

## ORIGINAL RESEARCH

# Assessment of severity and mineral composition of saliva in schoolchildren with molar-incisor hypomineralization (MIH)

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**Abstract**

The aim of the study was to evaluate the severity of molar incisor hypomineralisation (MIH), related oral health and investigate salivary mineral composition. The study was conducted with 50 participants aged between 6–15 years who were effected with MIH and 50 without MIH. The International Caries Detection and Assessment System (ICDAS) scores, Decayed, Missing, Filled Teeth/Surface (DMFT/S), dft/s and gingival/plaque indices were evaluated. The pH, flow rate, buffering capacity and mineral composition of saliva was measured. “Student *t*” test, one-way analysis of variance in repeated measurements of groups, and Tukey multiplex in subgroup comparisons was used. Kruskal-Wallis, Mann-Whitney U, Wilcoxon and chi-square tests were used to analyze qualitative data and compare groups. A total of 100 children (57 females 43 males, mean age  $10.12 \pm 1.85$ ) participated in the study. There was no difference between ICDAS, DMFT/S scores, but dft/s index values were statistically significant ( $p = 0.001$ ). The simplified oral hygiene index of MIH patients were statistically higher, but no significant differences were found in modified gingival indices ( $p = 0.52$ ). Although the salivary pH and flow rate of the patients in the study group were lower, the buffering capacity was higher than those in the control group, but no significant difference was observed ( $p = 0.64$ ). The mean values of phosphorus, carbon and calcium content in the saliva samples of MIH patients were higher than those of patients without MIH, and this difference was low for phosphorus ( $p = 0.76$ ) and carbon ( $p = 0.74$ ), but significantly higher for calcium. To the best of our knowledge, this is the first study to evaluate the association between calcium, phosphate and carbon levels in saliva of children with MIH. The significantly high amount of calcium in the saliva of patients with MIH suggests that further investigations are needed.

**Keywords**

Molar-incisor hypomineralisation; Saliva; Calcium; Phosphate; Carbon

## 1. Introduction

Molar incisor hypomineralization (MIH) is a clinical manifestation of systemic enamel hypomineralization that often affects the permanent incisors along with one or more first permanent molars [1]. The color and size of the defects can vary widely, ranging from white, creamy or yellow to brown [2]. Although the etiology of MIH is not precisely known, many different causes have been suggested [3]. Mineralization of first permanent molars and incisors begins in the last trimester of pregnancy and is completed in the first four years of life. The etiology of MIH is associated with complications that occur during the mineralization period [4]. Despite the association of several diseases of prenatal, perinatal or postnatal periods, the causes of MIH are yet to definitively determined.

The prevalence of MIH is estimated to be around 13% worldwide and varies from country to country. The prevalence

of MIH is highest in South America and Oceania, with rates of 18% and 16.3%, respectively. The prevalence is lower in Europe, Asia and Africa, with rates of 14.3%, 13% and 10.9%, respectively [5]. In Western Australia, the prevalence of demarcated opacities was 22%. The lowest prevalence of MIH in the literature was found in Hong Kong (2.8%). The highest reported prevalence of MIH was among Brazilian children (40.2%) [6].

Therefore, hypomineralized enamel has more porous structure than healthy enamel. This makes it more susceptible to tooth substance loss and plaque accumulation. Plaque accumulation is additionally enhanced by the sensitivity of teeth. As a result, patients with MIH often experience tooth decay and sensitivity, as well as challenges with dental treatment [7].

The mechanical, structural and chemical properties of MIH-affected enamel have been described in the literature [8–13]. Another study investigated the protein composition of saliva

in patients with MIH [14]. A study has found that the protein composition of saliva in patients with MIH may exhibit characteristic changes that could be associated with the clinical symptoms of the disease. In a previous study [15], buffering capacity, sialometry, calcium and phosphate concentration in all stimulated saliva and gingival crevicular fluid were compared with the caries severity and activity of school-aged children. A significant relationship was found, and a correlation between dental caries, buffering capacity and buccal calcium and phosphate was found [15].

Another study showed that mineral element concentrations in saliva may be a useful tool for assessing periodontal health and disease [16].

A study was conducted to investigate the mineral composition of mixed saliva from patients with dental fluorosis. The study found that the mineral composition of saliva changed depending on the level of enamel demineralization in the groups treated with two different dental fluorosis treatments [17]. Another study estimated salivary sodium, potassium, calcium, phosphorus and urea levels patients with type II diabetes. The findings revealed that there were discernible changes in salivary composition even among patients with well-controlled diabetes when compared with healthy controls [18].

The levels of calcium and phosphate in saliva are regulated by a number of factors, including genetic factors. Genes that regulate enamel formation may be associated with changes in the levels of calcium and phosphorus in saliva. Calcium and phosphorous concentrations in saliva and plaque play a key role in modulating the tooth demineralization and remineralization dynamics. According to a previous study, genetic variations in the genes Amelogenin (AMELX), Ameloblastin (AMNB) and Estrogen-related receptor beta (ESRRB) were associated with calcium levels in saliva. A weak association was observed between Enamelin (ENAM) allele distribution and phosphate levels in saliva. This study provides the first evidence that genetic variations can influence calcium and phosphorus levels in saliva [19]. A recent study found that the variant allele Kallikrein (KLK4) rs2235091 was associated with MIH [20].

The aim of this study was to determine the severity of MIH in children and adolescents diagnosed with MIH and to evaluate the salivary flow rate, pH and buffering capacity in these patients. The study also measured the amount of minerals in the saliva, evaluated the dental caries and periodontal status of the patients, and compared the results with the control group.

## 2. Material and methods

### 2.1 Study design and population

The sample size was determined based on the power analyses (The minimum sample size required to be included in the study with the lowest effect size ( $d = 3.31$ ), a 0.05 error rate, and a power of 0.95 was calculated as a total of 100 ( $n_1$  and  $n_2 = 50$ ) for the Independent Samples  $t$  test). The study group consisted of 50 children with MIH and the control group consisted of 50 children without MIH in their teeth. Both groups were applied Akdeniz University Faculty of Dentistry, Department of Pedodontics. A total of 100 children (57 girls and 43 boys)

between the ages of 8 and 15 were included in this study. All clinical examinations were performed by a single investigator.

The European Academy of Paediatric Dentistry (EAPD) criteria were used to diagnose MIH in the MIH group. These criteria included the presence of demarcated opacities, post-eruptive enamel breakdown, atypical restorations and extraction at least one first permanent molar due to MIH. The patients were also asked whether they had sensitivity in their teeth.

Inclusion criteria:

- children and adolescents aged 8–15 years,
- at least one molar with MIH,
- patients of the same age without MIH in their teeth for the control group.

Exclusion criteria:

- systemic diseases,
- long-term medication,
- remineralization therapy (such as fluoride, Casein Phosphopeptide-Amorphous Calcium Phosphate CPP-ACP, resin infiltration system (ICON) and similar) in the last 6 months.

All children and their families were informed verbally and in writing about the study. After the parents signed the informed consent form, clinical procedures were performed. The purpose of the study, the procedures to be applied, the possible benefits and risks were explained to the patients and their parents.

A survey form was completed asking for demographic data from patients. This form also asked whether the mother had a feverish illness during pregnancy, and whether the child had used antibiotics up to the age of 4. In addition, the form asked about the patients' birth time (normal/premature), birth type (normal/cesarean), and birth weight (normal/low). This form also asked whether any other family members had teeth with MIH.

### 2.2 Saliva collection

Saliva samples were collected between 9–12 AM, two hours after breakfast. The subjects were asked to chew the paraffin tablet for 30 seconds and swallow their first saliva. Then, for five minutes, the participants chewed paraffin tablets and the saliva was collected into sterile millimeter-graduated tubes. The pH of resting saliva, flow rate and buffering capacity of stimulated saliva were determined using Saliva-Check Buffer kit (GC, Alsip, Illinois, America).

Mineral analyzes were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Stable Isotope Ratio Mass Spectrometer (IRMS).

### 2.3 Oral health status

After the questionnaire was administered to the schoolchildren, an oral examination was performed using a light source, plane mirror (#5) and World Health Organization (WHO)-type probe to evaluate the oral health status of the participants. The International Caries Dental Assessment System II (ICDAS) was used to evaluate caries in both dentitions [21]. Caries experience was evaluated using the DMFT epidemiological index (decayed, missing and filled permanent teeth) and dft (decayed and filled) (WHO, 1997), which allow the evaluation

of the amount of teeth with caries in permanent and mixed dentition [22]. The simplified oral hygiene index [23] and modified gingival index [24] parameters were also calculated. Malocclusion was also evaluated in patients during the clinical examination.

DIAGNOdent Pen (Kavo, Biberach, Germany) device was also used to detect teeth with MIH. To prevent false positive results, the plaques on the teeth were removed by polishing and the teeth were air-dried for 5 seconds. The sapphire tip of the device was placed perpendicular to the hypomineralized surface, then the tip of the instrument was rotated around its own axis. Measurements were repeated from 3 different places on the hypomineralized surface, and the highest recorded value was the laser fluorescence value.

## 2.4 Statistical analysis

The obtained data are entered into the SPSS package program (SPSS 18.00 Statistical Package for the Social Sciences, IBM, Armonk, NY, USA), descriptive statistics (minimum, maximum, mean, standard deviation, *etc.*), correlation analysis and comparison tests were carried out. In the comparison of quantitative (quantitative) data, in the case of providing parametric conditions (“Kolmogorov-Smirnov Test” for Normal Distribution, “Levene’s Test for Homogeneity of Variances”), “Student *t*” test, one-way analysis of variance in repeated measurements of groups, and Tukey HSD multiplex in subgroup comparisons was used. In cases where parametric conditions could not be met, Kruskal-Wallis, Mann-Whitney U, Wilcoxon and chi-square ( $\chi^2$ ) tests were used to analyze qualitative data and compare groups. The results were evaluated at the 95% confidence interval, at the  $p < 0.05$  significance level.

## 3. Results

A total of 100 children participated in the study, with 57 females and 43 males. The mean age was  $10.12 \pm 1.85$  years.

The permanent first molars were more frequently affected by MIH (49.01%) than the permanent incisors (41.69%), permanent canines (3.6%), premolars (2.30%), permanent second molars (2%), and primary teeth (1.4%).

A total of 355 teeth were affected by MIH. Of these, 56% had limited opacities, 22% had enamel breakdown, 20% had atypical restorations and only 2% had to be extracted due to MIH. Additionally, 86% of the patients complained of tooth sensitivity.

Table 1 shows the correlation results between the number of teeth affected by MIH. A significant positive correlation was found between anterior teeth with MIH and the number of other permanent teeth with MIH, as well as the total number of teeth with MIH. Similarly, a significant positive correlation was found between the number of posterior teeth with MIH and the number of primary teeth with MIH as well as the number of other permanent teeth with MIH.

When the patients’ antibiotic use up to the age of 4 was investigated, the study group was found to have used significantly more antibiotics than the control group. Likewise, the patients in the study group were found to have had significantly more diseases in the first 4 years than the control group ( $p <$

0.05). There was also a significant difference between the two groups in terms of the diseases that the mothers had during pregnancy ( $p < 0.05$ ). When other etiological factors were compared, no statistically significant differences were found. Furthermore, 26% of patients had other family members who had been affected with MIH.

In this study, the prevalence of dental malocclusion was also evaluated in both groups of patients. The prevalence of malocclusion was significantly higher in patients with MIH ( $n = 30$ ) compared to those without MIH ( $n = 12$ ) ( $p < 0.05$ ).

The caries and periodontal status of patients with and without MIH was shown in Table 2. There was no significant difference in DMFT/S scores between the two groups. However, dft/s index values were significantly higher in patients with MIH ( $p < 0.05$ ). The simplified oral hygiene index (OHI-S) of MIH patients was also significantly higher ( $p < 0.05$ ), but no significant differences in modified gingival indices (MGI) were found between two groups.

According to the results of ICDAS scores, there was no statistically significant difference between two groups. The mean DIAGNOdent Pen value obtained from a total of 160 MIH teeth in the study group was calculated as 21.2.

Although the salivary buffering capacity and flow rate of the patients in the study group were higher, the salivary pH was lower than in the control group. However, no significant difference was observed between the two groups (Table 3).

The mean values of phosphorus, carbon and calcium content in the saliva samples of the patients with MIH were higher than those of the patients without MIH, with the difference being low for phosphorus and carbon, but significantly higher for calcium ( $p < 0.05$ ) (Table 3).

## 4. Discussion

The aim of this study was to determine the severity of MIH, evaluate the salivary flow rate, pH and buffering capacity in these patients, measure the amount of some elements in the saliva, evaluate the dental caries and periodontal status of the patients, and compare them with healthy controls.

Studies have shown that the presence of a severely affected first permanent molar in the mouth, is likely to affect the symmetry of the teeth, and more teeth, including first permanent molars and incisors are likely to be affected [25, 26]. However, other studies have found no relationship between the number of affected first permanent molars or the number of severely affected first permanent molars and defects in the incisors [27, 28]. The relationship between first permanent molar and hypomineralized second permanent molar has also been mentioned in several studies [29, 30]. As the severity of molars is affected, the risk of affecting the incisors also increases relatively [31, 32]. We found that the number of affected incisors was correlated with the number of affected first permanent molars and other permanent teeth. Similarly, we found a significant relationship between the posterior teeth with MIH and hypomineralized second permanent molar/other permanent teeth with MIH.

The etiology of MIH is still uncertain, and the studies suggest that it is a multifactorial condition with both environmental and genetic factors involved [3].

**TABLE 1. Correlation analysis findings of affected teeth with MIH.**

	Teeth with MIH	Anterior teeth with MIH	Posterior teeth with MIH	Primary teeth with MIH	Other permanent teeth with MIH
Anterior teeth with MIH					
<i>r</i>	0.878	1	0.290	-0.230	0.346
<i>p</i>	0.001		0.839	0.108	0.014
Posterior teeth with MIH					
<i>r</i>	0.490	0.290	1	0.327	0.589
<i>p</i>	0.001	0.839		0.020	0.001
Primary teeth with MIH					
<i>r</i>	-0.410	-0.230	0.327	1	0.060
<i>p</i>	0.779	0.108	0.020		0.630
Other permanent teeth with MIH					
<i>r</i>	0.581	0.346	0.589	0.060	1
<i>p</i>	0.001	0.014	0.001	0.630	

MIH: Molar incisor hypomineralization.

**TABLE 2. Clinical findings of patients.**

n = 100	Mean		SD		<i>p</i>
	Study	Control	Study	Control	
DMFT	3.70	3.42	2.50	2.84	0.60
DMFS	6.42	4.58	4.41	4.88	0.05
dft	4.06	2.32	3.42	3.06	0.001
dfs	7.66	4.28	6.52	5.86	0.001
OHI-S	2.11	1.31	0.80	0.61	0.001
CI	0.20	0.04	0.28	0.15	0.001
DI	1.91	1.24	0.65	0.51	0.001
MGI	0.73	0.79	0.47	0.39	0.52

DMFT: Decay, missing, filling teeth for permanent teeth; DMFS: Decay, missing, filling surface for permanent teeth; dft: Decay, filling teeth for primary teeth; dfs: Decay, filling surface for primary teeth; OHI-S: The simplified oral hygiene index; CI: Calculus index; DI: Debris index; MGI: Modified gingival index. SD: Standard deviation.

**TABLE 3. Saliva findings of patients.**

n = 100	Mean		SD		<i>p</i>
	Study	Control	Study	Control	
pH	6.89	7.0	0.27	0.36	0.11
Flow rate (mL/minute)	6.75	6.7	2.89	1.96	0.90
Buffering capacity (mmol H+)	8.42	8.22	2.36	1.99	0.64
Phosphorus (mg/L)	35.16	34.59	11.52	7.36	0.76
Calcium (mg/L)	20.42	1.67	6.57	0.72	0.001
Carbon (mg/L)	5.97	5.53	9.18	3.28	0.74

SD: Standard deviation.

There is little evidence of an association between prenatal factors and MIH. Some studies have shown a significant relationship between maternal diseases during pregnancy and MIH [33, 34], while others have found no such association [35–38]. In our study, we found a significant difference in the prevalence of diseases during pregnancy between the study

group and the control group. This situation may be related to the small size of our sample. Low vitamin D levels in pregnant mothers have been linked to an increased incidence of MIH in their children's molar teeth at 7–9 years of age [39].

The correlation between diseases in early childhood, up to the age of 3 or 4 years, and MIH has been subject to investiga-

tion, revealing notable associations between specific ailments and MIH [20, 40, 41]. According to our study, patients in the study group exhibited a significantly higher incidence of childhood disease during the initial 4 years than those in the control group. Although parents pay close attention to the health of their children during early infancy, recollecting illnesses from 7–8 years past can be challenging, especially when dealing with multiple children.

The use of antibiotics may also be a factor in the etiology of MIH. Studies evaluating this relationship have reported a significant relationship with the use of amoxicillin in the first year of life [33, 34, 42]. In our study, we found that antibiotic use was significantly higher in patients with MIH. However, it is not yet clear whether the use of antibiotics or the underlying diseases that require antibiotic use are responsible for MIH [41, 43].

According to the results of our study, no significant differences were found in other etiologic factors (the patients' birth time, birth type and birth weight) that may cause MIH.

It is important to note that most clinical studies on the etiology of MIH are retrospective. Information on the etiology of MIH is obtained through questionnaires or interviews, which can lead to inaccuracies due to individual memory bias. The importance of obtaining information on putative factors in early childhood from medical records or medical archives has been emphasized before, in order to increase reliability [40]. Another limitation of clinical studies on the etiology of MIH, same in our study is the small number of participants.

Teeth afflicted by MIH have been reported to display heightened sensitivity to stimuli such as cold, hot, sweet foods and drinks, as well as tooth brushing. This heightened sensitivity can increase the risk of dental caries if adequate oral hygiene is not maintained [44, 45]. In our study, 86% of the patients with MIH reported tooth sensitivity, whereas the remaining 14% did not.

Nonetheless, the relationship between MIH and dental caries remains unclear. Certain studies have identified a significant association between these two conditions [46, 47], whereas others have not [48, 49]. Ghanim *et al.* [50] found that patients with MIH had significantly higher DMFT and dft scores than the control group, suggesting that MIH may be a risk factor for dental caries. In our study, the mean DMFT/S values were higher in the study group than in the control group; however, this difference was not statistically significant. The mean dft/s values in the study group were significantly higher than those in the control group. These findings suggest a potential link between MIH and dental caries; however, further studies are required to confirm this finding.

Although many studies have demonstrated that MIH is a risk factor for dental caries, dental caries has rarely received substantial attention in the majority of epidemiological studies. Consequently, evaluating the extent of limited opacities and caries simultaneously is advisable.

When we compared both groups, there was no significant difference in terms of ICDAS II scores. In regions with a high prevalence of caries, MIH may go undetected because of the rapid progression caries, which can mask the signs of hypomineralization [51]. Nevertheless, studies have shown that children with MIH require more dental treatment in their

first permanent molars than their counter parts without MIH [52, 53].

One study found that teeth with increased DIAGNOdent readings in the absence of visible signs of dental caries may have enamel hypomineralization. This is because the less mineralized enamel scatters more laser light, which can lead to an increase DIAGNOdent reading [54]. We found that the mean DIAGNOdent pen value of 160 teeth with MIH was 21.2, which is in the range for deep enamel caries (21–29). This suggests that teeth with MIH may be more susceptible to caries than teeth without MIH.

In our study we also evaluated the periodontal status of patients. The simplified oral hygiene index (OHI-S) of patients with MIH was statistically higher ( $p < 0.05$ ) than patients without MIH. However, there were no significant differences in modified gingival indices (MGI) between two groups. This suggests that patients with MIH-related teeth had worse oral hygiene, as was also concluded in another study [55]. However, Praptiwi *et al.* [56] reported that most children with MIH had good or normal oral hygiene. This may be due to the regular implementation of caries prevention programs in schools or easier access to dental treatment opportunities in dental clinics.

However, no significant differences in MGI values were observed between the two groups. Similar results were documented in a study by Ulusoy *et al.* [57], which investigated oral health parameters in children aged 8–11 years with MIH using plaque and gingival indices. The plaque and gingival index values did not differ significantly between the MIH and control groups; however, the MIH group tended to have poorer gingival health [57]. Poor oral hygiene is a risk factor for an increased incidence of caries in patients with MIH. Therefore, imparting oral hygiene education with early diagnosis in this patient group, evaluating the results of this education periodically, and raising awareness among parents and patients about this issue holds paramount importance.

The mean salivary pH, flow rate, and buffering capacity values were calculated. Both patient groups exhibited low pH and buffering capacity, as well as moderate flow rate values. Although the salivary buffering capacity and flow rate of the patients in the study group were comparatively higher, their salivary pH was lower than that of the control group. However, no significant difference was found between the two groups. Ghanim *et al.* [50] reported that the saliva of patients with MIH displays altered physicochemical properties, such as varying flow rate, viscosity, pH and acid buffering capacity. Further investigations regarding salivary parameters in patients with MIH are required.

X-ray microanalysis (XRMA), X-ray diffraction (XRD), secondary ion mass spectroscopy (SIMS), and energy dispersion X-ray spectrometry (EDS) were used to evaluate the decrease in mineral content in teeth with MIH compared to healthy enamel [58–60]. When evaluating the Calcium/Phosphorus ratio, some studies found no significant difference between healthy enamel and enamel in teeth with MIH [59–62], while others reported a significant decrease in the Calcium/Phosphorus ratio in teeth with MIH [58, 63]. In another study, the authors analyzed the Calcium, Phosphorus, Oxygen, Carbon and Calcium/Phosphorus ratios

in hypomineralized second permanent molar teeth and found similar results to those in teeth with MIH [64].

Regarding carbon and carbonate content, research indicates an increase in carbon and carbonate concentration in MIH-affected enamel compared with healthy enamel [58, 61–63].

Studies evaluating protein content have reported that MIH-affected enamel has a higher protein content than healthy enamel [62, 65]. The increased protein content renders hypomineralized enamel less resistant to treatment than healthy enamel [66]. Another study found that individuals with low caries experience were found to possess significantly higher concentrations of phosphorus and calcium in their saliva [67].

It is well known that high concentrations of calcium phosphate salts in the oral cavity are dissolved in saliva. Saliva plays a crucial role in the integrity of the dental enamel and caries susceptibility. This is because saliva needs to be supersaturated with calcium and phosphate ions to prevent the dissolution of enamel apatite. Therefore, saliva helps to stabilize and protect the enamel [68].

According to the results we obtained from the available literature, there was limited data on the analysis of calcium, phosphorus and carbon in the saliva of patients with MIH. As we mentioned before, it was observed that the calcium/phosphorus ratio decreased and the carbon/bicarbonate ratio increased in MIH teeth compared to healthy teeth [58, 63]. In this study, we aimed to compare the amount of these minerals in the saliva of patients with and without MIH. According to the results we obtained, it was determined that the mean values of calcium, phosphorus and carbon content in the saliva samples of the patients in the study group were higher compared to the patients in the control group. However, the difference was small for phosphorus and carbon, but significantly higher for calcium ( $p < 0.5$ ). There are a number of possible explanations for this difference. One possibility is that MIH affects the way that the body absorbs and metabolizes calcium. Another possibility is that patients with MIH are more likely to have other medical conditions that affect calcium levels, such as hypoparathyroidism or renal failure. It is also possible that the difference in calcium levels is due to environmental factors, such as diet or exposure to toxins. Further research is needed to confirm the findings of our study and to investigate the possible mechanisms underlying the difference in calcium levels between patients with MIH and controls.

In a study, it was shown that some genes involved in enamel development are associated with calcium and phosphorus content in saliva [19].

Studies have shown that variations in genes involved in enamel development are associated with changes in the levels of calcium and phosphorus in saliva [19, 20]. The calcium level was associated with variations in the AMNB, AMELX and ESRRB genes. These genes are involved in the mineralization of enamel, and mutations in them can lead to the Amelogenesis Imperfecta phenotype (OMIN). This supports the link between these genes and enamel alterations and mineralization. The AMNB gene is also located in the calcium-binding phosphoprotein gene cluster on chromosome 4 [19]. In a previous study the role of ESRRB in the oral tissues was explored and found to be relationship with likelihood of developing cavities. The study also found that ESRRB is

expressed in the salivary gland and in the enamel development [69]. Although ESRRB has been shown to be expressed in salivary glands, its role in salivary function is still unknown. Therefore, it is possible that the association between ESRRB and calcium levels in saliva is through the salivary gland function.

One study found that genetic variations in the Estrogen Receptor Alpha (ESR1) and microRNA17 (miRNA17) genes are associated with salivary calcium and phosphorus levels [70]. However, it is unknown how this situation changes in patients with MIH. To the best of our knowledge, our study is the first to evaluate the amount of calcium, phosphorus and carbon in the saliva of patients with MIH teeth. We found that the amount of Ca was significantly higher in the saliva of patients with MIH.

These findings suggest an association between MIH and altered salivary mineral levels. However, further studies are needed to validate these outcomes and unravel the underlying mechanisms.

## 5. Conclusions

To the best of our knowledge, this is the first study to evaluate the levels of calcium, phosphorus and carbon in the saliva of patients with MIH. Our study found no significant difference in the levels of calcium and phosphorus in saliva between patients with MIH and controls. However, the significantly high amount of calcium in the saliva of patients with MIH was detected. Further research is needed to confirm the findings of our study and to investigate the possible mechanisms underlying the association between MIH and salivary composition.

## AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

## AUTHOR CONTRIBUTIONS

NI, OEG and HK—contributed equally to the work by analyzing and interpreting the data and revising the study.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was ethically approved with the decision of the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee, dated 12 March 2020 and numbered 221. All participants and their parent or guardian were freely invited, and those who accepted signed and informed consent approved.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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