a (demarkirani opacitet) (tablica 2.).

Vrjebila koja je uvrštena u model, ali nije zadovoljila adekvatnost regresijske analize, je lokalacija inicijalne promjene (demarkiranog opaciteta). Kontingencijski nalaz pokazuje da ne postoji ni jedan slučaj progresije bolesti kada je inicijalna promjena (demarkirani opacitet) na vestibularnoj ili oralnoj strani, pa taj parametar nije razmatran u regresijskoj analizi.

Rasprava

Najraniji pokušaj analiziranja kliničkih obilježja demarkiranih opaciteta jest epidemiološka oopservacijska studija Kocha i suradnika iz 1987. godine (6). To je bila prva studija u kojoj je analizirana doba kohorta u istoj populaciji na reprezentativnom uzorku ispitanika. Metodologija toga istraživanja temeljila se na podjeli zuba na jedinice. Okluzalna površina promatrana je kao jedna jedinica, a glatke plohe bile su podijeljene na tri jedinice (iniczina, srednju i gingivalnu). Analizirane su dvije karakteristike – boja zuba (bijela, žuta, smeda) i promjene na površini cakline (hrapava, abrađirana, dezintegritirana, netipični ispun). Zubi se smatralo hipomineraliziranim samo ako je promjena u boji ili strukturi zahvaćala više od jedne trećine zubne jedinice. U studiji je prepoznato značenje površine zuba zahvaćenog promjenom, zatim boje demarkiranog opaciteta, ali distribucija promjena u boji nije prikazana, kao ni distribucija najčešće zahvaćenih površina zuba.

Histomorfološka i biokemijska obilježja hipomineralizirane cakline na uzorku od 73 hipomineralizirana zuba (9) pokazuju da je u slučaju tamnih opaciteta hipomineralizaci-
thickness of the enamel, while light-colored opacity was a result of hypomineralisation in subsurface area of the enamel. In both kind of opacities, dark and light-colored ones, the surface of the enamel was glossy and solid. This discovery explained why dark-colored opacities were more prone to post-eruptive enamel breakdown. The possibility that chalky enamel is not a consequence of previously investigated etiological factors, but rather of a primary causal event during amelogenesis, has been under consideration. A biochemical analysis revealed the presence of extracellular serum albumin in the hypomineralised enamel. Recent findings indicate that blood-derived albumin infiltrates the immature enamel and directly blocks its mineralization. “The molecular timestamping” approach revealed traces of fetal albumin in intact opacities, signifying that localized exposure of the enamel to serum albumin during amelogenesis, rather than systemic injury to ameloblast, constitutes the crux of MIH pathogenesis (17).

In our sample of tooth observed, light-colored opacities were dominant (n=35) over dark-colored ones (n=8). There was a possibility that dark-colored opacities progressed in post-eruptive enamel breakdown during the examination. The color of the hypomineralised enamel, as assessed visually or by laser fluorescence, might be used clinically to reflect the severity of the defect. This seemed to play an important role on the PEB occurrence and should be considered as a potential predictor (8, 18). A strong positive correlation between the MIH progression and dark color of demarcated opacities was confirmed in our investigation.

The size of demarcated opacities was registered differently in available published studies. Some authors from Finland thus divided opacities in 3 categories, based on a size less than 2 mm, 2 to 4 mm and larger than 4 mm (14). The actual measured size of demarcated opacities expressed in millimeters was reported in several studies (19,14). Most of the MIH surveys registered only those opacities that were larger than 2 mm (9, 14, 19, 20), with the recommended usage of graduated periodontal probe (20). European Academy of Pediatric Dentistry (EAPD) later recommended registration of opacities larger than 1 mm (21). In a recently developed MIH index, lesion extension was registered in 3 stages, such as: less than one-third of the tooth affected, at least one-third but less than two-thirds of the tooth affected, and at least two-thirds of the tooth affected (13). In our study, the size of demarcated opacities was expressed as a percentage of tooth surface affected, and it was ranged from 1.33% to 56.56%. A correlation between size of demarcated opacity and MIH progression was not detected in our research.

Demarcated opacities were located mostly on occlusal surfaces, followed by buccal ones, while lingual surfaces were rarely affected (14). The EAPD defined that demarcated opacity could be located on occlusal and buccal surfaces but a need for separate registration according to location was not mentioned (21). In our research, demarcated opacities were registered on all tooth surfaces, and only one was located on the lingual surface of upper permanent first molar.

In our study, the number of teeth with demarcated opacities located on surfaces not exposed to masticatory forces was higher than those on occlusal/incisal ones (32 vs. 11), but ja prisutna u cijeloj debljini cakline, za razliku od svjeđljih u kojima je hipomineralizirano područje subpovršinsko. U oba slučaja hipomineralizirano područje prekriveno je sjajnom i čvrstom površinskom caklinom. Time je objašnjeno zašto su tamniji opaciteti skloniji posteruptivnom puknuću cakline, što je uočeno i u praksi.

Trenutačno se razmatra je li moguće da je razmeškana caklina prouzročena primarnim uzročnim događajima tijekom amelogeneze, te da nije posljedica razmatanih etioloških čimbenika. Biokemijskim analizama otkriven je ekstrecelularni serumski albumin u hipomineraliziranoj caklini. U novijim istraživanjima autori ističu da albumin podrijetlom iz krvi infiltrira nezrelu caklinu i izravno blokira njezinu mineralizaciju. Traagi fetalnog albumina u intaktnim opacitetima, otkriveni tijekom molekularnog mapiranja vremena, upućuju na to da je lokalizirano izlaganje cakline serumskim albuminima tijekom amelogeneze osnova patogeneze MIH-a prije negoli sistemsko oštećenje ameloblasta (17).

U našem uzorku promatran je veći broj zuba s demarkiranim opacitetom svijetle boje (n = 35) u odnosu prema tamnijima (n = 8). To se može tumačiti, i amajući na umu navedena istraživanja, kao mogućnost da su opaciteti tamne boje već progredirali u PLC u trenutku pregleda. Boja hipomineralizirane cakline, procijenjena vizualno ili primjenom laserske fluorescencije, može se koristiti u kliničkoj procjeni težine defekta. Boja DO-a važna je u nastanku PLC-a i treba se uzeti u obzir kao potencijalni prediktor progresije (8, 18). U našem istraživanju utvrđena je korelacija tamne boje demarkiranog opaciteta i progresije bolesti.

Veličina demarkiranog opaciteta u dostupnim istraživanjima obrađena je na različite načine. U studiji finskih istraživača svrstan je u tri kategorije (manji od 2 mm, od 2 do 4 mm i veći od 4 mm) (14). Stvarna veličina demarkiranih opaciteta dosad je zabilježena u nekoliko studija, a na osnovi veličine izražene u milimetrima (19, 14).

Kao kriterij za demarkirane opacitete u većini istraživanja navodi se da se promjene uočavaju ako su veće od 2 mm (9, 14, 19, 20), uz preporuku da se upotrebljava millimeterski gradirana parodontna sonda (20). Europska akademija za pedijatrijsku stomatologiju (EAPD) preporučuje da se demarkirani opaciteti bilježe ako su veći od 1 mm (21).

U nedavno predloženom indeksu MIH-a, veličina promjene sadržava 3 kategorije – zahvaćeno je manje od jedne trećine zuba, zahvaćeno je više od jedne trećine, ali manje od dvije trećine zuba i treća je kategorija kada su zahvaćene minimalno dvije trećine zuba (13).

U našem istraživanju zabilježena je veličina promjene na temelju vrijednosti postotne zahvaćenosti zubne površine hipomineralizacijom. Raspon veličina demarkiranih opaciteta na ispitivanim zubima bio je velik – od 1,33 % do 56,56 %. Korelacija između postotnih vrijednosti demarkiranih opaciteta i progresije bolesti nije ustanovljena.

Demarkirani opaciteti najčešće se uočavaju na okluzalnim i bukalnim površinama zuba, a rijetko na lingvalnim (14). Europska akademija za pedijatrijsku stomatologiju (EAPD) navodi da se demarkirani opaciteti nalaze na okluzalnim i bukalnim površinama zuba, bez napomene li se kao takvi i registriraju (21). U našem istraživanju evidentira-
2/3 of our sample were incisors. The post-eruptive enamel breakdown was rarely seen on incisors (1, 10). Examination in earlier age of patients should enable registration of hypomineralisations in initial stage (demarcated opacity). However, in some of our study participants, the post-eruptive enamel breakdown was registered on first permanent molars that were not fully erupted. It seemed that in our sample population the PEB presence could happen even before a tooth was exposed to masticatory forces. A possible explanation for that was that the post-eruptive maturation of the permanent tooth enamel was compromised due to inadequate oral hygiene and high caries rates in primary dentitions of our examinees. The absence of well mineralized surface above the hypomineralisation area was making tooth less resistant. The post eruptive breakdown (PEB) in our sample (n=7) was registered after one year, mainly on first permanent molars with an exception of one permanent incisor in which demarcated opacity was located on the incisal edge. The location of demarcated opacity on occlusal/incisal surfaces was proven to be a reliable independent predictive factor.

The importance of the number of teeth affected by hypomineralisation and the severity of molar incisor hypomineralisation were well documented (22-24). A chance of permanent incisor affection by hypomineralisation increased when larger numbers of first permanent molars were affected (24, 25). 70% of children with progressive stages of hypomineralisation on first permanent molars had demarcated opacities on permanent incisors (22, 23). The severity of MIH presence was associated with the number of teeth with MIH existence, meaning that participants with opacities only had a lower mean number of teeth involved compared to those with severe lesions (i.e. PEB, AF, and EX) cases of MIH progression (13). Our research findings confirmed a negative correlation between MIH progression and the number of affected teeth. Progression was noted only on the teeth of subjects that had less than four teeth affected. This could not be interpreted in a way that smaller numbers of teeth affected meant a higher risk of progression, since it was opposite to findings in other research. In our sample, first permanent molars with demarcated opacities, which were followed for one year, were mostly present in subjects with post-eruptive enamel breakdown or atypical filling on remaining first permanent molars. Also, permanent incisors with demarcated opacities observed were present in subjects with first permanent molars on which progressive stages were already present.

The location of demarcated opacities was not analyzed in regression model since all demarcated opacities that progressed after one year were located on surfaces exposed to masticatory forces. The abovementioned stressed the importance of location together with a high correlation of DO progression and DO location on occlusal/ incisal surfaces, which was an important finding in our research.

The size of tooth surface affected by demarcated opacities (expressed as a percentage) did not enter in final prediction model, which could be due to small number of teeth observed.

Limitations of our study were that age of observed population (from 6 to 9 years) did not allow registration of initial
stage on index teeth. Age of 8 years was recommended as optimal one for MIH examination (26). There are studies that included younger population but with goal to achieve a necessary sample size in cross sectional study (27). But, in populations with medium and high caries rate in primary dentition, such as the one studied in this research, registration should be performed even earlier. Thus, in such populations, monitoring of first permanent molars should be performed even during eruption.

The presence of Hypomineralised Second Primary Molars (HSPM) is also frequently investigated and considered as a predictive factor for MIH (13, 26). A new MIH/HSPM index was introduced recently in order to grade the clinical status, amount of tooth surface area affected, and other enamel defects comparable to MIH presence (13). Atypical caries was added as criterion of clinical status registration and lesion extension criteria was also registered (3 stages: less than one-third of the tooth affected; at least one-third but less than two-thirds of the tooth affected; at least two-thirds of the tooth affected) (13). The registration of location of demarcated opacities was not included in this index.

After conduction of our research a new index, the MIH Treatment Need Index (MIH-TNI) was introduced. It was designed to assess and plan treatment needs, providing information about the severity of MIH in populations and individuals. The index is based on essential parameters of demarcated opacity and symptoms which are clinically considered to be the most important ones with respect to MIH: hypersensitivity and PEB (28). A significant hypersensitivity of MIH teeth can cause discomfort and pain during oral hygiene performance. Additionally, it can affect eating habits (9, 10). Dental fear and behavior management problems in children with severe enamel hypomineralisation are also reported (29). In previous studies of MIH conducted in Bosnia and Herzegovina, no hypersensitivity of teeth affected was reported (16, 30). In a recent study, which was conducted to test different protocols for reducing sensitivity of hypomineralised teeth, the tactile and an air blast examination was used to register hypersensitivity score (31). If the presence of sensitivity is to be routinely registered in MIH studies, a clear methodology of such registration should be agreed.

**Conclusions**

Monitoring of first permanent molars during eruption is needed in populations with medium and high rates of caries in their primary dentition. Screensings for MIH in our population should be performed at age 5-6, along with a subsequent examination of Second Primary Molars. Dark color and localization of demarcated opacity should be determined as significant factors, which could have an impact in predicting demarcated opacity progression within molar incisor hypomineralisation. Further research into the occurrence of demarcated opacities and their role in predicting disease progress is recommended.
Conflict of interest
None declared

Compliance with ethical standards
Ethical approval: Research is conducted in accordance with the ethical standards and 1964 Helsinki declaration and was approved by Faculty of Dentistry, University of Sarajevo (Scientific and Educational Committee, 09-263-1/2010).
Informed consent: The informed consent was obtained from children’s parents.

Author’s contributions
A.A. - Conception and design; A.A., N.M. and L.B.R. - Acquisition, analysis and interpretation; A.A., E.B. - Drafting the article; A.A., N.M., E.B. - Revising it critically for important intellectual content; A.A., N.M., E.B., L.B.R. - Approved final version of the manuscript.

Sukob interesa
Autor nisu bili u sukобu interesa.

Etički standard
Istraživanje je provedeno u skladu sa etičkim standardima i Helsinkom deklaracijom, odobreno od Stomatološkog fakulteta, Sveučilišta u Sarajevu (nastavno znanstveno vijeće 09-263-1/2010).

Doprinos autora
A. A. – koncept i dizajn; A. A. M. i L. B. R. – prikupljanje, analiza i interpretacija podataka; A. A., E. B. – nacrt rukopisa; A. A., N. M. i E. B. – ključne preinake u dopuni intelektualnog sadržaja; A. A., N. M., E. B. i L. B. R. – odbranje završne verzije rukopisa.
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