Research Article

Assessment of the salivary level of glutathione and the feeding pattern in molar incisor hypomineralization among 7-9 years of age: an analytical cross-sectional study

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Abstract: Background: The study aimed to assess salivary glutathione levels and the pattern of feeding on molar incisor hypomineralization among healthy children aged 7-9 years. Materials and Methods: The study was a cross-sectional study. A total of 90 children sample was further subclassified into two groups: the study group, including 60 children with molar incisor hypomineralization, classified into two subgroups, 30 children with mild type and 30 children with severe type of molar incisor hypomineralization. In addition to the control group involving 30 children without incisor hypomineralization and free of caries. Examination and diagnostic criteria for hypomineralization of the molar incisor and assessment of severity were according to European Academy of Pediatric Dentistry (EAPD) guidelines in 2003. The salivary glutathione ELISA kit was used to assess the amount of glutathione in unstimulated saliva samples. After an oral examination, the parents of each child completed a self-administered questionnaire that included information on the child's feeding pattern during the first year of life. The questionnaires were returned to the researcher the following day. The child who received a mixed feeding pattern was excluded from the study. Results: The ordinal logistic regression model (Overall model test: $p < 0.001$) presented that salivary glutathione ($p < 0.001$), saliva pH ($p = 0.014$) and saliva flow rate ($p = 0.009$) were significant predictors of hypomineralization of the molar incisor. The feeding pattern ($p = 0.625$) and gender ($p = 0.394$) were not significant predicators for hypomineralization of the molar incisor hypomineralization. Conclusion: In children aged 7 to 9 years, salivary glutathione level is a negative predicator for the degree of hypomineralization of the molar incisor. Moreover, child feeding pattern, and gender, are not significant predicators of hypomineralization of the incisor hypomineralization.

Keywords: Hypomineralization, molar incisor, feeding pattern, breastfeeding, salivary glutathione, saliva pH, saliva flow rate.

Introduction

A qualitative developmental abnormality of enamel that affects one or more permanent first molars (PFM) in children and adolescents, whether or not the incisors are involved, is known as hypomineralization of the molar incisor (MIH). It is also called hypomineralized permanent first molars, idiopathic enamel hypomineralization, non-fluoride hypomineralization, and dysmineralized PFM [1]. MIH etiology is thought to be complex. Several risk factors have been proposed in recent systematic reviews of the literature focused on MIH. These include maternal illness and psychological stress during pregnancy, perinatal factors such as hypoxia, difficulties in delivery (like caesarean section) and prematurity, postnatal factors like diseases in early childhood diseases or fever and antibiotic use, and general exposure to environmental toxins and/or epigenetic factors [2,3,4]. The clinical manifestation of MIH can vary according to its severity. It may manifest as yellow-brown opacities, white-cream opacities, or post-eruptive enamel breakdown leading to atypical caries or restorations in one or more FPMs to be classified as MIH, it must be more than 1 mm [5].

A significant intracellular antioxidant is glutathione (GSH), which. Reduced GSH levels in saliva associated with several implications for oral health implications such as reduced neutrophil chemotaxis in periodontitis [6]. oral lichen planus [7], mental disorder [8], dental caries [9], and others [10].

The feeding pattern and duration of feeding can affect oral health. It has been shown that there is a strong relationship between duration of breastfeeding and MIH [11]. On the contrary, another study showed among exclusively breastfed children that no significant relationship was found between the existence of MIH and breastfeeding during the first 4 months of the life cycle and earlier [12]. In addition, feeding pattern has been reported to have not a significant effect on primary tooth eruption pattern [13]. The aim of this study is to evaluate the level of salivary glutathione and the pattern of feeding in incisor hypomineralization among healthy children aged 7-9.

Materials and Methods

On 28 December 2022, the ethical committee of the University of Baghdad's College of Dentistry accepted the study protocol (number 72032). The cross-sectional study carried out in Al kut city in Iraq during the period from the beginning of November 2022 until the middle of March 2023. Within this time, the schoolchildren were examined at school. Child's informed consent was obtained from their parents before examination. Dental examinations were performed using a disposble mouth mirror and a dental probe. For MIH, the teeth were examined and recorded on a specially designed patient research data sheet. The four permanent first molars and eight permanent incisors were examined for demarcated opacities and atypical restorations under a sun light source the unstimulated salivary samples were collected, and the salivary samples were analysed for special biochemical laboratory work in a laboratory.

The total sample consisted of 90, 7–9 years old children, divided into two groups: the study group, which included 60 children with MIH, classified into two subgroups: 30 children with mild MIH and 30 children with severe MIH. In addition to the control group that involved 30 children without MIH and free of caries. Before participating, each individual in the study and control groups gave their informed consent.

Inclusion criteria include: 7-9 year old healthy children living in Alkut city, having at least one first permanent molar erupted. Exclusion criteria include: Children with systemic diseases such as diabetes and cardiovascular disease, long-term prescription drugs,hypomineralized molar as a result of other health issues such as imperfect amelogenesis, enamel hypoplasia, fluorosis, opaque white spot, cavities or restorations in study teeth,children who did not return the questionnaire or who did not complete it and children received a mixed feeding pattern. For the control group, using the same inclusion and exclusion criteria, children of the same age range who are caries-free and do not have MIH.

Before clinical assessment, the participating children were asked to thoroughly wash their teeth with tap water. Subsequently, the child was placed on a desk or a table in the school.Then the children's teeth were examined. Sterilised cotton was used to dry the teeth and was examined under sun light. The diagnostic criteria for MIH were according to the guidelines of the European Academy of Paediatric Dentistry (EAPD) in 2003.

The unstimulated salivary sample was collected by drooling in the test tube (Shenzhen Mande Lab, China) under constant circumstances [14]. It was carried out according to the instructions constructed using the drooling method (Navazash and Kumer) [15]. Then, PH was measured by pH metre, and flow rate calcaulated in(ml/min)by dividing the total volume of saliva collected in millimeters by the time of collection in minutes. Saliva was centrifuged in the laboratory for 10 minutes at 3,000 rpm after the salivary flow rate and ph were calculated. The supernatant was then stored in a deep freezer at -20 ° C until analysis was done.

The salivary glutathione kit used the enzyme-linked immunosorbent assay (ELISA)technique to measure the amount of GSH in saliva which is based on the double antibody sandwich method, to measure human GSH..(YL Biont, Shanghai, China).

Each child received a self-administered questionnaire introduced in Arabic to obtain information on the age of the children, their sex, their medical and surgical history before entering the school, and their type of food in the first year of birth. The next day, the parents of the child returned the completed questionnaire to the researcher.

Statical analysis

Data were analysed via linear model ANOVA, Pearson's Chi-square test, and ordinal logistic regression model using R software version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 515 students were examined, 415 students lack inclusion criteria, 10 of parents of students refused to participate their children in the study, and 90 students included in the study. Descriptive data related to salivary glutathione level, saliva pH, saliva flow rate, feeding pattern, gender, and age are shown in figure and table 1. The ordinal logistic regression model (Overall model test: $p < 0.001$) showed salivary glutathione level ($p < 0.001$), saliva pH ($p = 0.014$) and saliva flow rate ($p = 0.009$) were significant predictors of hypomineralization of the molar incisor (Table 2). The feeding pattern ($p = 0.625$) and gender ($p = 0.394$) were not significant predicators of hypomineralization of the incisor molar (Table 2).

Table 1: Cross table for Dependent Variable, Molar incisor hypomineralization

	Free (N=30)	Mild (N=30)	Sever (N=30)	Total (N=90)	p value
Age					0.614[1]
Mean (SD)	8.1 (0.9)	8.1 (0.8)	8.3 (0.7)	8.2 (0.8)	
Range	7.0 - 9.0	7.0 - 9.0	7.0 - 9.0	7.0 - 9.0	
Salivary glutathione level					< 0.001[1]
Mean (SD)	8.2 (2.3)	6.1 (1.6)	4.1 (1.8)	6.2 (2.5)	
Range	3.1 - 15.0	3.0 - 10.4	0.3 - 8.4	0.3 - 15.0	
Saliva flow rate					< 0.001[1]
Mean (SD)	0.5 (0.2)	0.4 (0.1)	0.3 (0.2)	0.4 (0.2)	
Range	0.1 - 0.9	0.1 - 0.6	0.1 - 0.9	0.1 - 0.9	
Feeding pattern					0.875[2]
bottle	15.0 (50.0%)	17.0 (56.7%)	16.0 (53.3%)	48.0 (53.3%)	
natural	15.0 (50.0%)	13.0 (43.3%)	14.0 (46.7%)	42.0 (46.7%)	
Gender					0.875[2]
female	15.0 (50.0%)	16.0 (53.3%)	17.0 (56.7%)	48.0 (53.3%)	
male	15.0 (50.0%)	14.0 (46.7%)	13.0 (43.3%)	42.0 (46.7%)	

Linear Model ANOVA

Pearson's Chi-square test

Table 2: Outcomes of Ordinal Logistic Regression Model Assessing Predicators Related to the Molar Incisor Hypomineralization.

				95% Confidence Interval	
Predictor	**Estimate**	**P**	**Odds ratio**	**Lower**	**Upper**
Salivary glutathione	-0.861	< .001	0.42294	0.301	0.563
Saliva PH	-0.842	0.014	0.43104	0.213	0.823
Saliva flow rate	-4.607	0.009	0.00998	2.49E-04	0.284
Feeding pattern: natural – bottle	0.242	0.625	1.27352	0.485	3.422
Gender: male – female	-0.427	0.394	0.65218	0.239	1.73

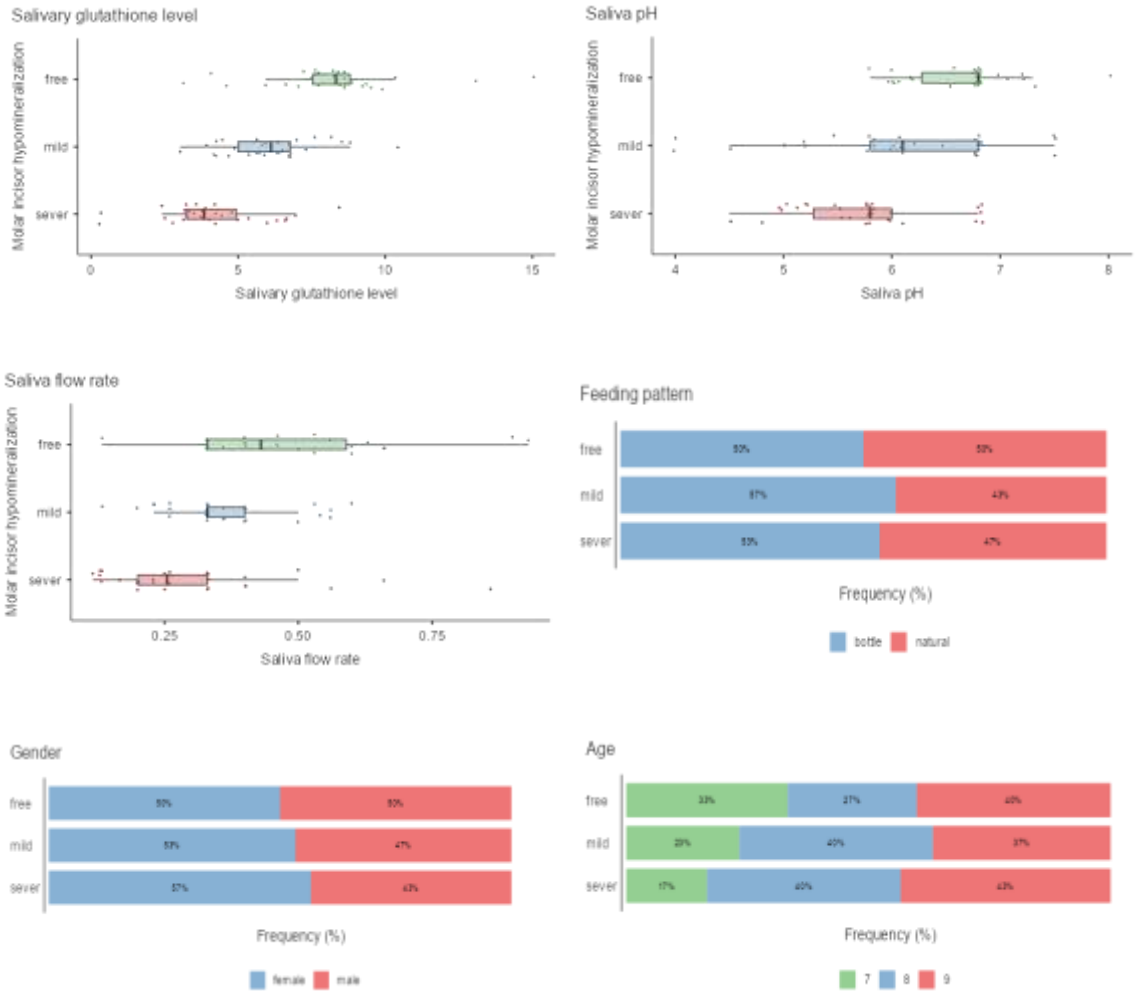

Figure 1: The box and the whisker plots and stacked bar plots showed descriptive data of the study variables.

Discussion

Hypomineralization of the molar incisor is characterized by enamel abnormalities that begin in the developmental stage of the process and may affect one to four permanent molars and permanent incisors [2,16]. Although the exact causes of MIH are still unknown, the majority of research indicates that prenatal such as maternal smoking, illness, perinatal such as prematurity, birth complications, and postnatal factors such as pneumonia, asthma, and Fever as well as environmental and genetic variables

are associated with the disorder. Other concerns mentioned include the prolonged use of antibiotics and infections of the respiratory system in the first year of life [17,18].

The results of this study showed feeding pattern was not a significant predicator for MIH. Fatturi *et al.* conducted a comprehensive review and meta-analysis that included five publications. The results of the meta-analysis indicated that breastfeeding was not associated with greater risks of MIH (OR = 1.15; 95% CI 0.95-1.40) [19].

The study findings indicate a significant negative relationship between the severity of MIH and salivary glutathione levels ($p < 0.05$). This can be explained by oxidative stress, which is defined as an imbalance between the amount of reactive oxygen species (ROS) produced and the body's capacity to detoxify ROS. ROS can damage the cellular component, including ameloblasts [20]. Furthermore, the condition's associated oxidative stress of the condition may have an impact on glutathione metabolism or the body's antioxidant capacity. This may result in changes in the amounts of glutathione in saliva and other tissues.

A literature search showed that several studies have studied the connection between glutathione levels and tooth caries, but to our knowledge no prior research has examined the relationship between salivary glutathione levels and MIH. Salivary GSH levels were shown to be significantly lower in caries-affected individuals than in non-carious individuals (Oztürk LK *et al*.) and the number of decayed, missing, and filled permanent teeth (DMFT)score was strongly correlated with GSH. This may be explained by the protective effect of salivary GSH against the development of dental caries. Research indicates that the inclusion of a sulfhydryl group (-SH) in the structure of glutathione, which helps neutralize free radicals, is a defense mechanism against increased oxidative stress, and the severity of dental caries increased in tandem with salivary levels of salivary glutathione [8].

This study found that girls were more likely than boys to have MIH, but there was no statistically significant relationship between gender and MIH ($p > 0.05$). This finding agreed with that of Allazzam et al., showed the predominance of the male gender with no significant differences [21]. Ofi and Salih reported that MIH was more prevalent among girls. However, the difference was not statistically significant [22]. Salih and Khalaf stated that MIH was not a gender-dependent phenomenon [23]. Furthermore, Hali et al. and Ismail et al. reported no significant difference between sexes in the incidence of MIH [24,25].

It is well known that saliva pH and flow rate and oral heath are closely related, and a change in one can affect others. This study showed that saliva pH and flow rate were significant predictors of MIH, which may be due to factors such as reduced saliva buffering capacity or increased acid production in the oral cavity. Saliva plays a crucial role in maintaining a neutral pH balance in the mouth, which helps protect teeth from acid attacks and aids in remineralisation. Any disruption in saliva composition or function can potentially affect the mineralisation process and contribute to the development of dental enamel defects. Similarly, the decrease in saliva flow observed in some individuals with region of the molar hypomineralization could be the result of underlying salivary gland dysfunction or reduced saliva production. The same result was also reported by Samuel et al. [26] while disagree with other study[27] which found salivary pH was lower in study group, but no significant difference was found between the two groups To our knowledge, the effect of saliva flow rate and saliva pH on MIH is rarely assessed in the literature.

Some limitations were noted in this study, as the information on the type of feeding in the first year of life was based on memories of mothers. Furthermore, MIH is multifactorial condition, genetic predisposition, and epigenetic influences are also likely to play a role in the putative multifactorial etiology of the condition.

Conclusion

In conclusion, according to the study findings, among 7-9 years old children, salivary glutathione level is a negative predicator for the degree of MIH. Moreover, child's feeding pattern and gender, are not significant predicators for MIH. However, in view of the inherent limitations of cross-sectional studies, more longitudinal clinical research is required to establish the cause-and-effect relationship between salivary glutathione levels and MIH severity.

Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

AHS and AAQ; study conception and design. AHS; data collection. AHS and AAQ; Methodology. JK and AHS; statistical analysis and interpretation of results. AHS; original draft manuscript preparation. AAQ and JK; Writing, review & editing. Supervision; AAQ. After reviewing the findings, each author gave their approval for the manuscript's final publication.

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تقييم مستوى الجلوتاثيون في اللعاب ونوع الرضاعة على نقص تمعدن الاضراس والقواطع الامامية بين الأطفال الأصحاء الذين تتراوح أعمارهم بين 7-9 سنوات دراسة تحليلية

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المستخلص:

الخلفية الدراسة تهدف إلى تقييم مستوى الجلوتاثيون في اللعاب ونوع الرضاعة على نقص تمعدن الاضراس والقواطع الامامية بين الأطفال الأصحاء الذين تتراوح أعمارهم بين 7-9 سنوات. المواد والطرق: كانت الدراسة عبارة عن دراسة مقطعية. تم تصنيف عينة مكونة من 90 طفلاً إلى مجموعتين: مجموعة الدراسة، بما في ذلك 60 طفلاً يعانون من نقص تمعدن القاطعة الرحى، وتم تصنيفهم إلى مجموعتين فرعيتين، 30 طفلاً من النوع الخفيف و30 طفلاً من النوع الشديد من نقص تمعدن القاطعة الرحى. بالإضافة إلى المجموعة الضابطة التي تضم 30 طفلاً لا يعانون من نقص تمعدن الاضراس والقواطع الامامية و لا يعانون من تسوس الاسنان ، كان النطاق العمري 7-9 سنوات. معايير الفحص والتشخيص لنقص تمعدن الاضراس والقواطع وتقييم خطورتها كانت وفقاً للقواعد الارشادية للأكاديمية الأوروبية لطب أسنان الأطفال في 2003. تم جمع عينات من اللعاب غير المحفزة لقياس مستوى الجلوتاثيون اللعابي بواسطة مجموعة الجلوتاثيون اللعابية .تلقى كل طفل استبيانًا ذاتيًا، يتضمن الرضاعة في السنة الأولى من العمر، وتم ملؤه بالكامل من قبل والدي الطفل، وتم إعادته إلى الباحث في اليوم التالي بعد الفحص الشفهي. (الطفل الذي تلقى رضاعة مختلطة تم استبعاده من الاختبار). النتائج :اظهر نموذج الانحدار اللوجستي الترتيبي (اختبار النموذج الشامل: وجود فرق ذات دلالات احصائية في مستوى الجلوتاثيون اللعابي ودرجة الحموضة في اللعاب ومعدل تدفق اللعاب وكانت من العوامل الهامة لنقص تمعدن الاضراس والقواطع الامامية, اما نوع الرضاعة، والجنس لم تكن مؤشرات هامة لنقص التمعدن في الاضراس والقواطع. الاستنتاج بين الأطفال الذين تتراوح أعمارهم بين 7-9 سنوات، يعتبر مستوى الجلوتاثيون اللعابي مؤشرا سلبيا لدرجة لنقص تمعدن الاضراس والقواطع الامامية علاوة على ذلك، فإن نوع رضاعة الطفل والجنس ليسا مؤشرين مهمين لنقص تمعدن الاضراس والقواطع