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# Ghrelin hormone might have a potential role in amelogenesis

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## Abstract

**Aims:** Amelogenesis imperfecta and generalised enamel hypoplasia are developmental dental anomalies that affect dental enamel. While amelogenesis imperfecta results from various gene mutations, the exact underlying mechanisms of the etiopathogenesis of both remain unclear. This study aims to evaluate Ghrelin hormone levels in children with generalised enamel hypoplasia to establish whether Ghrelin might have a potential role in enamel hypoplasia's etiology. The second purpose is to determine the correlations among the blood levels of Ghrelin, growth hormone (GH), insulin-like growth factor-1 (IGF-1), bone alkaline phosphatase (BALP) and osteocalcin (OC) that are vital in dental development.

**Material and methods:** Study was designed with two study groups, AI (hypoplastic amelogenesis imperfecta) (n = 15; mean-age 10.36 ± 1.90) and GEH (idiopathic generalised enamel hypoplasia) (n = 15; mean-age 10.42 ± 1.84), and a healthy control (n = 15; mean-age 10.39 ± 1.91) group. After fasting for 10-12 hours, simultaneous blood samples were collected; then, after centrifugation, serum and plasma were stored at −80°C until the day of analysis. Total Ghrelin levels of plasma and serum levels of GH, IGF-1, BALP and OC were measured using commercial ELISA kits.

**Results:** Ghrelin levels of AI and GEH groups were significantly lower ($P < .01$) than the control group.

**Conclusion:** This is the first study to reveal the decreased levels of Ghrelin in plasma of children with generalised enamel hypoplasia, suggesting a potential role for Ghrelin in amelogenesis. In order to determine its function in enamel formation, further studies should be carried out. The result of the present study suggests that paediatricians refer children with abnormal Ghrelin levels to a paediatric dentist to contribute to appropriate prophylactic and therapeutic interventions. Generalised enamel hypoplasia may also indicate possible abnormalities in Ghrelin levels for paediatricians. Therefore, paediatricians' knowledge about the clinical appearance of generalised enamel hypoplasia should be increased.

### What's known

- Generalised enamel hypoplasia, the underlying mechanism of which has not been clarified, continues to be a challenge of dentistry.
- It has been shown that Ghrelin is expressed in the dental developmental stages.
- However, up to date, the physiological importance of Ghrelin in dental tissue was not elucidated.

  

**What's new**

- This is the first study to reveal the decreased levels of Ghrelin in plasma in children with generalised enamel hypoplasia, suggesting a potential role for Ghrelin in amelogenesis.

## 1 | INTRODUCTION

Dental enamel, the outer layer of the dental crown, is the most hardened tissue of the human body. Enamel development occurs in a three-step process called amelogenesis divided into secretion, calcification and maturation stages respectively. Ameloblasts, which are specialised ectodermal cells, secrete proteinaceous enamel matrix in the secretory phase. Enamel formation is meticulously controlled in ameloblasts via the interaction of a series of enamel matrix molecules.[1-8] Therefore, impairment in ameloblast functions as a result of systemic or local factors in the secretory phase causes enamel hypoplasia. Enamel hypoplasia is characterised by enamel surface defects and a reduction in the amount of dental enamel.[1-3,7] If ameloblastic activity is impaired over an extended period, irregular or defective enamel formation occurs in more teeth and larger areas of teeth referred to as generalised enamel hypoplasia (GEH).[2] In GEH, the dentition is affected partially or wholly, and the defects can be seen on any surface of enamel but generally do not cover all surfaces of the crown.[9]

GEH may adversely affect the individual's life quality by causing consequences such as caries, tooth wear and sensitivity, poor aesthetic and psychological disturbances.[1,2,7,8,10-13] Hence, well-known possible aetiological factors, multidisciplinary treatment approach and timely diagnosis are essential for the comprehensive and adequate treatment of these patients.[7,8]

Although the etiology of GEH is not clearly understood, it may occur as a result of systemic, environmental and genetic factors.[1,2,7,14,15] Up to date, specific causes underlying the occurrence of particular types of enamel hypoplasia have not been identified.[3] However, those with unknown etiology that occur independently of heredity and teratogenic factors are called idiopathic GEH (IGEH).[7] The most common GEH caused by toxicity is dental fluorosis, which occurs when individuals are exposed to high fluoride levels (more than 1 part per million).[15] GEHs of genetic origin are classified under a group of developmental enamel defects known as amelogenesis imperfecta (AI).[2,7,16,17] AI is a group of conditions, genomic in origin, which affects the structure and appearance of enamel of all or nearly all the teeth without reference to chronology, which may be associated with morphologic or biochemical changes elsewhere in the body.[4]

Ghrelin is a novel acylated 28 amino acid peptide that is produced predominantly by the stomach.[18-22] It is a potent releaser of Growth hormone (GH) that actively participates in controlling energy balance and food intake regulation.[18-21] Ghrelin has been shown in the odontoblasts and the dental pulp by Aydin et al,[23] suggesting a possible role for dentinogenesis and the mineralisation process. It has been presented in tooth organs throughout tooth development stages, particularly in ameloblasts and odontoblasts with little spatiotemporal expression differences.[24] Nonetheless, the potential regulative roles of Ghrelin in development of tooth still requires validation by functional studies.[23,24]

The formation of teeth is closely related to the bone in several ways, including composition and formation.[2,25] It is likely that some of the same regulatory mechanisms involved in bone development could also apply to tooth development.[26] Most of the bone matrix proteins are also expressed during tooth formation.[2,25-27]

Ghrelin is involved in bone mineralisation and vascular calcification as a regulator by influencing the GH/Insulin-like growth factor-1(IGF-1) axis, bone alkaline phosphatase (BALP), osteocalcin (OC), runt-related transcription factor 2 (Runx2), bone morphogenetic protein-2 (BMP-2) and extracellular signal–regulated kinase (ERK) signalling pathway.[22,28-32] These factors are known to play a role in the development of the dental hard tissues.[5,6,25,27,33,34]

It may be assumed that possible deviations in Ghrelin hormone levels could be attributed to the consequences mentioned above. Hence, the present study aimed to evaluate Ghrelin, GH, IGF-1, BALP and OC levels in plasma in a group of children with AI and IGEH compared with healthy controls. The present study is the first study that compares blood Ghrelin levels in patients with GEH and demonstrates the correlations between GH, IGF-1, BALP and OC.

## 2 | MATERIAL AND METHOD

### 2.1 | Patients and healthy controls

Individuals with GEH according to the developmental defects of enamel (DDE)-modified index[35] during intra-oral examinations of children aged 0-14 years who applied with complaints of dental caries, hypersensitivity and aesthetics concerns to Dentistry Faculty Pedodontics Clinics between 02/2011 and 02/2012 were included in the study.

The study was designed with two study groups, AI (n = 15; mean age 10.36 ± 1.90) and GEH (n = 15; mean age 10.42 ± 1.84), and a healthy control (n = 15; mean age 10.39 ± 1.91) group.

Both control and the study groups were intra-orally examined in accordance with WHO criteria.[36] DMF-T/df-t [Decay (D/d), Missing (M), Filling (F/f)] index and plaque index (PI) of all subjects were recorded.

A comprehensive history was obtained for differential diagnosis from the subjects. According to the anamnesis, if the etiology of GEH was related to the genetic aspect, the individual was included in the AI group, and if it could not be associated with any factor, the GEH group. Individuals diagnosed with another disease, having

medication, and has GEH related to an etiology other than genetics, were excluded from the study.

The control group consisted of children who were followed up at the Medical Faculty Healthy Pediatric Clinic, referred to our clinic for a consultation to confirm their orodental health and found healthy by an oral examination.

### 2.2 | Sample collection, preparation, storage and analysis for total Ghrelin

By the standard procedure described by Hosoda et al,[37] following an overnight fast (at least 10-12 hours), all blood samples were collected between 08.30 and 10.00 into ethylenediaminetetraacetic acid (EDTA)-coated tubes. The samples were instantaneously cooled and centrifuged at 4°C for 15 minutes at 3000 rpm. Plasma was stored at −80°C until the assay was performed. All samples were stored without adding any preservatives.

### 2.3 | Sample collection, preparation and storage for GH, BALP, IGF-1 and OC

Following an overnight fast, all blood samples were collected between 08.30 and 10.00 into dry tubes. After coagulation was achieved, samples were centrifuged at 4°C for 15 minutes at 3000 rpm. Each serum sample was divided into five aliquots and stored at −80°C until the assays were performed.

### 2.4 | Laboratory methods

Serum GH, IGF-1, BALP, OC and plasma Ghrelin levels were measured using commercially available enzyme-linked immunosorbent assays [DRG hGH ELISA Kit, no. EIA-3552; DRG International, Inc, New Jersey, USA; Assaypro AssayMax Human Insulin-like Growth Factor 1 (IGF-1) ELISA Kit, no. EI1001-1; Assaypro LLC, Missouri, USA; TSZ ELISA Human Bone Alkaline Phosphatase (BALP) ELISA Kit, no. HU9176, TSZ ELISA, MA, USA; DIAsource hOST-EASIA Kit, no. KAP1381, DIAsource ImmunoAssays SA, Louvain-la-Neuve, Belgium; Millpore Human Ghrelin (total) ELISA Kit, no. EZGRT-89K; EMD Millipore Corporation, Missouri, USA; respectively] according to the manufacturers' instructions, based on the direct sandwich technique. All measurements were performed using an ELISA plate reader (Elx800 Bio-Tek Instruments Inc, USA) at the 450 nm wavelength. The concentrations are given in pg/mL for Ghrelin, μIU/mL for GH and ng/mL for IGF-1, BALP and OC.

### 2.5 | Statistical analysis

Statistical analysis was performed with Statistical Package for the Social Sciences software for Windows (version 15.0, SPSS Inc, Chicago, IL, USA). The data are expressed as arithmetic means ± standard deviation (SD). Kolmogorov-Smirnov test was used to determine the normal distribution of the data. The student's *t*-test was used to compare the means of the variables measured in both groups, and the relationships between values were analyzed by Pearson analysis. P values less than 0.05 were considered significant.

## 3 | RESULTS

### 3.1 | Characteristics of patients and healthy children as controls

Patients and controls showed no significant group differences regarding gender and age (Table 1).

### 3.2 | Total Ghrelin, GH, IGF-1, BALP and OC levels of study and control groups

Total plasma Ghrelin concentrations were significantly low in AI and GEH groups (735.53 ± 242.35 pg/mL; 776.06 ± 175.32 pg/mL respectively) at an advanced level ($P < .01$) as compared with healthy controls (940.68 ± 124.58 pg/mL). Mean total Ghrelin, GH, IGF-1, BALP, OC levels and their SD values of patients with AI and GEH and control groups were given in Table 2.

Serum GH concentrations were decreased and IGF-1 concentrations were elevated in AI patients (5.58 ± 4.34 μIU/mL; 157.83 ± 30.79 ng/mL respectively) than in healthy controls (7.70 ± 4.90 μIU/mL; 133.11 ± 54.90 ng/mL respectively) but the differences were not statistically significant ($P > .05$).

The mean values and standard deviations of DMFT/dft and plaque index (PI) of the two study groups are given in Table 3.

## 4 | DISCUSSION

Numerous studies have been conducted on the etiology of enamel hypoplasia; however, it has not been fully elucidated yet.[7] Factors that cause GEHs are may be genetic (AI), teratogenic (dental fluorosis)

**TABLE 1** Characteristics of patients and healthy children as controls. Data are expressed as arithmetic means ± standard deviations (SD)

| | n | Age (years) Mean ± SD | *P* | Gender ratio (male:female) |
|---|---|---|---|---|
| AI | 15 | 10.36 ± 1.90 | .977 | 8:7 |
| CONTROL | 15 | 10.39 ± 1.91 | | 8:7 |
| GEH | 15 | 10.42 ± 1.84 | .965 | 8:7 |
| CONTROL | 15 | 10.39 ± 1.91 | | 8:7 |

*Note:* Student's *t*-test

**TABLE 2** Mean total Ghrelin, GH, IGF-1, BALP and OC levels and their SD values of study and control groups

| | AI (n = 15) | | GEH (n = 15) | | CONTROL (n = 15) |
|---|---|---|---|---|---|
| | Mean ± SD | *P* | Mean ± SD | *P* | Mean ± SD |
| Ghrelin Plasma | 735.53 ± 242.35 | .008** | 776.06 ± 175.32 | .006** | 940.68 ± 124.58 |
| BALP | 350.93 ± 88.63 | .555 | 319.60 ± 80.35 | .669 | 332.36 ± 81.39 |
| OC | 60.72 ± 20.66 | .347 | 54.01 ± 17.81 | .983 | 54.14 ± 16.74 |
| IGF-1 | 157.83 ± 30.79 | .140 | 129.36 ± 42.78 | .836 | 133.11 ± 54.90 |
| GH | 5.58 ± 4.34 | ,220 | 7.06 ± 6.14 | .755 | 7.70 ± 4.90 |

*Note:* Student's *t*-test.
**$P < .01$.

**TABLE 3** Mean DMFT/dft and PI and their SD values of AI and GEH groups

| | AI (n = 15) | GEH (n = 15) | |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | *P* |
| DMFT/dft | 3.93 ± 3.11 | 4.43 ± 3.74 | .68 |
| PI | 1.58 ± 1.01 | 1.33 ± 0.83 | .38 |

*Note:* Student's *t*-test.

or idiopathic (IGEH), and sometimes they occur as a secondary manifestation of a systemic disease (eg celiac disease).[7,15]

It is essential to obtain a detailed history in order to determine the differential diagnosis among hypoplasic AI, IGEH and dental fluorosis.[7,15] Up to date, at least 33 gene mutations are known to cause AI, while 19 have been associated with isolated AI.[12] Despite this, it is reported that 51% to 72% of AI cases are not genetically diagnosed.[1] AI diagnosis involves establishing a possible inheritance pattern, recognising the phenotype and correlation with tooth formation dates.[4] Besides that, the exclusion of external factors such as environmental or any others and chronological developmental disorders is a part of the differential diagnosis of AI. Therefore, rather than a genetically differential diagnosis of AI, a very detailed history was obtained from the participants that could determine the possibility of genetic transmission and excessive fluoride consumption.

The formation of dental hard tissues, namely enamel, dentin and cement, is substantially similar to bone development in several ways, including expressing proteins such as GH, IGF-1, OC and BALP.[2,25-27,33,38,39] Multifunctional hormone Ghrelin directly regulates bone development in relation to these proteins.[22,28-30,40-42]

Odontogenesis occurs as a result of sequential and reciprocal interactions between the oral ectoderm and the neural crest-derived mesenchyme. Tooth crown development morphologically starts with the thickening of the epithelium and progresses through bud, cap and bell forms creating by this thickened epithelium. During the bell stage, epithelial cells differentiate into ameloblasts and secrete enamel matrix, whereas mesenchymal cells into odontoblasts and secrete dentin matrix. This process is followed by mineralisation.[5-7,24]

The only research investigating the expression pattern of Ghrelin during odontogenesis showed a spatial and temporal expression at different developmental stages. It has been demonstrated that Ghrelin is predominantly present in the epithelium prior to differentiation of ameloblasts and odontoblasts and less in the surrounding mesenchyme. Ghrelin expression has been shown in secretory and mature ameloblasts and odontoblasts by slightly weak staining intensity than the prior early bell stage. It was specified that expression has become relatively stronger in ameloblasts in the secretory phase, although persistently in both cell types producing the matrixes. It was reported that in the consequent stages, no staining had been detected in odontoblastic processes in the dentinal tubules and immature enamel and dentin. The significant and persistent expression of Ghrelin in ameloblasts was underlined, indicating the possible functional role of Ghrelin in amelogenesis.[24] This study was designed to evaluate the plasma Ghrelin levels of patients with GEH in order to investigate whether this potential role of Ghrelin in amelogenesis appears clinically.

Developmental enamel defects can be seen in many cases associated with Ghrelin disturbances (eg hypothyroidism, hypoparathyroidism, cardiac disease, chronic renal failure and birth prematurity).[22,40,43-45] Vice versa, both hyper- and hypo-ghrelinemia have also been shown in syndromes with developmental enamel defects such as celiac disease,[46,47] Prader-Willi syndrome,[48,49] McCune Albright syndrome,[50,51] Seckel syndrome[52,53] and Alström syndrome.[54]

Depending on these existences, it can be hypothesised that there is a strong relationship between deviations of Ghrelin levels and enamel hypoplasia development. However, this relationship has not been studied and remains unclear. Therefore, in the present study, it was aimed to determine whether there is any deviation of Ghrelin levels in the plasma of individuals with GEH.

Celiac disease can be considered as a good starting point to explain the basis of this hypothesis. It is known that Ghrelin levels are high before starting a gluten-free diet and decreasing the transition to the gluten-free diet in patients with celiac disease.[47] Moreover, dental enamel hypoplasia occurs secondary to celiac disease in the teeth that develop in the period of gluten intake coinciding with amelogenesis.[46]

Controversy with the results of research on subjects with celiac disease,[47] the significant consequence of the present study was that

there were statistically significantly lower plasma Ghrelin levels in AI and GEH groups compared with the controls. This finding supports the view that Ghrelin may play a role in developmental enamel defects since enamel hypoplasia might be seen in conditions either low and high Ghrelin levels.

It is noteworthy to point out here; interestingly, Ghrelin enhances the expression of osteoblastic genes in the bone while reducing vascular calcification despite both tissues developing through the same mechanisms.[22,23,28-32,40-42,55-60]

It has been reported that osteoblastic differentiation and calcification of vascular smooth muscle cells are inhibited by administering high doses of Ghrelin but not at low Ghrelin levels.[30]

On the contrary, Maccarinelli et al[32] reported that although Ghrelin stimulates osteoblast proliferation at the maximal level at low doses ($10^{-10}$ M), it is not affected at higher doses ($10^{-8}$ and $10^{-9}$ M). Researchers proposed the hypothesis that the stimulatory effect with low concentration could be rehabilitated by recognising peptides to different sub-inhibitory receptors when the dose was increased.[32]

Different binding affinities of Ghrelin and its synthetic analogue to the growth hormone-releasing receptor (GHS-R) have been demonstrated. Besides, stimulating or inhibitory effects on cell proliferation have been reported for these peptides.[28,30,32,60,61] Based on these situations, it was suggested that Ghrelin might create the physiological response in only certain ranges, and when the given dose is exceeded, a resistance mechanism has evolved to Ghrelin.[32]

In bone development, Ghrelin stimulates GH release from the pituitary by signaling through the growth hormone secretagogue receptor (GHS-R). Ghrelin-induced GH production supports bone development via the GH/IGF-1 axis.[22,31,32,60,62] There was no statistically significant difference in serum GH and IGF-1 levels in the groups compared with controls in the present study. This result is consistent with the study that found the serum GH and IGF-1 levels of Ghsr-null mice were comparable with wild-type mice.[63]

Furthermore, Ghrelin was found to modulate the proliferation and differentiation of osteoblasts by increasing or suppressing OC and BALP expression.[31,32,41] No correlation was detected between the Ghrelin levels in plasma and serum OC levels in any group in the present study. Yet, the plasma Ghrelin and serum BALP levels were correlated positively in the control group. Consistent with this study's findings, it was reported that Ghrelin had not affect OC in any of three different cell cultures, reflecting different stages of osteoblastic development. However, Ghrelin has been stated to increase BALP in highly undifferentiated cells as an essential modulator.[61]

In conclusion, this is the first study to reveal the significant decrease in Ghrelin hormone levels in the plasma of children with AI and IGEH. The present study confirmed the crucial role of Ghrelin in the amelogenesis stage. Early detection of Ghrelin level deviations can avoid enamel defects causing aesthetic and functional obstacles. This could be succeeded by working in collaboration with paediatricians and pedodontists. It seems convenient for paediatricians to refer children with abnormal Ghrelin levels to a paediatric dentist to provide exhaustive dental management. GEH may also indicate possible abnormalities in Ghrelin levels for paediatricians. Therefore, the level of knowledge of paediatricians about the clinical appearance of GEH should be increased.

The drawback of the present study is the limited sample size. However, isolated dental hard tissue defects that are not related to any syndrome or systemic diseases are not common. The results need to be confirmed with a larger number of samples having dental hard tissue anomalies that are isolated or a part of a systemic disease.

Up to date, the physiological importance of Ghrelin in dental tissue was not elucidated. Immunohistochemical studies are needed in which the expression patterns in odontoblastic differentiation and mineralisation stages to understand the possible role of Ghrelin in tooth development. In order to determine its function in enamel formation, further studies should be carried out using ameloblast-like cell lines and Ghrelin-knockout mice.

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## DISCLOSURE

The authors declare that they have no conflict of interest.

## ETHICAL APPROVAL

The study was approved by the Clinical Research Ethics Committee of Medical Faculty of Istanbul University, Turkey, 13.01.2011/129. Subjects were entered into the study after obtaining written informed consent from the parents and, if appropriate, from the patients.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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