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REVIEW

- 1 Effects of energy and sports drinks on tooth structures and restorative materials

Erdemir U, Yildiz E, Saygi G, Inan Altay N, Eren Mert M, Yucel T

MINIREVIEWS

- 8 Concepts and challenges of alveolar ridge preservation and augmentation

Munhoz EA, Cardoso CL, Bodanezi A, Mello MB, Yaedu RYF, Ferreira Junior O

ORIGINAL ARTICLE

Retrospective Study

- 15 Tooth agenesis and craniofacial morphology in pre-orthodontic children with and without morphological deviations in the upper cervical spine

Jasemi A, Sonnesen L

ABOUT COVER

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Effects of energy and sports drinks on tooth structures and restorative materials

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Abstract

Sports and energy drinks are consumed by more people

than ever. Sports and energy drinks may enhance physical resistance, stimulate metabolism, prevent rehydration and replace electrolytes during high activity efforts. However, these drinks often have a low pH and are acidic, which can erode enamel and dentin, and increase dentine hypersensitivity. In addition to the adverse effects of sports and energy drinks on tooth structures, they often have the potential to damage restorative materials. These drinks often contain artificial colors which have potential to discolor resin composite materials and glass ionomers. The acidic nature of these drinks could also lead to a degradation, increase in wear, and roughening of the surface of the restorative materials. Many of the negative consequences of sport and energy drinks can be related to their over-consumption among children and teenagers. Patients should be advised to have a healthy diet, and consume soft and energy drinks in moderation, to avoid any negative dental or health consequences. The over-consumption of sports and energy drinks which are high in sugar and have the lowest pH are most likely to cause avoidable dental problems.

Key words: Energy drinks; Sports drinks; Dental caries; Dental erosion; Discoloration; Microhardness; Surface roughness

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Core tip: Dentists have a duty to their patients to give them instructions on the consumption of drinks or foods which can damage dental health. Most food and drinks have little noticeable effects on dental health. Among the drinks that are most likely to damage teeth and restorative materials are sports and energy drinks which contain sugar to feed oral bacterial, and drinks which have a low pH which can erode teeth and increase their sensitivity. Patients who suffer poor oral health because of their over-consumption of sports and energy drinks need to be made aware of the likely causes of their dental problems.

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INTRODUCTION

Intake of sports and energy drinks has gained popularity markedly in recent years^[1]. The consumption of energy and sports drinks mainly by professional and amateur athletes, sportsmen, and adolescents has been on the rise with extensive advertisements since 1997, with the debut of Red Bull^[2-7]. The purposes for producing each of these drinks are different, and the ingredients are mostly distinctive.

While sports drinks are consumed to boost performance, prevent rehydration, and replace electrolytes during high-activity sports^[2,3,8-10], energy drinks are used to enhance physical resistance and a state of alertness, increase the rate of giving responses, supply greater concentration, and stimulate the metabolism during sports, driving, and leisure activities^[11,12].

Sports drinks, which are used largely by consumers, contain great amounts of "carbohydrates, such as glucose, fructose, sucrose, and synthetic polymer maltodextrins that are also known as glucose polymers^[13,14]. On the other hand, energy drinks are composed of "caffeine, herbal extracts such as guarana, ginseng, and ginkgo biloba, B vitamins, amino acids such as taurine, amino acid derivatives such as carnitine, and sugar derivatives"^[10,11].

It is crucial to know the difference between energy drinks and sports drinks^[9]. The main active ingredient in energy drinks is caffeine^[15]. Caffeine-containing energy drinks have not been produced to support rehydration^[16]. Energy drinks mainly have higher carbohydrate contents than sports drinks do^[9]. Energy drinks also have both sugar-containing and sugar-free versions^[11].

Problems related to consuming sports and energy drinks can be inspected in dental clinics. However, it may be difficult to distinguish the etiological factor because dentists generally do not think about soft drink users, who are very common in the population. In this section, the clinically visible effects of sports and energy drinks are evaluated for dental professionals to look over.

EVALUATION OF DENTAL CARIES AND DENTAL EROSION CORRELATED WITH SPORTS AND ENERGY DRINKS

Many research studies have indicated that sugar-containing beverages can cause dental caries^[13,17]. The sugars in drinks are metabolized by plaque microorganisms to compose organic acids that initiate demineralization^[18].

However, a number of studies also support that sports drink consumption is not directly associated with dental caries^[13,14]. Sports drinks have had the same cariogenicity as fruit juices, drinks, and carbonated beverages. The "diet" or "light" soft drinks have a reduced risk of caries compared to that of sugared drinks due to the lack of sugar^[19]. Thus, although there is no precise evidence supporting a relationship with dental caries, the caries-causing potential of these drinks should be carefully considered^[13].

Evidently, acidic drinks play a significant role in the pathogenesis of dental erosion^[2,17,20-25]. Dental erosion is the loss of the outmost surface of enamel and exists when the surface pH reduces below to critical threshold value of 5.5^[18,26]. When the critical pH reaches 5.5, the hydroxyapatite crystals begin to dissolve and the enamel begins to be at risk of decalcification^[2,6]. Although a few studies with regard to the erosive potential of sports drinks have reported a lack of relationship between consumption of sports drinks and dental erosion^[2,13,18], many other studies have stated that sports drinks and energy drinks have the potential to cause enamel surface loss and surface softening, leading to dental erosion due to their low pH and the existence of citric acid^[6,14,26-30]. In a recent study, human tooth enamel samples were immersed in sports and energy drinks for repeated short exposure times. Researchers ensured that drinks had eroded the enamel layers. In the results of the study, it was also indicated that energy drinks had higher titratable acidity values than sports drinks. Besides the fact that titratable acidity is an important predictor of enamel dissolution, samples immersed in energy drinks also showed higher enamel loss. Unfortunately, the tissue loss is irreversible^[31]. Another study investigating enamel loss showed that sports and energy drinks had the most aggressive enamel dissolution compared to Coca Cola and soft drinks^[1].

Frequency, duration, temperature, and time of exposure to acidic drinks have been shown to affect the severity of erosion^[32,33]. It has been revealed that prolonged contact time between a beverage and the enamel or root surface increases the possibility of dental erosion occurrence^[13,34]. Therefore, according to their properties, sports and energy drinks can be highly detrimental for teeth if consumed frequently and improperly. Furthermore, it is inevitable to prevent dental erosion in patients at risk because of many etiological factors such as reduced salivary flow or buffering capacity, beverage-holding habits, or mouth breathing. Individuals with these conditions are at increased risk of erosion when consuming sports and energy drinks frequently^[28]. Temperature differences also have an effect on the erosive potential of acidic drinks. It has been previously stated that acidic drinks are less erosive at lower temperatures^[35]. It is obviously advantageous that sports and energy drinks are generally consumed at lower temperatures (*i.e.*, 4 °C).

In comparison to soft drinks, sports drinks do not

contain much more acid than a wide variety of other drinks such as fruit juices, beer, and wine^[13]. According to Cavalcanti *et al.*^[36], energy drinks have a high erosive potential, as they have low pH and a high non-reducing sugar content. The erosive potential of sports drinks is increased when they are consumed during periods of dehydration and low salivary flow, which could occur during exercising or sports activities^[3]. It has also been revealed that sports drinks can reduce the surface hardness of enamel^[29], and this could be an etiological factor for cervical dentine hypersensitivity, which can be severe and debilitating^[37,38]. Another clinically relevant situation about sports and energy drinks is thought to be the increase in surface roughness at dental tissues. A recent study revealed that Gatorade, a sports drink, and Red Bull, an energy drink, showed significantly higher roughness results in enamel samples, compared to Coca Cola and coffee^[39]. It is obviously seen from the above mentioned studies that sports and energy drinks are more responsible from initiating dental erosion than caries. It is clear that lowering the plaque pH to cause demineralization is in low possibility to happen when compared to their acidic potential which may soften, roughen and discolor the teeth.

Moreover, the erosive potential of sports drinks also depends on the type of acid and the ingredients in the formulation^[40,41]. Citric acid, which is also called International Numbering System for Food Additives 330 acidulant^[26], is commonly used in soft drinks^[42]. This acid is one of the most powerful ones due to its chelating capacity, which is responsible from calcium sequestration from saliva and teeth^[26,28]. The citrate anion has the ability to chelate calcium with an additive effect to the erosive potential of the proton ions released^[43]. Many studies have stated that beverages containing citric acid and having a low pH are thought to have the most erosive capacity^[44-48]. Likewise, Meurman *et al.*^[48] compared sports drinks, all of which contained citric acid or maleic acid. It was determined that drinks containing citric acid had a higher erosive potential than that of those containing maleic acid. The pH scores of several sports and energy drinks are given in Table 1.

On the other hand, saliva provides calcium and phosphate ions for remineralization and proteins for the development of a protective pellicle^[42]. The buffering capacity of saliva has also been considered to be important, even more than pH^[22,40,49]. The citric acid in acidic drinks can be modified by incorporating calcium, phosphate, and fluoride or by diluting the drinks by adding water or reducing the total sugar concentration to reveal a significant and protective effect against enamel erosion^[17,50].

Many additive materials are being used for reducing the damage done by sports and energy drinks. The addition of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) at 0.2% to soft drinks diminishes their erosive potential. The reason for that is the increased availability of calcium and phosphate ions at the enamel surface^[3,42,51,52]. In addition, treatment

Table 1 The pH scores of several energy drinks¹

Energy drink	Brand name	pH score
Powerade	The Coca-Cola Co. Atlanta GA, United States	3.79
Gatorade	The Gatorade Co. Chicago IL, United States	3.27
Burn	The Coca-Cola Co. Atlanta GA, United States	2.67
X-IR	Nice Trading Inc. Istanbul, Turkey	3.15
Red bull	Red Bull GmbH Am Brunnen, Austria	3.54
Isostar	Isostar, Wander AG, Switzerland	3.87

¹Scores were cited from; Erdemir U, Yildiz E, Eren MM, Ozel S. Surface hardness evaluation of different composite resin materials: Influence of sports and energy drinks immersion after a short-term period.

of eroded teeth due to acidic drinks with CPP-ACP has increased the hardness of the enamel^[53,54]. An *in vitro* study showed that the enamel erosion caused by sports drinks could be eliminated by adding 0.09% to 0.25% CPP-ACP due to a rise in pH and decline in titratable acidity of the modified sports drink^[3]. However, the modified beverages have lower palatability than more acidic sports beverages. Although product modification by adding calcium or phosphate can create a lower erosive potential in sports and energy drinks, consumers may refuse the altered palatability or texture and prefer the ones with lower palatability^[14,41]. Another adverse effect of the low pH of sports and energy drinks is the potential for damaging the properties of composites. Factors such as temperature changes, salivary enzymes, the ionic composition of food or beverages, and pH level might also effect the properties of restorations in the oral cavity^[55].

DISCOLORATION OF RESTORATIVE MATERIALS AND THE EFFECT OF SPORTS AND ENERGY DRINKS

As of now, a great spectrum of material diversity has been identified for practitioners. This diversity is not only for supporting aesthetic results of the anterior region but also for resisting the mechanical forces encroaching on the posterior region. However, regardless of which material is chosen, emphasis is generally on aesthetics. Moreover, color is one of the inseparable parts of the aesthetic view. Color stability of any dental material means to be able to maintain its original color. As the oral cavity is full of dynamic movements of microflora and intake of colored food and drinks, it is challenging to retain the colors of dental materials. After prolonged exposure to oral environment, discoloration of restorations is one of the major problems leading to failure of restorations^[35,56]. In this part, effects of consuming sports and energy drinks on color stability and other physical properties of various dental materials is discussed.

COMPOSITE RESINS

Color stability of resin composites is related to many factors. Both the structures of resins and the

Table 2 Example of some of the energy drinks and their colorants with properties

Energy drink	Brand name	Colorant	Properties
Powerade	The Coca-Cola Co. Atlanta GA, United States	Brilliant Blue	Water soluble food dye, gives bright blue color
Gatorade	The Gatorade Co. Chicago IL, United States	Brilliant Blue	Water soluble food dye, gives dark red color
Burn	The Coca-Cola Co. Atlanta GA, United States	Allura Red	Caramel can change the color from yellow to brown
Red bull	Red Bull GmbH Am Brunnen, Austria	Caramel, Riboflavin	
Britvic ginger ale/sour lemon	Britvic Soft Drinks Ltd. Chelmsford CM, England	Caramel, Chlorophyllin	
Reina premium	Alda Drinks Inc.Ltd., Turkey	Caramel	
Rockstar	Fruko Drinks Inc. Ltd., Istanbul, Turkey	Caramel, Riboflavin	Riboflavin gives yellow or yellow-orange color
28 black classic	Splendid Drinks AG Senningeberg, Luxembourg	Caramel	

external behaviors of the patients have an influence on discoloration^[57-59]. The discoloration of the resin composites may be caused by internal or external factors^[35,60-63]. External discolorations result from adsorption and absorption of water-soluble substances through the resin matrix. Internal discolorations are permanent and can be caused by the resin material itself. The resin matrix, the interface between the matrix and the fillers, the type and amount of fillers, and polymer quality has a considerable influence on discoloration^[64]. Studies have shown that internal discolorations are negligible after water storage when composites are completely polymerized^[65,66]. At the same time, colorants, chemical dyes, or inadequately polymerized composites caused significant color changes^[67]. Due to the hydrophobic/hydrophilic property of the resin matrix, the color susceptibilities of the composites vary^[56]. If the resin matrix of the composite is more likely to absorb water, water-soluble pigments such as juices, tea, coffee, and soft drinks will stain composites^[68,69]. Adversely, composites with low water sorption are more susceptible to discoloration by hydrophobic solutions^[59]. Moreover, it became obvious that resins containing bisphenol A glycidyl methacrylate have lower susceptibility to discoloration due to their hydrophilic hydroxide groups than urethane dimethacrylate (UDMA)-containing resins with less hydrophilic aliphatic chains. Properties of fillers do have an important role in discoloration. Some studies revealed that increased filler content improved color stability^[70-72]. Microhybrid composites with high organic filler content were shown to have more color stability than nanofilled and nanohybrid composites after two weeks immersion. The results were attributed to the filler size and morphology of the microhybrid composite^[72]. The poor matrix-filler linkage also resulted in discoloration^[56].

Certain dietary habits such as drinking coffee, tea, cola, red wine, and whiskey or oral habits like tobacco use in a pattern were found to stain composites in varying degrees^[63,73-80]. Energy drinks had a risk of causing discoloration due to their wide variety of ingredients. Varying amounts of caffeine, guarana extract, taurine, and ginseng are the main ingredients of energy drinks^[67]. Besides, the commonly used food dyes have the main responsibility for discoloration. These include Brilliant Blue, Allura Red, and Caramel, among others.

Colorants of commonly used energy drinks are shown in Table 2. All these colorants could be absorbed and penetrated into the organic matrix^[69,76]. There have been research effects about the colorant effect on restorative materials. Composite resins are the first to come up. Erdemir *et al.*^[35] showed that all tested sports drinks (Powerade, Red Bull, Burn) had clinically perceptible ($\Delta E > 3.3$) discoloration on varying composite discs after six months. In the same research, different types of composites had been investigated. At one- and six-month evaluations, nanofill composites showed lower discoloration than microhybrid composites. The smaller the particles of the resin matrix, the smoother the surfaces of the composites. Thus, larger particles were more prone to color stability than the small particles. That was the reason why microhybrid composites had lower color stability than nanofilled composites, which was explained in the study.

Indeed, the resin matrix of composites absorbs water from the environment and spreads it to the whole structure. However, inorganic glass fillers such as silica, SiO₂, are only able to absorb water over their surface^[81,82]. The excessive amount of absorbed water could cause several problems, such as resin matrix plasticization, silane hydrolysis, and micro-cracks formation^[65,66,82]. Through these cracks, colorants could penetrate, and discoloration may occur^[65,83].

The acidic nature of the energy drinks could negatively affect the surface degradation as well. The subsurface ions such as Ca, Al, and silicone would be lost and surface degradation could begin. This may lead to a decrease in wearing resistance and roughening of the surface^[57,65,84]. The roughening of the surface by wear and by chemical degradation may also affect "gloss" and consequently increase the extrinsic staining^[67,85]. A study has reported that resin materials are susceptible to surface roughness degradation after immersion in sports drinks^[63]. The pH's of energy drinks may tend composite resins to erode under acidic conditions. Acids inside these drinks could penetrate into the resin matrix and display the release of unreacted monomers to the environment. This may result in lower surface hardness scores of composite resins^[35].

In addition, surface hardness of a restorative material depends on the duration of exposure time and the composition of the material^[55,86]. However, previous

studies have shown that these beverages potentially cause dental erosion^[2,13], and that may influence the material's mechanical and physical characteristics^[86,87]. Furthermore, the kind of acid in the solutions might have reduced the surface hardness of restorative materials. It has been reported that organic fillers can be damaged by citric acid found in many sports and energy drinks^[88-90]. Briefly, consuming both sports and energy drinks frequently, could damage the composites inside and may shorten the longevity of the restorations.

Other tooth-colored restorative materials

The effect of color stability of glass ionomers and resin-modified glass ionomers has been tested with coffee, red wine, tea, and cola^[65,91,92]. Glass ionomers have hydrophilic properties that may cause water sorption. On the other hand, resin-modified glass ionomers could show more color resistance than conventional glass ionomers due to their resin monomers inside. Gürdal *et al.*^[92] compared the level of color stability of a composite, a conventional glass ionomer, and a poly-acid modified resin composite. After being immersed in mouthrinses for 12 h, the composite resin showed the lower stability followed by conventional glass ionomer and poly-acid modified resin composite. The reason for a poly-acid modified resin composite showing the highest color stability was thought to be the matrix filled with UDMA inside. However, many other studies had conflictive results in favor of both composites and glass ionomers^[65,93,94]. Ceramics have higher susceptibility to discoloration than composites due to their more hydrophobic nature^[56]. Because of this reason, ceramics have not compared with resin composites generally.

CONCLUSION

Dentists have a duty to their patients to give them instructions on the consumption of drinks or foods which can damage dental health. Most food and drinks have little noticeable effects on dental health. Among the drinks that are most likely to damage teeth and restorative materials are sports and energy drinks which contain sugar to feed oral bacterial, and drinks which have a low pH which can erode teeth and increase their sensitivity. Patients who suffer poor oral health because of their over-consumption of sports and energy drinks need to be made aware of the likely causes of their dental problems.

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Concepts and challenges of alveolar ridge preservation and augmentation

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Abstract

The loss of the post-extraction alveolar ridge vertical and horizontal volume constitutes an irreversible process and presents a considerable impact on the prosthetic rehabilitation, particularly when implant-supported. Therefore, alveolar ridge resorption has become a challenge in contemporary clinical dentistry and alveolar ridge preservation and augmentation are an interesting therapeutic approach. The employment of biomaterials, as a therapeutic alternative to preserve bone in height and volume, has been frequently studied over the years, due to its conceptual attractiveness and its simple technique. The purpose of this paper is to review and discuss current methods to optimize the alveolar bone repair while maintaining its horizontal and vertical dimensions. This paper is based on scientific studies published in English including systematic reviews and also animal and human studies that were searched using the keywords "alveolar ridge preservation," "bone substitute", "biomaterials", "bone graft" and "grafting". Either autogenous bone as xenogenic and alloplastic materials, platelet rich plasma and use of membrane are alternatives. It becomes fundamental to understand that alveolar bone loss is still a clinical challenge and alveolar ridge preservation techniques can minimize, but not completely, eliminate the resorption process. The goal of alveolar ridge preservation and augmentation is to use a combination of bone or biomaterials to create bone which is sufficient for dental implant placement. Freeze-dried bone is generally recognized as giving more predictable treatment outcomes than synthetic materials or platelet rich plasma, and membranes must always be used to separate hard and soft tissues to promote optimal tissue healing.

Key words: Alveolar ridge preservation; Tooth extraction; Bone substitute; Bone regeneration

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Core tip: The placement of dental implants generally requires the preservation and augmentation of the alveolar ridge with freeze-dried bone or bone substitutes. Our analysis of animal studies, clinical trials, reviews and meta-analyses has revealed that freeze dried bone, despite its limitations, is still among the most predictable of all the available biomaterials for creating high quality bone that can support dental implants.

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INTRODUCTION

The alveolar healing process can be conceptualized as a combination of biological events which occur from the extraction, aiming the total filling of the dental socket with bone tissue. Immediately after extraction, the alveolus is filled with blood clot mainly composed of erythrocytes and platelets attached to a fibrin system, which will be replaced by a highly vascularized granulation tissue to start the bone formation process inside the alveolus^[1,2]. Preclinical and clinical studies in the absence of bone augmentation have shown that post-extraction alveolar ridge volume loss constitutes an irreversible process which involves both horizontal and vertical reduction, thus, the buccal wall becomes more affected than the lingual wall^[3-5]. Alveolar ridge atrophy can cause a considerable impact on the prosthetic rehabilitation, particularly when implant-supported^[5,6]. Therefore, the alveolar ridge preservation has become a key component of contemporary clinical dentistry^[5,6].

The use of freeze-dried bone and synthetic bone arose in the 80s, as a therapeutic alternative for the maintenance of tooth root, in order to preserve bone density. This approach has gained popularity over the years, due to its conceptual attractiveness and simple technique^[5,7]. However, there are several discussions about the employment of materials for graft procedures, either for autogenous bone as allogenic, xenogenic materials or alloplastic constitute alternatives to be employed.

In previous studies, grafting is reported in reference to autogenous bone, for its osteogenic capacity, considering that it does not trigger a specific immune response^[8]. Frequently, the disadvantages associated with this approach are related to the necessity of a second surgical site, risks of vascular and neurological injuries and postoperative morbidity^[9].

Due to these factors, improvements on the technological development of biomaterials have been made, in

an attempt to influence selectively the tissue response of the receptor site^[10]. This paper aims to review and discuss current methods to optimize the alveolar bone repair while maintaining its horizontal and vertical dimensions.

LITERATURE REVIEW

This paper is based on scientific researches published in English including systematic reviews, including animal and human studies. Case reports and discussion articles were excluded. Studies published in English from 1960 to 2015 were searched in MEDLINE (PubMed) and Bireme databases. The keywords "alveolar ridge preservation," "bone substitute", "biomaterials", "bone graft" and "grafting" were employed for searching.

THE ATROPHY OF THE ALVEOLAR RIDGE AFTER TOOTH EXTRACTION

After tooth extraction, the alveolar bone undergoes an additional atrophy as a result of the natural remodeling process^[11,12]. This process begins immediately after extraction and may result in up to reabsorption of 50% of ridge width within 3 mo^[4]. Studies show higher oral absorption as compared to the lingual wall, and the influence of some factors, such as age, on the amount of horizontal and vertical ridge reabsorption^[12,13]. A recently published systematic review^[14] reported a higher horizontal reduction of the alveolar ridge (29%-63%, 3.79 mm) than vertical bone loss (11%-22%, 1.24 mm in vestibular, 0.84 mm mesial and 0.80 mm distal) in 6 mo. In a long-term study in 2000, Ashman^[15] reported a reduction of 40%-60% alveolar bone height and width, within the first 2-3 years^[2,15,16].

For oral rehabilitation after tooth loss, the preservation of the alveolar ridge is extremely important. These vertical as well as horizontal dimensional changes of the alveolar ridge may complicate the rehabilitation procedure when dental implants are used^[17]. Over recent years, the theme of alveolar ridge preservation has been studied, which was defined as "any procedure undertaken at the time, or following an extraction, designed to minimize external reabsorption of the ridge and maximize bone formation within the socket"^[18]. Many studies using different techniques have been performed.

ALVEOLAR RIDGE PRESERVATION

The autogenous graft and the employment of various synthetic materials are often contraindicated^[19,20] and xenogenous grafts^[21,22] are being widely employed, but some properties of these materials for bone neoformation are being studied, for instance, the employment of different preparations of mineral bovine bone and the period of bone tissue formation^[20]. However, some studies demonstrated histological findings which present partial reabsorption of the material, questioning their

potential for absorption^[23,24].

Therefore, when the objective consists on the preservation of the alveolar ridge, certain factors are critical for the selection and indication of the material, such as, the type of mucosal closure required; gain of horizontal and vertical bone tissue; time required for installing implants; and success rate of implants in the grafted area and the remaining material.

As for bone tissue gain, a systematic review has demonstrated that to preserve the alveolar ridge with bone graft, by employing techniques, is effective, both horizontally and vertically, but loss of bone volume should always be expected^[5,25-32].

In a review study comparing the blood clot with the employment of materials and barrier to preserve the alveolar ridge, it was clinically observed that the mean variation of width in the ridge preservation group was between -1.0 and -3.5 ± 2.7 mm^[12]. In the control group, the variation of width was between -2.5 and -4.6 ± 0.3 mm. These outcomes revealed a statistically lower decrease in the preservation groups (in five out of seven studies). Regarding the ridge height, the average clinical change in the preservation groups was $+1.3 \pm 2.0$ to 0.7 ± 1.4 mm and in the control groups: -0.8 and $-3.6 \pm 1.6 \pm 1.5$ mm^[12]. These results showed that the height reduction of the conservation groups was significantly lower in six out of eight trials^[12]. This study concluded that reabsorption in alveolar ridge may be limited, but cannot be totally eliminated by the employment of grafts or membranes^[12].

In a systematic review and meta-analysis, Avila-Ortiz *et al.*^[5] (2014) observed that alveolar ridge preservation is effective in limiting physiologic ridge reduction, when compared with tooth extraction only. The clinical magnitude of the effect was 1.89 mm in the buccolingual width, 2.07 mm in the midbuccal height, 1.18 mm in the midlingual height, 0.48 mm in the mesial height and 0.24 mm in the distal height. The flap elevation, membrane utilization, and the application of a xenograft or an allograft are associated with superior outcomes, particularly on midbuccal and midlingual height preservation^[5].

Recently, Jambhekar *et al.*^[33] (2015) showed in a systematic review that randomized controlled clinical trials observed the lowest loss of buccolingual width for xenografts (1.3 mm). The allografts showed 1.63 mm, followed by the alloplasts with 2.13 mm, and sockets without any bone substitute: 2.79 mm^[33]. Regarding the loss of buccal wall height, the lowest results were represented by xenografts (0.57 mm) and allografts (0.58 mm). The alloplast and sockets did not demonstrate any grafting (0.77 mm and 1.74 mm, respectively)^[33]. Microscopic evaluation revealed the highest vital bone content for sockets grafted with alloplasts (45.53%). The sockets with no graft material demonstrated 41.07% of vital bone content followed by xenografts and allografts showing 35.72% and 29.93%, respectively^[33]. Regarding the amount of remnant graft material, sockets grafted with allografts demonstrated

the highest value (21.75%), followed by xenografts (19.3%) and alloplasts (13.67%)^[33]. Also, the sockets with no grafting (52.53%) revealed the highest connective tissue content at reentry time, followed by allografts (51.03%), xenografts (44.42%), and alloplasts (38.39%)^[33].

INORGANIC BOVINE BONE GRAFT

Kotsakis *et al.*^[34] (2014) compared the blood clot with inorganic bovine bone and bioactive glass ceramics in human alveolar sockets and analyzed the preservation of the alveolar ridge width, considering that higher alveolar preservation was observed in the group with inorganic bovine bone (1.39 ± 0.57 mm) followed by the group with ceramic bioactive glass (1.26 ± 0.41 mm) and lower preservation in the control group, filled only by blood clot^[34], although this difference did not seem to be clinically significant.

Another randomized study of inorganic bovine bone was suggested in order to compare to a control group^[27]. Histological and histomorphometric analyses were performed from biopsies of 40 sites, 7 mo after the surgery^[27]. As a result, higher horizontal reabsorption was observed in the control group (4.3 ± 0.8 mm) when compared to the bovine graft group (2.5 ± 1.2 mm). The reduction in the ridge height of the vestibular side was 3.6 ± 1.5 mm for the group without graft, whereas it was 0.7 ± 1.4 mm for the graft group. Moreover, the vertical change in the lingual thickness was 0.4 mm in the graft group, and 3 mm in the group without graft^[27]. Histologically, the presence of structured trabecular bone mineralization, as well as particles of grafted material was observed in all samples. The bone formed in the control sites was well structured, with a lower percentage of mineralized bone. The amount of connective tissue was significantly greater in the group without graft than in the graft group^[27]. The approach for ridge preservation, employing bovine bone in combination with collagen membrane, has limited significantly the hard tissue reabsorption, after tooth extraction^[27]. In addition, 7 mo after tooth removal, the histological analysis showed a significantly higher percentage of total trabecular bone mineralized tissue at preservation sites, referring only to the extraction sites^[27].

Munhoz *et al.*^[35,36] (2011 and 2012) evaluated the biomechanical response of previously grafted bone with inorganic bovine bone graft, to titanium implants in rabbits mandible. After periods of 2 and 6 mo, the force necessary to retrieve implants was quantified and no significant difference in removal torque was observed^[35]. Also titanium implants were inserted in the studied areas and after 0, 30, 60, and 180 d the sides were analyzed radiographically and histomorphometrically^[36]. No significant differences were detected in radiographic vertical bone height or bone area. Histologically, bone to implant contact was statistically lower in the control group on day 0 (same day of implantation); however,

a significant increase was observed after 60 and 180 d^[36]. The use of an inorganic xenograft prior to insertion of a titanium implant did not interfere in the course of osseointegration^[36].

In 2000 and 2001, Artzi *et al.*^[23,37] assessed clinically and pathologically, during 9 mo, the behavior of the porous inorganic bovine bone (PBBM) in human alveoli, after extraction. The results of this study clearly show that 9 mo after material insertion into the alveolus, the particles were still in place, even in the apical portion^[23,37]. The studies concluded that the spongy PBBM is a biocompatible filling agent in extraction sites and an acceptable graft to preserve the toothless ridge in sites prepared to receive osseointegrated implants. Besides that, additional studies are necessary to determine the reabsorption capacity, as well as the nature and the importance of the PBBM amorphous organic substance, observed in the grafted particles^[37].

ALLOPLASTIC MATERIALS

The use of bioactive glass was evaluated for alveolar ridge preservation in humans by Clozza *et al.*^[38] in 2012. Subjects who needed titanium implant therapy after tooth extract were grafted and assessed after 1 wk and 3 mo. Alveolar sites treated demonstrated preservation of about 77% of the original width dimensions, with a mean width loss of 1.8 ± 1.1 mm. Moreover, it was observed that vertical loss of the buccal bone was 2.7 ± 1.1 mm, while loss of the lingual bone was 1.9 ± 1.2 mm^[38].

Another study, which demonstrated successful implantation in areas with inorganic bovine bone graft and also with bioactive glass, was carried out by Kotsakis *et al.*^[34] (2014), which showed an overall success rate of 94.1% (16 out of 17 implants were successful), considering that no implant was lost in the bioactive glass group, and one implant failed in the inorganic bovine bone group. Additionally, the study analyzed the torque and primary stability of the implant for each group and concluded that the bioactive glass may be more appropriate to achieve primary stability of implants placed 5-6 mo after extraction^[34]. Conversely, Avila-Ortiz *et al.*^[5] (2014) in a meta-analysis study observed that the use of a xenograft or an allograft presented a beneficial effect in midbuccal alveolar bone height preservation when compared to alloplastic materials^[5] and another study^[33] in 2015 demonstrated that xenografts and allografts revealed lower loss values than any bone substitutes or sockets without grafting^[5,33].

Brkovic *et al.*^[39] studied the preservation of the alveolar bone ridge with β -tricalcium phosphate, with and without the employment of type I collagen membranes, and observed after 9 mo that the horizontal dimension of the alveolar bone ridge had decreased significantly in the group without membrane; there was no significant difference in bone formation between the two groups, with the presence of bone marrow and beta-tricalcium

phosphate^[39]. Both groups demonstrated significant amounts of bone and morphology for implant placement, after a healing period of 9 mo^[39].

Another study evaluated bone regeneration after teeth extraction^[40], comparing histologically bioactive glass ceramic with inorganic bovine graft. Nineteen patients underwent 20 tooth extractions. Ten sites were grafted with bioactive glass and the other 10 with inorganic bovine bone^[40]. The evaluation of bone regeneration and the installation of implants were performed after 4-6 mo of surgery. During the installation procedure of the implants, bone biopsies were taken^[40]. The histomorphometric evaluation revealed that graft residual values were significantly higher in the inorganic bovine graft group (25.60 ± 5.89) in comparison with the bioactive glass group (17.40 ± 9.39)^[40]. The amount of new bone regenerated also was statistically higher in the bioactive glass group (47.15 ± 8.5) in comparison with the inorganic bovine graft group (22.2 ± 3.5)^[40]. The study suggests that bioactive glass seems to be a desirable graft, in addition to increasing bone regeneration, when compared to xenotransplantation of inorganic bovine bone^[40].

PLATELET-RICH PLASMA

The employment of platelet-rich plasma (PRP) constitutes an innovative technique to improve bone healing. Some studies have been conducted in order to verify the effectiveness of PRP with different bone substitutes. These studies have found that PRP, when combined with autogenous bone, provides considerably faster bone regeneration results, radiographically and histomorphometrically, besides indicating denser bone^[41]. Variable successful results have been demonstrated when the PRP is added to an allograft^[42].

A study of Kaur *et al.*^[43] in 2013, evaluated by radiographs the employment of PRP with a hydroxyapatite compound with beta tricalcium phosphate, and compared to a control group. The results suggest radiographic evidence of early bone formation and maturation^[43].

Another animal study performed inferior premolars extraction in 12 beagle dogs and the alveoli were filled with Cerasorb, on the control side, and a Cerasorb mixture of PRP on the test side^[44]. Samples from bilateral biopsies were removed from graft insertion sites 6, 12, and 24 wk after surgery. Six weeks after grafting, proliferation of osteogenic cell mesenchyme was more abundant in the test group^[44]. Histomorphometric data revealed a significantly higher percentage of bone area in the test group (45.9%) than in the control group (30.8%) ($P < 0.05$)^[44]. Twelve weeks after grafting, the test group still presented some advantages over the control group, in terms of bone regeneration (52.5% of bone in the test group vs 49.4% in the control group, $P < 0.05$)^[44]. Twenty-four weeks after grafting, bone forming activity was almost identical in both groups, and the bone area in the two groups did not differ

significantly (62.9% and 61.9%, respectively; $P < 0.05$)^[44]. The results histomorphometrically suggested stronger bone regeneration in the early healing phase, after PRP topical application use^[44].

MUCOSAL CLOSURE

Regarding the mucosal closure in the surgery, healing is ideally recommended. However, Meloni *et al.*^[45] (2015) showed in their study that inorganic bovine bone graft can be maintained either with epithelial conjunctive tissue flap or with collagen matrix, which showed no difference in the results. Although there were no statistically significant differences between the groups, after 5 mo of tooth extraction, it is noteworthy to emphasize that the collagen matrix is more appropriate, because a second surgical site is not required in this case^[45]. The same study reports a 100% implant success rate, considering that the 30 patients showed no failure or complications after one year of the installation procedure^[45]. Furthermore, there were no statistically significant differences between the two groups in peri-implant marginal alterations at bone level (difference: 0.07 ± 0.11 mm; 95%CI: $-0.02-0.16$, $P = 0.41$) after one year of implant placement^[45].

Alveolar ridge preservation with membrane utilization resulted in statistically significantly less reabsorption in ridge width and height, compared to socket for natural healing^[12,32,46]. The association of bone graft and membrane resulted in statistically significantly less reabsorption, horizontally^[26,27] and vertically^[26] in comparison to naturally socket healing. The histological evaluation demonstrated new bone formation with presence of graft particles^[26,27].

Avila-Ortiz *et al.*^[5] (2014), in a meta-analysis study, also observed that membrane use had a strong beneficial effect on the preservation of midbuccal and midlingual alveolar bone height.

In another systematic review study^[12] comparing sockets left healing naturally and the use of membranes, it was observed clinically that alveolar bone width variation was between -1.0 and -3.5 ± 2.7 mm for experimental groups and -2.5 and -4.6 ± 0.3 mm for control groups, leading an important preservation of the experimental groups (five of seven studies)^[12]. The experimental groups demonstrated a change in alveolar bone height from $1.3 \pm 0.7 \pm 1.4$ and 2.0 mm and between -0.8 and $-3.6 \pm 1.6 \pm 1.5$ mm, in the control groups^[12]. Regarding the preservation of height, the experimental groups were significantly higher when evaluated six of eight studies^[12]. The conclusion of this review was that the resorption of the alveolar edge may be reduced, however cannot be completely eliminated by the employment of membranes or grafts^[12].

Hoffman *et al.*^[47] investigated clinically alveolar socket regeneration using polytetrafluoroethylene (PTFE) membranes of high density (dPTFE) without the use of graft materials. A total of 276 alveolar sockets were obtained, which were flaps and a dPTFE

membrane was placed on the site. Primary closure was not obtained with the use of these membranes^[47]. Cemento-enamel junctions of adjacent teeth were used as reference points. The measurements have been taken immediately post extraction and 12 mo after surgery in the same areas. Hard tissue biopsies were taken from 10 representative cases after 12 mo, during implant placement^[47]. A strict oral biofilm control scheme was applied in all individuals during the observation period. The study showed that there was a significant preservation of the volume, indicating that the newly formed tissue in the extraction sites was essentially bone^[47]. In addition, the study pointed out that there was no influence of gender, smoking, age or clinical bone level before treatment on the results. The study concluded, then, that the use of dPTFE membranes was effective in preservation of soft and hard tissues in alveolar sockets after teeth extraction^[47].

Still, regarding the use of membranes, a systematic review was conducted to evaluate the efficacy of barrier membranes in alveolar bone preservation^[48,49]. A total of 3986 manuscripts were found in the initial search and 34 studies met the inclusion criteria^[48]. Four animal studies concluded that the use of membrane increases the amount of bone (mean difference of 0.32 mm). The qualitative results about horizontal bone augmentation were controversial^[48]. The membranes have not increased the risk of inadequate healing, according to both human (odds ratio, 5.67) and animal studies (odds ratio, 3.35)^[48]. This study concluded that there is limited evidence for the effectiveness of barrier membrane in the treatment of bone defects. Most of the results are based on animal studies^[48]. More randomized clinical trials are needed to measure objectively the effectiveness of membranes in alveolar ridge preservation^[48].

TIME OF IMPLANTATION AFTER ALVEOLAR RIDGE PRESERVATION

The time of implantation after alveolar ridge preservation varies across studies. Studies in rabbits using inorganic bovine bone graft showed a lower time of implantation, after 2^[35,36] and 3 mo^[24].

Human studies filled with the same graft showed time of implantation of 6^[50,51] and 7 mo^[52]. Allografts showed the same period of 6 mo^[53] and alloplastic materials varied from 3^[54] to 6 mo^[40,55]. The use of grafting materials for alveolar ridge preservation seems to delay the rehabilitation process in 6 mo at most cases.

CONCLUSION

The goal of alveolar ridge preservation and augmentation is to use a combination of bone or biomaterials to create bone which is sufficient for dental implant placement. Freeze-dried bone is generally recognized as giving more predictable treatment outcomes

than synthetic materials or platelet rich plasma, and membranes must always be used to separate hard and soft tissues to promote optimal tissue healing.

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Retrospective Study

Tooth agenesis and craniofacial morphology in pre-orthodontic children with and without morphological deviations in the upper cervical spine

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Abstract

AIM: To analyze differences in prevalence and pattern of tooth agenesis and craniofacial morphology between non syndromic children with tooth agenesis with and without upper cervical spine morphological deviations and to analyze associations between craniofacial morphology and tooth agenesis in the two groups together.

METHODS: One hundred and twenty-six pre-orthodontic children with tooth agenesis were divided into two groups with (19 children, mean age 11.9) and without (107 children, mean age 11.4) upper spine morphological deviations. Visual assessment of upper spine morphology and measurements of craniofacial morphology were performed on lateral cephalograms. Tooth agenesis was evaluated from orthopantomograms.

RESULTS: No significant differences in tooth agenesis and craniofacial morphology were found between children with and without upper spine morphological deviations (2.2 ± 1.6 vs 1.94 ± 1.2 , $P > 0.05$) but a tendency to a different tooth agenesis pattern were seen in children with morphological deviations in the upper spine. In the total group tooth agenesis was associated with the cranial base angle (n-s-ba, $r = 0.23$,

$P < 0.01$), jaw angle (ML/RLar, $r = 0.19$, $P < 0.05$), mandibular inclination (NSL/ML, $r = -0.21$, $P < 0.05$), mandibular prognathia (s-n-pg, $r = 0.25$, $P < 0.01$), sagittal jaw relationship (ss-n-pg, $r = -0.23$, $P < 0.5$), overjet ($r = -0.23$, $P < 0.05$) and overbite ($r = -0.25$, $P < 0.01$).

CONCLUSION: Etiology of tooth agenesis in children with upper spine morphological deviations was discussed. The results may be valuable for the early diagnosis and treatment planning of non syndromic children with tooth agenesis.

Key words: Children; Tooth agenesis; Upper cervical spine morphology; Craniofacial morphology

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Core tip: Tooth agenesis and craniofacial morphology was examined in non syndromic children with upper cervical spine morphological deviations. No significant differences in tooth agenesis and craniofacial morphology were found between children with and without upper spine morphological deviations, but a non-significant tendency of a different tooth agenesis pattern between the groups was seen. In the total group significant associations between tooth agenesis and craniofacial morphology were found. A different aetiology for tooth agenesis in children with morphological deviations in the upper spine was suggested. The results may be valuable for the early diagnosis and treatment planning of non syndromic children with tooth agenesis.

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INTRODUCTION

Tooth agenesis is a common congenital malformation that can occur either as an isolated finding or as part of a syndrome^[1]. The complex and multifactorial etiology behind tooth agenesis is yet to be fully understood^[2,3]. Tooth agenesis can occur as a result of mutations in genes involved in normal tooth development. Defects in the MSX-I and Sonic Hedgehog genes have been identified as causing tooth agenesis^[2]. Furthermore, normal tooth development is dependent on the maturation of the bone surrounding the tooth germ and the nerve innervation of the teeth^[3,4].

The prevalence of tooth agenesis among a healthy Danish population is between 7.8% and 8.2%^[3,5]. Agenesis of the mandibular second premolar is most often observed (4.1%), followed by the maxillary

second premolar (2.2%), the maxillary lateral incisors (1.7%) and the mandibular central incisors (0.2%)^[6].

Previous studies have found an association between tooth agenesis and craniofacial morphology in non syndromic individuals^[5,7-12]. It is generally agreed that tooth agenesis affects the craniofacial morphology in the sagittal and vertical dimension and that the deviation in the craniofacial morphology is associated with the prevalence and pattern of tooth agenesis^[5,7-11]. In patients missing more than 12 teeth the prognathia of the mandible was more pronounced and the face was more squared compared to patients with less tooth agenesis^[10].

The craniofacial morphology is also associated with upper cervical spine morphology in non syndromic individuals. In patients with severe skeletal malocclusion traits such as skeletal deep bite, skeletal open bite, skeletal maxillary and mandibular overjet, the prevalence of morphological deviations in the upper cervical spine was significantly higher compared to subjects with neutral occlusion and normal craniofacial morphology^[13-16]. The pattern of morphological deviations in the upper cervical spine in these patients with severe skeletal malocclusions indicated fusion between the second and third cervical vertebra, block fusion between the second, third and fourth cervical vertebrae, occipitalization as assimilation of the first cervical vertebra with the occipital bone and partial cleft of the first cervical vertebra^[13-16]. Furthermore, deviations of the upper cervical spine morphology were significantly associated with a large cranial base angle, retrognathia of the jaws and a large inclination of the jaws^[13-16].

As associations between tooth agenesis and craniofacial morphology and associations between craniofacial morphology and upper cervical spine morphology have been described there may be an association between upper cervical spine morphology and tooth agenesis. To our knowledge the relation between upper cervical spine morphology and tooth agenesis has not yet been investigated.

Therefore, the aims of the present study are: (1) to analyze the differences in prevalence and pattern of tooth agenesis and craniofacial morphology between non syndromic children with tooth agenesis with and without upper cervical spine morphological deviations; and (2) to analyze the associations between craniofacial morphology and tooth agenesis in the two groups together.

MATERIALS AND METHODS

The materials included cephalograms and orthopantomograms from non syndromic pre-orthodontic children registered between 1966 and 1997 at the orthodontic clinic, Muncipal Dental Service of Farum, Denmark. All the children with tooth agenesis that met the below inclusion criteria were included in the study: Children between 8 and 18 years old referred for orthodontic treatment before the orthodontic treatment began;

Table 1 Mean (X-bar) and SD of number of tooth agenesis and craniofacial morphology in children with and without upper spine morphological deviations

Variable	With upper spine deviations (n = 19)		Without upper spine deviations (n = 107)		Group P
	X-bar	SD	X-bar	SD	
Age	11.9	1.2	11.5	2	NS
No. ageneses	2.2	1.6	1.94	1.2	NS
n-s-ar	123.4	4	123.5	4.7	NS
n-s-ba	130	4.3	130.2	4.4	NS
ML/RLar	121	4.9	123.1	6.5	NS
s-n-ss	81.5	3.4	80.5	4	NS
s-n-pg	79.5	3.2	78.7	3.9	NS
ss-n-pg	2	1.7	1.8	2.6	NS
NSL/NL	8	2.7	7.1	3.6	NS
NSL/ML	30.4	4.5	31.6	5.6	NS
NL/ML	22.4	4.3	24.5	5.4	NS
Overjet	5	2.4	4.4	2.2	NS
Overbite	3.8	2	3.2	1.7	NS

NSL: Nasion-Sella line; NL: Nasal line; ML: Mandibular line; NS: Not significant, unpaired *t* test.

one orthopantomogram and one lateral cephalogram before orthodontic treatment; agenesis of at least one permanent tooth, excluding the third molars; the first five cervical vertebrae visible on the lateral cephalogram. The exclusion criteria were: Children with known craniofacial or other syndromes; children with no tooth agenesis, excluding the thirds molars; children with insufficient medical records and X-rays.

A total of 126 children met these criteria and were included in the present study: 62 girls (aged 8-16 years, mean 11.32 years) and 64 boys (aged 8-16 years, mean 11.7 years) with an overjet ranging between -2.5 and 11 mm (mean 4.5 mm) and with an overbite ranging between -5 and 8 mm (mean 3.3 mm). According to the upper cervical spine morphology the children were divided into two groups: One group with upper cervical spine morphological deviations consisted of 19 children, 12 boys and 7 girls aged 9-14 years (mean age 11.9) and one group without upper cervical spine morphological deviations consisted of 107 children, 52 boys and 55 girls 8-16 years (mean age 11.4).

The study was approved by the Danish Data Protection Agency (No. 2013-54-0509).

Tooth agenesis was registered on orthopantomograms and the craniofacial and upper cervical spine morphology was registered on lateral cephalograms.

Registration of tooth agenesis

The registration of tooth agenesis was performed by visual assessment of the orthopantomograms. Only the permanent dentition was analyzed and the third molars were excluded from the study. Each registration on the orthopantomogram was compared with the individual child's medical record and available information of the dentition. Only tooth agenesis where a tooth and its tooth bud was missing from the orthopantomogram and no history of extraction could be found in the

Table 2 Pattern of tooth agenesis in children with and without upper spine morphological deviations

Variable	With upper spine deviations (n = 19)		Without upper spine deviations (n = 107)		Group P
	n	%	n	%	
Gender					
Male	12	63.2	52	48.6	NS
Female	7	36.8	55	51.4	NS
Multiple ageneses	1	5.3	5	4.7	NS
Agenesis localization					
Mandible	16	84.2	81	75.7	NS
Maxilla	8	42.1	52	48.6	NS
Both jaws	5	26.3	26	24.3	NS
Agenesis tooth					
Incisor	3	15.8	25	23.4	NS
Canine	0	0.00	2	1.9	NS
Premolar	17	89.5	85	79.4	NS
Molar	2	10.5	3	2.8	NS
Several tooth groups	3	15.8	7	6.5	NS

NS: Not significant, Fisher's exact test.

corresponding medical record was registered. The registration included: Number of missing teeth; registration of multiple tooth agenesis (more than 4 missing teeth^[10]); location of the tooth agenesis with regards to which jaw; agenesis pattern with regards to which tooth group (Tables 1 and 2).

Registration of upper cervical spine morphology

The cephalograms were studied for deviations in the morphology of the first five cervical vertebrae by visual assessment according to Sandham^[17] and divided into two groups: Posterior arch deficiencies (PAD) and fusion anomalies. PAD consists of partial cleft and dehiscence. Partial cleft is defined as lack of fusion of the posterior arch^[18] (Figure 1). Dehiscence is defined as inadequate development of a portion of the vertebra^[18]. Fusion anomalies consist of fusion, block fusion and occipitalisation. Fusion is defined as fusion of two vertebrae at the articular facets, the posterior arch or the transverse process (Figure 2). Block Fusion is defined as fusion of more than two vertebrae at the vertebral bodies, the articular facets, the posterior arch or the transverse processes. Occipitalisation is defined as partial or complete fusion of the atlas (C1) with the occipital bone^[17,18]. Morphological deviations were only registered if they were visible on all the cephalograms available in the medical record of the child. If, in the visual assessment of a cephalogram, any doubts occurred about the presence of morphological deviations, the subject was registered as having no morphological deviations in the upper spine. All cephalograms were reviewed together with supervisor LS.

Registration of the craniofacial morphology

The craniofacial morphology was registered on lateral cephalograms of the children standing in the standardized head posture with their teeth in occlusion according to Siersbæk-Nielsen *et al*^[19]. Twelve reference



Figure 1 Illustration of partial cleft of C1 marked by circle.

points were digitalized on cephalograms using the TIOPS™ software (Tiops 2005, Version 2.12.4) and nine angular measurements were measured according to Siersbæk-Nielsen *et al.*^[19]. Because the cephalograms were not scanned in a 1:1 scale, the overbite and overjet was measured by hand on analog cephalograms and taking into account the magnification of 5.6%. The points and lines are illustrated in Figure 3 and the mean values are shown in Table 1.

Reliability of the method

The reliability of the variables describing the cranial base and the vertical and sagittal craniofacial dimensions was assessed by re-measuring 25 lateral cephalograms selected at random from the previously evaluated cephalograms. The lateral cephalograms were marked and measured again, and paired *t* test found significant differences between the two sets of recordings related to the measurement of NSL/NL, NL/ML and ML/RLar. Since the pterygomaxillary point (Pm) is included in both NSL/NL and NL/ML, the location of the point was discussed and redefined. Subsequently, paired *t* test found no significant differences between the two sets of recordings. The method errors calculated by Dahlberg's formula ranged from 0.01 to 1.32 degrees^[20] and the Houston reliability coefficient from 0.89 to 1.00^[21]. The reliability was within the average range as traditional film-based radiographs^[22]. The reliability of the visual assessment of the morphological characteristics of the cervical vertebral units has previously been reported ($k = 0.82$)^[23].

Statistical analysis

Regarding the craniofacial dimensions, the effect of age was assessed by linear regression analysis and for the occurrence of morphological deviations of the cervical column by logistic regression analysis. Differences in means of the craniofacial dimensions and number of tooth agenesis between genders and between the groups were assessed by unpaired *t* test. Differences in tooth agenesis pattern between genders and between the groups were assessed by Fisher's exact test. Associations between tooth agenesis and craniofacial morphology and the possible effect of age and gender



Figure 2 Illustration of fusion between the second and third vertebrae at the articular facet marked by circle.

were tested by linear regression analyses. The results were considered significant at *P* values below 0.05. The statistical analyses were performed using SPSS 20.00 (Inc., Chicago, Illinois, United States).

RESULTS

No significant age and gender differences were found between children with and without morphological deviations in the upper cervical spine (Tables 1 and 2). In the group of children with morphological deviations in the upper spine (15.1% of the total group) the deviations occurred only as fusion between the second and third vertebra (42.3%) and partial cleft of the atlas (63.2%). Both morphological deviations occurred in 5.3% of the children with morphological deviations in the upper spine.

No statistically significant differences in tooth agenesis and craniofacial morphology were observed between children with and without morphological deviations in the upper spine. However, in children with morphological deviations in the upper spine a tendency to a different tooth agenesis pattern was seen as a larger occurrence of molar agenesis and agenesis of several tooth groups compared to the children without morphological deviations in the upper spine (Table 2).

In the total group, statistically significant associations were found between tooth agenesis and craniofacial morphology (Table 3). Multiple agenesis was positively associated with the gonial angle (ML/RLar; $P < 0.05$) and significantly negatively associated with horizontal overjet ($P < 0.05$) and vertical overbite ($P < 0.01$; Table 3). Agenesis of incisors was negatively associated with the sagittal jaw relationship (ss-n-pg; $P < 0.01$). Agenesis of premolars was significantly positively associated with the cranial base angle (n-s-ba, $P < 0.01$) and the sagittal jaw relationship (ss-n-pg, $P < 0.05$; Table 3). Agenesis of the molars was significantly positively associated with the mandibular prognathia (s-n-pg, $P < 0.01$) and significantly negatively associated with the sagittal jaw relationship (ss-n-pg, $P < 0.05$) and the mandibular inclination (NSL/ML, $P < 0.05$; Table 3).

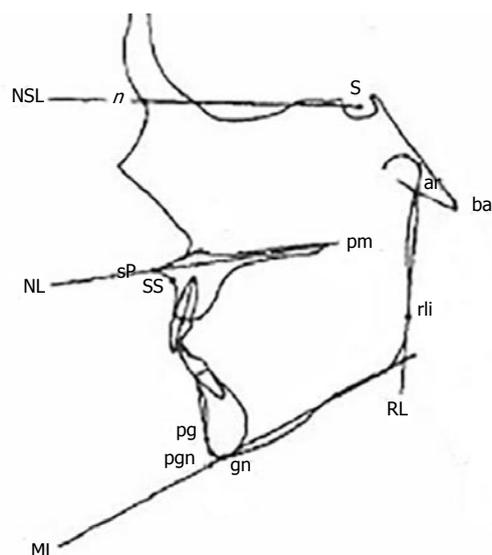


Figure 3 Illustration of the points and lines according to Siersbæk-Nielsen *et al*^[19]. NSL: Nasion-Sella line; ML: Mandibular line; NL: Nasal line.

DISCUSSION

The present study has analyzed the differences in tooth agenesis and craniofacial morphology in pre-orthodontic children with tooth agenesis with and without upper cervical spine morphological deviations. To our knowledge this has not previously been reported in the literature. Additionally, the associations between tooth agenesis and craniofacial morphology in the two groups together were investigated.

In the total group of 126 non syndromic children with tooth agenesis, 15.1% had morphological deviations in the upper cervical spine which is in agreement with previous reported occurrence of morphological spine deviations in healthy adults with neutral occlusion, no tooth agenesis and normal craniofacial morphology (14.3%)^[18]. Previous studies have shown that patients with severe skeletal malocclusions such as large overjet and overbite had a significantly higher occurrence of upper spine morphological deviations compared to controls^[13-16]. Therefore a higher occurrence of morphological deviations in the upper spine was expected in the present study of children with tooth agenesis. One explanation for the relatively low occurrence of morphological deviations in the upper spine could be that the mean values for the overjet and overbite in the present study was within normal range and therefore children with severe malocclusion was few.

In the present study, no statistically significant differences in prevalence or pattern of tooth agenesis were found between non syndromic children with and without upper cervical spine morphological deviations. However, the non syndromic children with morphological deviations in the upper spine did show a tendency to have a greater percentage of molars agenesis and agenesis of several tooth groups compared to children without upper spine morphological deviations. In a healthy non syndromic population agenesis of the third

Table 3 Significant associations tested for age and gender effect between tooth agenesis and craniofacial morphology in the total group

	n-s-ba	ML/RLar	s-n-pg	ss-n-pg	NSL/ML	Overjet	Overbite
Multiple ageneses		0.19 ^a				-0.23 ^a	-0.25 ^b
Agenesis of incisor				-0.24 ^{b,c}			
Agenesis of premolar	0.23 ^b			0.22 ^a			
Agenesis of molar			0.25 ^b	-0.23 ^a	-0.21 ^a		

^a*P* < 0.05, linear regression; ^b*P* < 0.01, linear regression; ^c*P* < 0.05 gender effect. NSL: Nasion-Sella line; ML: Mandibular line.

molars are often seen, but agenesis of first and second molars as reported in children with morphological deviations in the upper spine in the present study almost never occurs^[2,5] because normal tooth development is dependent on the maturation of the bone surrounding the tooth germ and the nerve innervation of the teeth^[3,4]. Therefore it may be hypothesized that the etiology of tooth agenesis could be different in non syndromic children with morphological deviations in the upper spine as the tooth agenesis does not follow the normal pattern of tooth agenesis according to the nerve innervation. Previously, an association between the craniofacial skeleton and the upper cervical spine has been established^[24-26]. An explanation for the association between the craniofacial skeleton including the jaws and teeth and the cervical spine could be found in the early embryogenesis. The notochord determines the development of the cervical spine, especially the vertebral bodies, and also the basilar part of the occipital bone in the cranial base which is the posterior part of the cranial base angle^[27-33]. The para-axial mesoderm forming the vertebral arches and remaining parts of the occipital bone is also formed from the notochordal inductions. Therefore, a deviation in the development of the notochord may influence the surrounding bone tissue in the upper spine as well as the posterior part of the cranial base to which the jaws including the teeth are attached^[24-26]. Only a non-significant tendency of differences in tooth pattern between children with and without morphological deviations in the upper cervical spine was found in the present study. This may be because the malocclusion and tooth agenesis were not extreme in the present sample and therefore a clear pattern could not be found.

Surprisingly, no statistically significant differences in the craniofacial morphology between the children with and without upper cervical spine morphological deviations were found. Previously, it has been shown that deviations of the upper cervical spine morphology were significantly associated with a large cranial base angle, retrognathia of the jaws and a large inclination of the jaws in non syndromic patients with severe skeletal malocclusion^[13-16,18]. Therefore it was expected to find a

difference in the craniofacial morphology between the two groups in the present study.

In agreement with previous studies^[5,7-12] an association between tooth agenesis and the craniofacial morphology was found in the present study. In general, it was found that tooth agenesis was positively associated with the cranial base angle, gonial angle and the mandibular prognathia and negatively associated with the sagittal jaw relationship (except from agenesis of the premolars), mandibular inclination, overjet and overbite in the present study. The pattern of the association between the craniofacial morphology and tooth agenesis was in agreement with previous studies of non syndromic individuals^[5,7-12].

In conclusion no significant differences in tooth agenesis and craniofacial morphology were found between the groups of children with and without morphological deviations in the upper spine, but a non-significant tendency to a different tooth agenesis pattern between the groups was seen. In the total group significant associations between tooth agenesis and craniofacial morphology were found. A different etiology for tooth agenesis in children with morphological deviations in the upper spine was suggested as these children may have a tendency for developing a different tooth agenesis pattern compared to children without upper spine morphological deviations. The results may be valuable in the early diagnosis and treatment planning of non syndromic children with tooth agenesis.

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COMMENTS

Background

Associations between tooth agenesis and craniofacial morphology as well as associations between craniofacial morphology and upper cervical spine morphology have previously been found. The relation between upper cervical spine morphology and tooth agenesis has not yet been investigated.

Research frontiers

Previously, an association between the craniofacial skeleton and the upper cervical spine has been established. An explanation for the association between the craniofacial skeleton including the jaws with the teeth and the cervical spine could be found in the early embryogenesis as a deviation in the development of the notochord.

Innovations and breakthroughs

As the relation between upper cervical spine morphology and tooth agenesis has not previously been investigated, the results of the present study may be a breakthrough in etiological and diagnostics considerations in non syndromic children with tooth agenesis.

Application

Children with upper spine morphological deviations may have a tendency for developing a different tooth agenesis pattern compared to children without upper spine morphological deviations. Therefore a different etiology for tooth agenesis in children with morphological deviations in the upper spine was suggested. The results may be valuable in the early diagnosis and treatment planning of non syndromic children with tooth agenesis.

Peer-review

This is a report of a well conducted study. The pattern of tooth agenesis in patients with or without upper cervical spine anomalies was investigated and presented.

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