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EDITORIAL

Controversy of silver amalgam as a restorative material

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Abstract

Silver amalgam contains mercury leading to concerns about the potential toxic effects of amalgam on the health of dental patients. The debate over the toxicity of silver amalgam restorations has divided the dental profession for over a century. The use of amalgam restorations for anterior teeth have been declining worldwide due to patient's safety concerns and preference for tooth colored restorations. Nevertheless, amalgam has served the dental profession for decades and benefited hundreds of millions of patients because of its longevity as a dental restorative material. Amalgam is still the World's most widely used restorative material for posterior teeth.

Key words: Esthetic resorations; Silver amalgam; Toxicity

Core tip: This editorial highlights the importance of silver amalgam restoration in restorative dentistry.

Ballal V. Controversy of silver amalgam as a restorative material. *World J Stomatol* 2015; 4(2): 35-36 Available from: URL: http://www.wjgnet.com/2218-6263/full/v4/i2/35.htm DOI: http://dx.doi.org/10.5321/wjs.v4.i2.35

INTRODUCTION

The most widely used dental restorative material for posterior teeth is silver amalgam. Amalgam is naturally adhesive to teeth and is long-lasting, for these reasons Amalgam has served the dental profession for decades. Although there has been scientific and political efforts to stop Amalgam being used as a dental restorative material (Minamata Convention on Mercury, January 2013)[1]. The continued use of Amalgam in dentistry is controversial since many people believe its use should be prohibited. However, attempts to completely replace Amalgam with composite resins and other dental materials has failed because no other materials can match Amalgam in terms of its low cost, ability to withstand wear and breakage, and its longevity as a restorative material. These are the properties which makes Amalgam the first choice of most of the Worlds dentists for posterior restorations. Silver amalgam has distinctive qualities which endears itself to the clinician. Considering its uniqueness as a substantial restorative material, compared to other commercially available tooth colored/esthetic materials, which can also be toxic[2,3]. The advantages of amalgam must generally outweigh its dangers, because it is still widely used, even though some countries have prohibited its use in dentistry. Very few articles in the, scientific literature associate dental amalgam with toxic effects

or damage to the health of patients, therefore it seems over-reactive to prohibit the use of Amalgam for the restoration of teeth. Instead, researchers should be developing improved formulations of Amalgam to reduce its potential for toxicity and to improve its clinical performance.

Toxic materials are sometimes needed in dentistry. Sodium hypochlorite is extremely toxic[4,5], but is the most widely used root canal irrigating solution in endodontics[4,5]. Radiation used to take x-rays can be dangerous in high doses. Some wavelengths of light, such us ultraviolet light can cause damage to eyes, and can be linked to deleterious health effects like cancer, depressions, heart disease, *etc*[6]. Despite the potential health risks, toxic materials and radiation are still commonly used in dentistry, because similar to Amalgam the risks can be managed to avoid causing harm to patients. This explains why even with so much controversy, that Amalgam is still the most widely used restorative material for posterior teeth.

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EDITORIAL

Periosteal pedicle graft for the treatment of gingival recession: A viable alternative to sub-epithelial connective tissue graft

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Abstract

Treating gingival recessions is important to satisfy the functional and aesthetic needs of the patients. Among various available techniques to treat gingival recessions, the subepithelial connective tissue graft technique is still considered to be the best despite its inherent disadvantages. The recent innovation utilising periosteum as a pedicle graft to treat gingival recession defects has drawn considerable attention and may provide a viable alternative to subepithelial connective tissue graft.

Key words: Periosteal pedicle graft; Gingival; Recession; Connective tissue; Graft

Core tip: The periosteal pedicle graft (PPG) is an emerging technique to treat gingival recession defects and has advantages over subepithelial connective tissue graft (SCTG). The technique not only provides a viable treatment option to manage gingival recessions without involving two surgical sites and additional cost but also produced results which have raised the question whether PPG can replace SCTG in near future?

Mahajan A. Periosteal pedicle graft for the treatment of gingival recession: A viable alternative to sub-epithelial connective tissue graft. *World J Stomatol* 2015; 4(2): 37-38 Available from: URL: http://www.wjgnet.com/2218-6263/full/v4/i2/37.htm DOI: http://dx.doi.org/10.5321/wjs.v4.i2.37

INTRODUCTION

Gingival recession defect (GRD) is among the most common condition for which the patients seek professional dental care. If neglected, gingival recession may not only result in functional problems like dentinal hypersensitivity and root caries but may also lead to unaesthetic facial appearance. The consequences of gingival recession defects are well recognized by the dental professionals and numerous treatment options have been suggested to resolve GRD. Among all the techniques utilised to treat GRD, ranging from restorative to prosthetic to surgical measures, the mainstay of treatment is still the periodontal plastic surgery. The surgical techniques applied to cover denuded root surfaces mainly utilize soft tissue autografts, which may be either free or pedicle and

harvested adjacent to the GRD or from the palate. Although many techniques have been proposed for the treatment of GRD, a detailed review of the scientific literature clearly rates the Sub-epithelial connective tissue graft (SCTG) better than all other techniques owing to the excellent post treatment aesthetic outcomes and sustained long term results associated with the SCTG[1]. Despite the fact that SCTG is considered to be the gold standard for the treatment of GRD the search for a technique which eliminates the inherent limitations associated with SCTG (two surgical sites, increased patient trauma, postoperative complications) is still on. The use of acellular dermal matrix graft and GTR membranes has also been proposed to improve patient centred outcomes in addition to clinical outcomes but the techniques have failed to gain the popularity due to the associated increased cost of treatment and uncertain predictability of these procedures[2]. Recently, the use of periosteum has been suggested for the treatment of GRD and has drawn considerable attention[3]. Although the use of periosteum in regenerative therapies is not new and it has been used successfully in the treatment of bony defects by the oral and maxillofacial surgeons,orthopaedicians and periodontal surgeons[4,5] but the idea to utilize the periosteum as a pedicle graft for treatment of soft tissue defects like GRD is innovative and interesting. The detailed technique utilizing the periosteum as a pedicle and the term "Periosteal Pedicle Graft (PPG) "for the treatment of single tooth GRD were first published in the Australian dental journal in 2009[3] and later the technique was successfully used to treat adjacent multiple gingival recession defects for the first time in 2011[6].

Since the invention of the PPG technique multiple studies have been done and have shown encouraging results both in terms of root coverage and patient satisfaction[7-11], the reasons suggested for the successful treatment outcomes include: (1) PPG can be harvested adjacent to the GRD eliminating the use of second surgical site thus minimising intra-operative trauma and postoperative complications; (2) There is no limitation to the amount of the graft that can be harvested in case of PPG hence PPG can be used effectively to treat multiple adjacent gingival recession defects; (3) Since periosteum is highly vascular and PPG is ideal for placement over avascular root surfaces; (4) Owing to the presence of stem cells in the periosteum there is an actual possibility of new attachment during the healing period; and (5) Patients are more satisfied with procedures which require minimum intra-operative trauma and postoperative complications hence PPG scores better in terms of patient satisfaction over SCTG.

Considering the above facts and current evidence it may be concluded that PPG has emerged as a viable option for the treatment of GRD with a great possibility to regenerate the lost periodontal tissues and form a new attachment at the treated gingival recession site, although it is still uncertain whether it will achieve the status at par with SCTG because for that to happen the technique will have to pass the test of time.

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REVIEW

Platelet preparations in dentistry: How? Why? Where? When?

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Abstract

The aim of this article is to review the outcomes of platelet preparations in dentistry. A structured electronic search discovered 348 articles, which described the use of autologous platelet concentrates with a relevance to clinical dentistry. Among these articles, 220 articles investigated platelet rich plasma, 99 investigated platelet rich fibrin, 22 investigated plasma rich in growth factors and 7 investigated the use of concentrated growth factors. Several studies reported beneficial treament outcomes in terms of enhanced bone and soft tissue regeneration.

Key words: Platelet concentrates; Platelet rich plasma; Platelet rich fibrin; Concentrated growth factors; Growth factors; Dentistry; Plasma rich in growth factors

Core tip: Autologous platelet concentrates (platelet-rich plasma, platelet rich fibrin, plasma rich in growth factors, concentrated growth factor), are blood derivatives, prepared from patient's own blood, reach in platelets, growth factors and cytokines, which can be used to promote guided tissue regeneration in dentistry and oral surgery.

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INTRODUCTION

Bone and soft tissue regeneration is frequently required in dentistry, mainly but not exclusively for implantology and periodontology. Tissue regeneration is a complex process of healing and tissue growth, which involves different biological elements and strategies. These include the use of bone grafts[1,2], biomaterials and growth factors[3], natural or synthetic scaffolds and more recently the use of stem cells[4,5]. Nowadays, a whole range of modern surgical procedures and a variety of dental materials are available. These are performed to reconstruct bony defects of the upper and lower jaw and for augmentation of lost structures of the residual alveolar ridge. Autologous platelet concentrates are a promising and innovative therapeutic approach in various medical fields, including dentistry[6-10]. Platelets play a crucial role not only in hemostasis, but also in the

wound healing process, as they are reservoirs of growth factors and cytokines, which in turn are key promoters for bone and soft tissues regeneration. After platelets are activated, they become trapped within a fibrin matrix and release growth factors. Together the fibrin can form a scaffold and the growth factors can stimulate tissue healing and regeneration repair responses. An improved understanding of the physiologic properties of platelets in wound healing over the last two decades, has led to more successful therapeutic applications, especially in oral surgery.

Platelet concentrates

Platelet concentrates are blood derivatives[11,12], prepared from the patient's own blood and containing autologous platelets, growth factors and cytokines involved in the key processes of tissue regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. Platelets are packed with secretory granules, which are necessary to fulfill their functions. There are three types of secretory granules, α granules are the most abundant and have a high protein content. The granules contain cytokines and growth factors, such as vascular endothelial growth factor, epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, hepatocyte growth factor and the insulin-like growth factor as well as several others. The release of these growth factors from activated platelets can promote healing in both soft and hard tissues.

Most platelet concentrate preparations used in guided tissue regeneration surgery are termed Platelet-Rich Plasma (PRP), even if they differ slightly according to their preparation from a patients peripheral blood. These variations include differences in centrifugation speeds and times, differences in adding chemicals, and differences in the selection of supernatants and precipitates. These variations can cause differences in fibrin network structures and in platelets, leucocyte and growth factors content. Therefore the term PRP alone can be non-specific, because it does not define the actual preparation protocol. Depending on the leukocyte content and fibrin architecture, five main categories of PRPs can be defined: (1) Pure Platelet-Rich Plasma, such as cell separator PRP, Vivostat platelet rich fibrin (PRF)[13] or Anitua's PRGF[14,15]; (2) Leukocyte and Platelet-Rich Plasma; (3) Pure Plaletet-Rich Fibrin, such as Fibrinet; (4) Leukocyte- and Platelet-Rich Fibrin, such as Choukroun's PRF; and (5) Concentrated growth factors (CGF). In the following paragraphs, the use of PRP, PRF, plasma rich in growth factors (PRGF) and CGF in dentistry and oral surgery will be reviewed.

PRP

PRP is blood plasma that has been enriched with platelets and it was the first generation of platelet concentrates to be used in clinical practice by Marx in 1998[16]. PRP has a platelet concentration of 1000 $\times$ 10^9/L in 5 mL of plasma, which is 3-5 times higher compared to the normal whole blood platelet count (150-400 $\times$ 10^9/L). PRP contains (and releases through platelet degranulation) several growth factors and cytokines that can stimulate bone and soft tissue healing[17-19]. PRP is prepared by drawing peripheral venous blood from a patient's arm. The fresh blood is immediately mixed with an anti-coagulant to prevent clotting and then the platelets are concentrated using a two-step gradient centrifugation method[20]. In this method, the first spin (called the hard spin) separates the red blood cells (RBCs) from the plasma containing platelets, leukocytes and clotting factors, the second spin (called the soft spin) is used to delicately separate the platelets and leukocytes, from the plasma. The soft spin produces PRP and separates it from the platelet-poor plasma (PPP), free from the interference associated with large number of red blood cells. Commonly, with commercially available systems, a one-step method is used to separate the RBCs, buffy coat and plasma into three distinct layers. The buffy coat contains platelets and leukocytes and is often collected as PRP. The top plasma layer is often called PPP, which is discarded, leaving the PRP to be injected into surgical sites to accomplish guided tissue regeneration.

PRF

PRF consists of an intimate assembly of cytokines, glycanic chains, structural glycoproteins enmeshed within a fibrin network, and is considered to be the second generation of platelet concentrates[21-23]. The cytokines, glycanic chains, structural glycoproteins can have synergetic effects on tissue healing processes. The PRF pioneers were Choukroun *et al*[24,25], who used it to promote the osseointegration of dental implants. Several studies have demonstrated the clinical effectiveness of autologous PRF to regenerate defects in hard and soft tissues. The preparation of PRF is similar to PRP and consists in collecting peripheral venous blood from the patient's arm. Except that no anti-coagulant is used during blood harvesting. After the blood is collected it is immediately centrifuged for 10 min to activate the platelets, leading to the initiation of a coagulation cascade. After centrifugation, the blood is separated into three different layers: acellular PPP (platelet poor plasma) on top, a PRF clot in the middle and RBCs at the bottom of the test tube. The PRF clot obtained after centrifugation is collected, 2 mm below the lower dividing line and the other layers are discarded. The clinical success of the PRF protocol is dependent on a quick collection of blood and its transfer to the centrifuge. Because no anti-coagulant is used, the blood sample begins to coagulate almost immediately, and a failure to accomplish the quick preparation of PRF could cause a diffuse polymerization of fibrin, which is not ideal for tissue healing.

PRGF

PRGF is prepared from peripheral venous blood drawn from a patient's arm. PRGF is prepared using

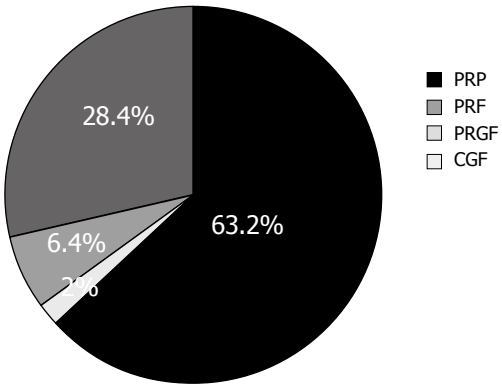

Figure 1 Number of studies with platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors in dentistry up to January 2015. PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

a modified PRP protocol developed by Anitua[26-28]. The difference between PRGF and PRP is that PRGF is optimized to deliver a more sustained release of growth factors. PRGF can create a three-dimensional fibrin scaffold which can be injected into a tissue defect, to maintain the regenerative space and can be used as a scaffold for cells to accomplish tissue regeneration. The Leukocyte content of PRGF is eliminated to prevent the pro-inflammatory effects of the proteases and acid hydrolases contained within these cells. PRGF is prepared from a small volume of patient's peripheral venous blood and is collected by a one-step centrifugation using sodium citrate as the anti-coagulant (Endoret System, Biotechnology Institut, Minano, Alava, Spain). After activation, PRGF progressively releases a pool of proteins and growth factors, which accelerate soft tissue healing as well as bone regeneration. Different formulations of PRGF with therapeutic potential can be obtained from a patient's blood depending on the degree of coagulation and activation of the samples. PRGF supernatant can be used as conventional eye-drop solution and cell culture media solution; liquid PRGF can be used to coat dental implant surfaces to promote osseointegration; the fibrillar and cellular scaffold-like PRGF can be used to fill tissue defects as part of ulcer treatment, sealing tooth sockets after tooth extraction, and promoting the epithelialization of soft tissues.

CGF

CGF, first developed by Sacco, in 2006, is an autologous fibrin network, rich in leukocytes and platelets[29,30]. CGF also contains autologous osteo-inductive growth factors derived from platelets and an osteo-inductive fibrin matrix. Similar to PRF, CGF is created using a one-step centrifugation method, but it requires a special programmed centrifuge (Medifuge MF200, Silfradent srl, Forli, Italy), which uses plastic tubes, coated with silica particles, and without the addition of exogenous substances. The final blood product is separated into three layers, two are discarded, and the CGF is collected from the buffy coat layer, which consists of a dense fibrin matrix that is rich in growth factors.

Table 1 Number of different studies published in the literature using platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors in dentistry

	Platelets concentrates			
Study type	PRP	PRF	PRGF	CGF
Clinical trials	116	50	13	1
Animal studies	46	13	5	1
In vitro studies	20	17	2	1
Technical report	3	1	0	2
Case report	35	18	2	2

PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

STUDY STRATEGY

A structured electronic search of scientific papers up to January 2015, was conducted using two medical databases (PubMed and the Cochrane Library) and specific keywords: "platelet concentrates in dentistry", "PRF" "Platelet rich fibrin Choukroun", "platelets in dentistry and maxillofacial surgery", "PRP", "CGF", "PRGF", "periodontal regeneration". For each of these platelet concentrate categories, their therapeutic potential in dentistry was evaluated according to the following inclusion criteria: (1) clinical trials; (2) animal studies; (3) *in vitro* studies; (4) case reports; and (5) technical reports. Subsequently, the articles for each type of platelet concentrate (PRP, PRF, PRGF and CGF) were classified according to these inclusion criteria and study type.

RESULTS

A total of 563 articles were identified as meeting the inclusion criteria of investigating the clinical use of autologous platelet concentrates in dentistry. However, after all the studies not relevant to dentistry, or containing no data were excluded, 348 articles were included in this review. Of the 348 articles, 220 articles (63.2%) were about PRP, 99 articles (28.4%) investigated PRF, 22 articles (6.4%) investigated PRGF and 7 articles (2%) were about CGF (Figure 1).

The articles were classified according to the type of platelet preparations in dentistry and the type of research performed in the article, which are briefly described below and summarized in Table 1: (1) PRP: from 220 articles, 116 were human clinical trials, 46 regarded animal studies, 20 were about *in vitro* investigations, 3 were technical reports and 35 were case reports; (2) PRF: from 99 articles, 50 were human clinical trials, 13 regarded animal studies, 17 were about *in vitro* experiments, 1 was a technical report about PRF general properties and 18 were case reports; (3) PRGF: from 22 articles, 13 were human clinical trials, 5 regarded

animal studies, 2 were about *in vitro* experiments and 2 were case reports; and (4) CGF: from 7 articles, 1 was a human clinical study, 1 regarded an animal study, 1 was about an *in vitro* study, 2 were technical reports about CGF properties and its application in dental implantology and 2 were case reports.

PRP studies in dentistry

PRP was used to treat periodontal intrabony defects in fifteen studies[31-45]. Nine studies described the use of PRP in cyst enucleations/periapical surgeries[46-54]. Forty-eight studies investigated PRP in sinus floor elevation treatments[55-101]. Twenty-two studies reported the use of PRP for the treatment of periodontal and periimplant defects[102-123]. Four studies used PRP for covering the roots of teeth[124-127]. Six studies investigated the efficacy of PRP for the treatment of gingival recession[128-133]. Four studies evaluated the benefits of using PRP to repair furcation defects[134-137]. Twenty-five studies investigated PRP for the repair of mandible/maxilla fractures[138-160]. Thirty-one studies investigated the use of PRP in endodontic surgery[161-188]. Eighteen studies investigated the use of PRP for dental extraction socket preservation before implant placement[189-206]. Twenty-two studies investigated the stimulating effect of PRP on alveolar bone regeneration and reconstruction[207-224]. Eight studies investigated the use of PRP to improve the healing and regeneration of soft tissues[225-231], mostly for periodontal ligament repair, and reducing the incidence of complications. Eight studies investigated PRP using *in vitro* protocols to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[232-237] (Figure 2).

PRF studies in dentistry

PRF was used in six studies to treat periodontal intrabony defects[238-243]. Four studies used PRF to regenerate tissue following cyst enucleations, and periapical surgeries[244-246]. Eleven studies investigated the ability of PRF to regenerate tissues following sinus floor elevation[247-256]. Eight studies investigated the use of PRF to treat periodontal and periimplant defects[257-263]. One study tested PRF as a potential root coverage repair treatment[264]. Two studies investigated the efficacy of PRF in gingival recession treatment[265,266]. Four studies investigated PRF to treat furcation defects[267-269]. Eight studies applied PRF to heal mandible or maxilla fractures[270-276]. Twenty one studies investigated the usefulness of PRF as part of endodontic surgery to repair periapical tissues[277-293] . Eleven studies investigated the ability of PRF to preserve tooth sockets after tooth extraction in preparation for dental implant placement[294-302]. Nine studies investigated the ability of PRF to stimulate alveolar bone regeneration and reconstruction[303-309]. Ten studies investigated the ability of PRF to improve the healing and regeneration of soft tissues, especially periodontal ligament, reducing complications[310-319]. Four studies investigated the *in vitro* effects of PRF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[320-323] (Figure 2).

PRGF studies in dentistry

PRGF was investigated in two studies to treat periodontal bone defects[324,325]. PRGF was investigated in two studies to regenerate tissues following cyst enucleations and periapical surgeries[326,327]. The potential of PRGF to heal tissues following sinus floor elevation treatment[328,329] was reported in two studies. Two studies reported that PRGF had a positive effect on the healing of periodontal and periimplant defects[330,331]. One study investigated the use of PRF to cover the roots of teeth[332]. One study investigated the efficacy of PRGF to heal tissues following gingival recession treatment[333]. Two studies investigated the benefits of PRGF for the treatment of furcation defects[334,335]. One study investigated the effectiveness of PRGF to heal mandible/maxilla fractures[336]. One study investigated the effectiveness of PRGF to heal periapical soft tissues following endodontic surgery[337]. Four studies investigated the clinical potential of PRGF to preserve tissue in tooth extraction sockets prior to dental implant placement[338-340]. One study investigated the stimulating effect of PRGF on alveolar bone regeneration and reconstruction[341]. One study investigated the ability of PRGF to improve the healing and regeneration of soft tissues, especially the periodontal ligament[342]. Two studies investigated the *in vitro* effect of PRGF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[343,344] (Figure 2).

CGF studies in dentistry

Compared to the other platelet articles, only a few had investigated the use of CGFs as part of dental treatment. A reason for the lack of CGF articles may be because it is newest of the platelet protocols and there has not been enough time for many articles to be published. Three studies were found which investigated CGF for tissue regeneration following sinus floor elevation[345-347]. One study investigated the *in vitro* effectiveness of CGF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[348]. One study investigated the healing effects of CGF for tissue repair following endodontic surgery[349]. Two studies investigated soft tissue and periodontal ligament healing after using CGF to accomplish guided tissue regeneration[350,351] (Figure 2).

DISCUSSION

Dentists have different types of biomimetic biomaterials to help guided bone and soft tissue regeneration. All these biomaterials have advantages and limitations and no single type of biomaterial has all the properties needed to be the universal dental regeneration biomaterial. A natural scaffold regeneration material is the blood clot, and several protocols have been

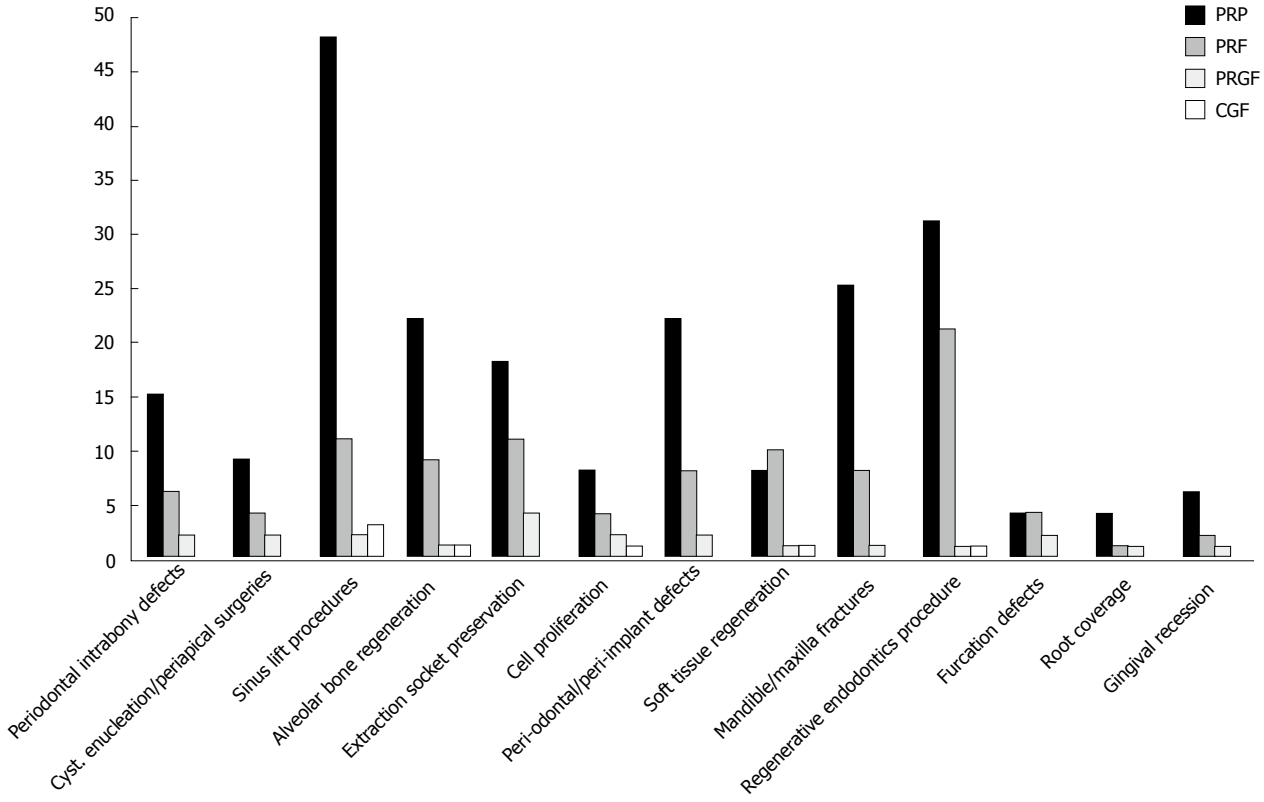

Figure 2 Platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors application in dentistry up to January 2015. PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

developed to improve the scaffold and growth factor properties of the blood clot (PRP, PRF, PRGF and CGF). These platelet rich preparations have been shown to improve healing, quicken tissue regeneration, improve the quality of tissues that are regenerated, and to reduce the incidence of complications. Alternatively, there are also many studies, which have shown that platelet rich preparations had little or no effect on tissue healing in comparison to biomimetic scaffolds. This explains the need to carefully investigate the uses of platelet concentrates as part of dental treatments.

After the careful analysis of the literature, the follow questions could be asked: (1) How is platelet rich fractions of blood prepared? (2) Why use platelet concentrates in dentistry? (3) Where to use platelet concentrates in dentistry? and (4) When to use platelet concentrates in dentistry?

The answer to the first question about how platelet rich fractions of blood are prepared, was answered in the previous paragraphs. All the techniques involve the centrifugation of the patient's peripheral venous blood and the use of fractions containing fibrin, platelets, leukocytes and growth factors. Red blood cells are discarded.

The answer to the second question about why platelet concentrates are used in dentistry is because they are cheap natural scaffolds and source of growth factors to stimulate tissue regeneration. Platelet concentrates are biocompatible and can sometimes offer potential benefits including rapid wound healing and bone regeneration. A controversial advantage is a reduction of postoperative pain and an unequivocal advantage is the lack of risk of infectious disease transmission. Sometimes platelet concentrates cannot be used where a patient does not want to donate their own blood, or when a special-needs patient or child refuses to cooperate with the collection of their blood.

The answer to the third question about why autologous platelet concentrates are used in oral and maxillofacial surgery and periodontal regenerative therapy is because of some promising results for tissue regeneration following sinus floor elevation (especially with PRP and CGF)[55-101,345-347], bone filling of periodontal intrabony defects[102-123,238-243,324,325], regeneration of alveolar ridges[207-224,303-311,343], dental extraction socket preservation[189-206,294-302,338-340], gingival recession treatment[128-133,265,266], mandibular and maxilla fractures[138-160,270-276,333]. Platelet concentrates have been used to manage bisphosphonate-related osteonecrosis of the jaw to enhance wound healing and bone maturation[271,272,352].

The answer to the fourth question about when to use platelet concentrates is the most difficult to reach for most dentists. A general rule of guidance is to use platelet concentrates, scaffolds, or biomaterials, in surgical situations where the prognosis for tissue repair is poor in the absence of a tissue regeneration scaffold and addition of growth factors[225-231,310-319].

CONCLUSION

In conclusion, platelets concentrates represents innovative tools in dentistry. The results, demonstrate that these concentrates are effective at improving bone and soft tissues healing. Moreover, well-enhanced bone regeneration can be obtained when PRP, PRF and CGF are used together with autogenous bone, with recombinant human growth factors such as recombinant BMP and also with other biomaterials (as for example Bio-Oss- *Geistlich-Switzerland* and Hydroxyapatite)[95-96]. However, the definition and validation of accurate protocols is a key issue for the long-term development of these techniques. So for further research is required to establish a standardized protocol for the use of these concentrates in the treatment of tissue regeneration.

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REVIEW

Salivary gland disorders: A comprehensive review

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Abstract

Salivary glands are complex in nature. They could be either tubulo acinar, merocrine or exocrine glands secreting mainly saliva. Salivary gland is one of the main soft tissue structures in the maxillofacial area. Saliva is a clear, slightly acidic muco serous fluid that coats the teeth, mucosa and thereby helps to create and maintain a healthy environment in the oral cavity. Salivary glands may be affected by a number of diseases: local and systemic and the prevalence of salivary gland diseases depend on various etiological factors. The glands may be infected by viral, bacterial, rarely fungal or its ductal obstruction which may cause painful swelling or obstruction, affecting their functions. The salivary gland may also be affected by a various benign and malignant tumours. This review article briefly describes about the various salivary gland disorders, diagnostic techniques and their management including the recent advances and the future perspective.

Key words: Salivary gland disorders; Xerostomia; Salivary biomarker; Salivary diagnostics; Exocrine glands

Core tip: The aim of this article was to analyse detailed aspects of various salivary gland disorders, their diagnostic and therapeutic advances in the prevention and management of salivary gland diseases of the oral cavity, including the recent developments and their future perspective.

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INTRODUCTION

A gland consists of specialized type of cells, wherein they produce products which are used elsewhere in the body. Salivary glands are complex, tubulo acinar, exocrine or merocrine glands secreting mainly saliva. Saliva is the product of the major and minor salivary gland dispersed throughout the oral cavity. It is a complex mixture of organic, inorganic components and water, carrying out several functions. There are three pairs of major salivary glands namely parotid, sub mandibular and sublingual glands in addition to numerous minor salivary glands in the oral cavity[1].

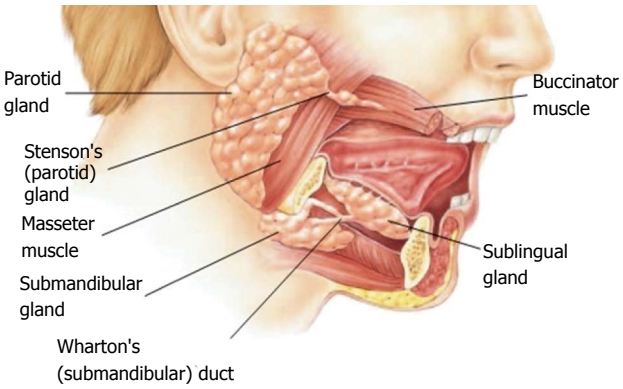

Figure 1 Major Salivary Glands and their related structures.

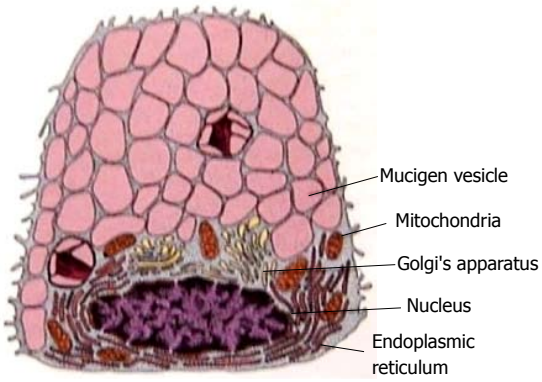

Figure 2 Mucous secreting cell showing Mucigen vesicle.

SALIVARY GLANDS DEVELOPMENT AND ANATOMY

The development of the parotid gland starts from 4-6th week, the submandibular gland at 6th week and the sublingual gland including minor salivary glands develops at 8-12 wk of embryonic life. The various developmental stages are: Bud formation, Epithelial cord formation, Branching and glandular differentiation, canalization and cyto differentiation. The parotid is ectodermal while the submandibular and sublingual glands are endodermal in their origins. The parotid represents the largest of the salivary gland which is situated between the external acoustic meatus between the ramus of the mandible and sternocleidomastoid muscle. Each gland is encapsulated and is composed of fat tissue and cells that secrete mainly the serous fluids. The major duct of each parotid gland is called Stensen's duct which opens into the vestibule of the mouth opposite the crown of the upper second molar tooth. The parotid gland being primarily serous in secretion secretes watery serous saliva[2].

The submandibular glands are located along the side of the lower jawbone in the anterior part of digastric triangle. Each gland has a major duct called Wharton's duct which opens on the floor of the mouth, on the summit of sublingual papilla at the side of frenulum of the tongue. Each of these glands is covered by a capsule which gives off mixed serous and mucous secretion in nature. The sublingual glands are the smallest of the major salivary glands which lies above mylohyoid and below the mucosa of the floor of the mouth. They are not covered by a capsule and are therefore more dispersed throughout the surrounding tissue. Their secretions are drained by many small ducts known as Rivinus's ducts that exit along the sublingual fold at the floor of the mouth. Sometimes, few anterior ducts may join to form a common duct called Bartholin's duct, their secretion being mixed in nature which empties into Wharton's duct. The sublingual and minor salivary glands are primarily mucous in nature.

Types

Salivary glands can be classified according to size as major and minor glands. The major salivary glands are of three pairs namely the parotid, submandibular and sublingual glands are shown in Figure 1. There are a numerous minor glands present in labial, buccal, glosso palatine, palatine and lingual areas in the oral cavity.

Based upon the type of secretion salivary glands may be predominantly serous, mucous or mixed depending on the type of secreting cells. Parotid and Von Ebners glands are purely serous while minor salivary glands like glosso palatine, palatine and anterior lingual glands are purely mucous. The mixed types of salivary glands are submandibular, sublingual, labial, buccal and posterior lingual glands.

Histology of salivary glands

Each gland has the secretory unit which is mainly composed of acinus, myoepithelial cells, intercalated duct, striated and excretory ducts. The acinus could be serous, mucous or mixed. These acini contain amylase granules in serous and granules with mucin in mucous glands and are responsible for producing primary secretion is shown in Figures 2 and 3. The secretory granule in mixed salivary glands contains serous demilunes, capping mucous acinar cells (Demilunes of Gianuzzi or Heidenham) producing sero mucous saliva. The ductal system of the salivary gland has a varied network. The three classes of ducts are intercalated, striated, and excretory each with different structure and function.

Saliva: It is mainly secreted and produced by the salivary gland. The total volume of saliva secreted daily in an adult person is 600-1000 mL out of which 60% is secreted by the submandibular glands, 30% by the parotid, 5% by the lingual and 7% by the minor salivary glands with a pH in the range of 6.0-7.0. However, the salivary secretion is a reflex action arising from the salivary centres dependent on afferent stimulation. The sublingual and minor salivary glands spontaneously secrete saliva though the bulk of this secretion is nerve

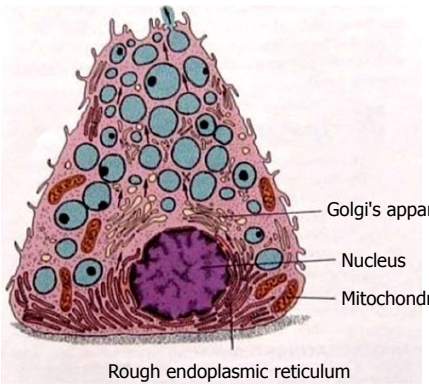

Figure 3 Serous secreting cell with secretory granules.

mediated. The normal average salivary flow rate ranges from 0.1-0.3 mL per minute[3].

Composition

Saliva is mainly composed of the following components. Electrolytes like sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphate, thiocynate, and fluoride. Secretory proteins/Peptides like Amylase, proline rich proteins, mucins, histatin, cystatin, peroxidase, lysozyme, lactoferrin, glycoproteins, lysozyme, defensins, and cathelicidin LL37. They also contain secretory immune globulins-(IgA), IgG, IgM, organic components like, glucose, amino acids, urea, uric acid, and lipid molecules. The other components that are present are epidermal growth factors, epithelial cells, insulin, cyclic adenosine monophosphate, binding proteins and serum albumin. In addition, biologically active peptides such as leptin, ghrelin and endothelin which are identified in saliva are of supreme importance to general health and also oral health in particular[4-9]. Functions: saliva mainly helps in lubrication for the movement of oral tissues against each other and the food, aids in digestion, in taste perception, neutralises by its buffering action the bacterial acids and thereby promotes remineralisation by reducing dissolution of enamel by inhibition of calcium phosphate precipitate. The saliva over all protects the teeth and the oral mucosa by the presence of immunoglobulin's tissue repair factors and antibacterial system[4].

Oral diagnostic approaches to the patients with salivary gland disorders: (1) Past and Present History: to enquire about the history of patient who had undergone any surgery/radiotherapy, or have any underlying systemic problems/the patient is under any medications, *etc.* A thorough medical history and physical examination are also essential; and (2) Clinical Examination; a study by Navazesh suggests four clinical measures to diagnose the hypo function in the salivary gland. They are dryness of the lips and buccal mucosa, absence of saliva produced by the gland, bimanual palpation, and DMFT scores[5].

For evaluations of Dry mouth and a salivary mass or enlarged salivary gland, the following diagnostic approached may be applied: (1) Imaging of salivary glands; (2) Sialography; (3) Special imaging; (4) Sialochemistry; and (5) Biopsy and culture.

Salivary gland disorders: (1) Developmental-Aplasia, Atresia, Aberrancy; (2) Functional Disorders-Xerostomia, Sialorrhea (Ptyalism); (3) Inflammatory-infectious conditions; acute and chronic bacterial infection; Sialadenitis, Viral infection; Mumps, Human immunodeficiency virus associated salivary gland disorder; Post irradiation Sialadenitis, chronic sclerosing Sialadenitis, cheilitis glandularis; (4) Traumatic/ Obstructive-Mucocele, salivary duct cyst (mucose retention cyst, Ranula), Nicotinic stomatitis, Sialolithiasis; (5) Autoimmune-Sarcoidosis, Sjogrens syndrome, Mikulicz's disease; (6) Neurological-Frey's syndrome; (7) Degenerative-idiopathic Sialolithiasis; (8) Non inflammatory non neoplastic-Sialadenosis; (9) Vascular-Necrotizing sialometaplasis; (10) Neoplastic-Benign: Papillary Cystadenoma Lymphomatosum, Pleomorphic Adenoma, BasalCell Adenomas, Oncocytoma, Canalicular Adenoma, Myoepithelioma, Sebaceous Adenoma, and Ductal Papilloma. Malignant: Adenoid Cystic Carcinoma, Hyalinising Clear Cell Carcinoma, Mucoepidermoid Carcinoma, Acinic Cell Carcinoma, Adeno carcinoma, Carcinoma[3,6]; and (11) Classification of Salivary Gland Tumours according to WHO 2005 is listed in Table 1.

Developmental disorders

Atresia is the congenital occlusion or absence of salivary ducts which leads to xerostomia or mucous retention cyst.

Aplasia is the complete absence of one or more salivary gland which leads to xerostomia, and affected patients are more susceptible to dental caries. This condition could be an isolated finding or associated with other disorder like hemi facial microsomia or Treacher Collins syndrome. Recent studies suggest that mutation in fibroblast growth factor 10 (FGF10) affecting the FGF receptor signalling, has been linked with this condition[7]. Enamel hypoplasia, extensive occlusal wear of teeth or congenital absence of teeth are other oral manifestations of salivary agenesis. However the treatment is supportive.

Aberrancy: it is an anatomic variant wherein the normal salivary gland develops at an abnormal position. Sometimes they are found adjacent to lingual surface of the mandible within a depression. Ex: Staphne's bone cyst or Staphne's bone cavity: It is thought to be created by an ectopic portion of salivary gland tissue which causes remodelling of the mandibular bone. This creates an apparent cyst like radiolucent area seen on the radiographs[8]. It appears below the inferior alveolar nerve canal in the posterior region of the mandible.

This lesion is not discovered during routine examination, as it causes no symptoms and do not require intervention. However, surgical intervention is recommended in atypical

Table 1 World Health Organization 2005 Classification of salivary gland tumors

Epithelial tumors
Benign epithelial tumors
Pleomorphic adenoma
Myoepithelioma
Basal cell adenoma
Oncocytoma
Canalicular adenoma
Warthins tumors
Cystadenoma
Papillary cyst adenoma
Mucinous cyst adenoma
Benign sebaceous neoplasm
Sebaceous adenoma
Sebaceous lymphadenoma
Ductal papilloma
Intraductal
Inverted ductal
Sialadenoma papilliferum
Malignant epithelial tumors
Mucoepidermoid carcinoma
Acinic cell carcinoma
Adenoid cystic carcinoma
Polymorphous low grade adenocarcinoma
Epithelial myoepithelial carcinoma
Clear cell carcinoma
Basal cell adenocarcinoma
Oncocytic carcinoma
Myoepithelial carcinoma
Adenocarcinoma NOS
Carcinoma ex pleomorphic adenoma
Metastatising pleomorphic adenoma
Carcinosarcoma
Salivary duct carcinoma
Cyst adenocarcinoma
Low grade cribriform cystadenocarcinoma
Sialoblastoma
Malignant sebaceous tumor
Sebaceous adenocarcinoma
Sebaceous lymphadenocarcinoma
Squamous cell carcinoma
Mesenchymal tumors
Benign
Haemangioma
Haemangiopericytoma
Lipoma
Neurofibroma
Schwannoma
Malignant
Fibrosarcoma
Malignant fibrous histiocytoma
Liposarcoma
Malignant lymphoma
Metastatic tumor

regions in which the diagnosis is unclear and a tumor is suspected.

Functional disorders

Xerostomia: It is defined as the subjective sensation of oral dryness that may or may not be associated with a reduction in salivary output. The condition may be transient, prolonged or permanent depending upon the duration of the condition.

Aetiology: Temporary causes are: (1) Psychological causes due to anxiety and depression; (2) Drug therapy-Drugs that exert anti-anticholinergic and decrease the volume of serous saliva are: anticholinergic ex:atropine, anti-hypertensive ex: reserpine, methyldopa, antihistamine ex: diphenhydramine, antidepressant: amitryptiline, antipsyschotics: diazepam, anti parkinsonian drugs: procyclidine, anti-emetics: hyoscine and antispasmodics: tizandine. Drugs that exert sympathomimetic action and produce more viscous mucinous saliva with less volume are: Nasal decongestants, appetite suppressants, bronchodilators, and amphetamines. Some drugs may also exert their neural effects in higher centres of the brain, by stimulation of adreno receptors in the frontal cortex that can produce inhibitory effects on salivary nuclei's; (3) Duct calculi: a blockage of the duct of a major salivary gland (submandibular) can produce dryness on the affected side with pain and swelling in the gland on stimulation. If left untreated it can cause progressive fibrosis of the gland and permanent xerostomia; (4) infections; Sialadenitis is the inflammation of the salivary gland, acute infections like mumps and post-operative parotitis, chronic conditions like swellings related to nutritional deficiency, and iodine hypersensitivity, wherein in all these conditions causes hypo salivation[3,9].

Permanent causes: (5) Salivary gland aplasia, Sjogrens syndrome: causes dry eyes, dry mouth and often associated with rheumatoid arthritis. Other systemic disorders like diabetes mellitus, Parkinson's disease, cystic fibrosis, sarcoidosis, vitamin A, riboflavin, nicotinic acid deficiencies and in anaemia's; (6) Surgery or trauma to the ducts may also impair secretion; and (7) Radiotherapy: hypo salivation occurs on exposure of major salivary glands to radiation bilaterally in head and neck cancer. At radiation doses > 3000 cGy, the patient is at risk if all major glands are in the field of radiation. Irreversible effects occur at a dose of 6000 cGy for 5 wk. Radiation causes acinar cell atrophy and fibrosis, changes in vascular connective tissue and neurologic function. The degree of salivary gland alteration depends on dose volume factor, patient age, and time of exposure to radiation. Serous acini are affected before mucous acini resulting in thick viscous secretion. Depending on the amount of salivary tissue in the field, xerostomia may resolve within 6 mo and sometimes may be permanent. There can also be changes in salivary composition, decreased secretory IgA and buffering capacity with increased magnesium, calcium, potassium and sodium chloride in post radiotherapy cases[9-11].

Signs and symptoms: Lips are often cracked, peeling and atrophic; Buccal mucosa may be corrugated and pale: (1) Tongue may be smooth and reddened, cracked or fissured, with loss of papillation; (2) Increase in erosion and caries, particularly decay on root surfaces and even cusp tip involvement; (3) Erythematous form

of candidiasis is frequent; (4) Lipstick sign: occurrence of shed epithelial cells on the labial surfaces of maxillary anterior teeth as the mucosa adheres to the teeth due to reduced saliva; (5) Tongue blade sign: when held against buccal mucosa, the tissue adheres to the tongue blade as it is lifted away; (6) Viscous sticky saliva with difficulty in speaking and swallowing; (7) Halitosis, altered taste and smell, gingivitis; (8) Complaint of burning mucosa, lips or tongue; (9) Ulceration of oral mucosa; (10) No accumulation of saliva in the floor of the mouth; (11) Poorly fitting prosthesis; and (12) Enlargement of salivary glands.

Xerostomia associated problems are: Dental Caries, Dry mouth, Dysgeusia, Dysphagia, oral Candidiasis, and Bacterial infections[12].

Treatment of xerostomia associated problems: Dental caries; use of fluorinated dentifrice (0.05% NaF)/fluoride gel in the concentration of 1% NaF, 0.4% Stannous fluoride application of 0.5% sodium fluoride varnish to teeth, regular use of re mineralising tooth paste. Dental examination every 6 mo and bitewing radiograph once a year for early diagnosis of dental caries. The recent advances in chair side diagnostics test kits are GC Salivary check-Buffer Kit that identifies, measures, and assesses patient for caries risk based on saliva conditions like hydration, consistency, pH of resting saliva and flow, and buffering capacity of stimulated saliva[13]. GC Saliva Check Mutans Kit is another chair side diagnostic kit used for rapid detection of high levels of S.mutans without the need for incubation is possible within 15 min. In a study, Gopinath *et al*[14] evaluated the effect of salivary testing in dental caries assessment using salivary testing kit (GC Asia Dental Pvt Ltd, Japan) and recommended adopting this test in patients with high caries risk.

A similar study conducted by Wennerholm *et al*[15] compared Saliva-Check Mutans and Saliva-Check IgA Mutans with the Cariogram for caries risk assessment and the data suggested that the combination of Saliva-Check Mutans and Saliva-Check IgA Mutans could be used for caries risk assessment.

Kanehire *et al*[16] aimed to develop a simple screening technique for the diagnosis of hypo salivation by estimation of capsaicin-stimulated salivary flow using filter paper. Five spots containing starch and potassium iodide on filter paper with or without capsaicin and a colouring reagent was designed in this assay system. The study suggested that this test would be useful for evaluating the retained functional ability of salivary glands and screening of hypo salivation with dry mouth.

Dry Mouth should be hydrated regularly using water or lozenges with citric acid to stimulate salivation, artificial salivary substitutes, lubricants such as lanolin based product Vaseline, olive oil, vitamin E or lip balm, oral gels such as oral balance, Dry mouth gel (GC Asia Dental Pvt Ltd, Japan) which can be applied on buccal and lingual surfaces of teeth and oral mucosa which can be applied any time during the day as needed. Even mouthwashes and sprays, sugar free gums, mints water or ice chips are recommended[6,12,17]. Sialogogues like pilocarpine 5 mg 3 times a day, cevimeline 30 mg 3 times a day, bromhexine, bethanecol, and anethole trithione are prescribed. Use of Salivary substitute's solutions mainly containing electrolytes stimulates natural saliva, example Salivart, Oralube, Xerolube, Plax may also be recommended.

Application, in children 1 spray whereas in adult 2-3 sprays should be directed into the back of the mouth and tongue for the relief of dry mouth symptoms. The characteristics features that these substitutes possess are that they have electrolytes and pH similar to saliva and low viscosity allowing electrolytes particularly calcium to travel through matrix of saliva substitute which helps in remineralisation process. Mucin containing saliva orthana and Glycerate polymer are also suggested for xerostomia. There are studies suggesting the role of acupuncture therapy for improvement in salivation as a treatment option for patients responding to muscarinic agonists[17].

Measuring biofilm activity is possible by using recently introduced simple chair side adenosine triphosphate (ATP) bioluminescence test, CariScreen (Oral BioTech, Albany, Ore) the caries susceptibility test to assess cariogenic bacterial activity and their levels in caries free and caries active patients in about 15 s measurement with a meter[18].

Inflammatory

Sialadenitis is an inflammation condition affecting the salivary glands. Parotid salivary glands are most commonly affected in adolescents and in children, debilitated adults, or patients with medication on tricyclic antidepressants and tranquilizers.

Aetiology: The main etiologic factors for sialadentis can be either infectious or non-infectious factors. Bacterial and viral agents can cause of sialadenitis. Bacterial sialadenitis is caused because of retrograde spread of infection secondary to decreased salivary flow or ductal obstruction. Decreased salivary flow can be secondary to medications, dehydration or debilitating conditions. Ductal obstruction can be due to sialolithiasis, strictures within the ductal system and common in submandibular salivary glands or due to pressure effect from adjacent tumors.

Staphylococcus aureus is the most common etiologic agent for acute bacterial parotitis in addition Staph. Pyogenes, Strep. Viridians and other microorganisms can also cause sialadenitis. Viruses causing sialadenitis include paromyxo viruses (mumps-most common), Coxsackie virus, cytomegalo virus, *etc.* The patient may present with fever and dehydration[19]. Clinical features: clinically there is sudden pain at the angle of the jaw which is unilateral with glandular enlargement

and tender to palpation with purulent discharge over Stensens duct.

Treatment includes administration of salivary stimulants, antibiotics and surgical drainage.

Acute postoperative parotitis: Aetiology is a form of sialadenitis which occurs after a major surgical procedure where in the patient depends only on intravenous fluids. In addition these patients are on atropine which is a pre anaesthetic medication for drying the secretions and this may contribute to dryness of mouth and subsequent inflammation of parotid salivary glands. Non-infectious causes of salivary gland inflammation are sarcoidosis and Sjogrens syndrome.

Clinical features: Parotid gland is the most common salivary gland involved in acute bacterial sialadentitis. The patient presents with painful, usually bilateral swelling of the parotid salivary glands with low grade fever. In addition the patient may also complain of difficulty in opening the mouth. On clinical examination the skin over the parotid region may be inflamed and intra orally purulent discharge may be observed from parotid duct. Treatment-The condition usually resolves in about 48 h. However, symptomatic treatment is recommended.

Mumps is an acute paramyxo virus induced infection of parotid salivary glands. Aetiology- It is a contagious infection spreading through airborne droplets or direct contact of saliva. The peak incidence of mumps is reported during winter and spring season[20]. Clinical features: The infectivity of the mumps virus ranges from 3 to 4 d after the onset of the disease[21,22]. During the prodromal phase of the disease, the patient may complain of low grade fever, muscle pain, head ache and malaise[20] followed by unilateral or bilateral enlargement of parotid salivary glands associated with pain which is severe during mastication. The inflammation of the salivary gland starts reducing by the end of 1st week and the patient returns to normal by 10 d[21]. Epididymoorchitis, Oophoritis, pancreatitis and acute meningitis are the complications of mumps[21]. Treatment is symptomatic and Mumps vaccination (MMR) may decrease the incidence of this infection and considered as preventive measure.

Chronic recurrent parotitis: The proposed etio pathogenesis for this disorder include, congenital[23,24] and acquired factors like ductal obstruction secondary to inflammation infection and autoimmune diseases[25,26].

Chronic sclerosing sialadenitis: Also known as Kuttner's tumour was identified by Kuttner in 1896. Aetiology-The condition is a chronic inflammatory reaction secondary to ductal obstruction and subsequent salivary stasis. However, salivary flow obstruction is proposed to be the main factor in the pathogenesis of this disorder[27].

Clinical feature: Clinically the condition presents as a painful, hard swelling of submandibular salivary gland. The pain and swelling may be present for a variable duration of time. The differential diagnosis includes chronic sialadenitis, sialolithiasis, and benign lymphoepithelial lesions. Treatment-The condition is managed by surgical excision of the involved gland and chances of recurrence of the lesion or changing into malignancy is found to be rare[27].

Hepatitis C virus associated sialadenitis: Aetiology-hepatitis C virus (HCV) is found to affect the salivary glands and cause the glandular inflammation. Clinical feature: The affected patients may present with mild swelling of the parotid gland with minimum or no symptoms of dry eyes and dry mouth[27,28]. The diagnosis of HCV is by the detection of HCV DNA and anti HCV antibodies. Treatment- Hepatitis associated sialadenitis is treated symptomatically[29].

Human immunodeficiency virus infection: In Human immunodeficiency virus (HIV) infected patients salivary gland lesions commonly occur which may be neo plastic or non-neoplastic in nature. AIDS related tumours such as lymphoma and Kaposis sarcoma and a Sjogrens syndrome like condition occurs in these patients and are described as "HIV salivary gland disease" (HIV-SGD) is considered to be due to reactivation of a latent virus. Various studies have expressed the strong association between salivary gland dysfunction seen in HIV affected patients and Human Cytomegalovirus (CMV) saliva. Clinical feature: The condition is characterised by xerostomia with unilateral or bilateral salivary gland enlargement with reduced tear production**.** Diagnosis is by biopsy of the major gland which shows the presence of hyperplastic lymph nodes with lymphocytes and cystic cavities obtained from patients affected from HIV-SGD[6,28]. Treatment-Administration of oral sialagogues/ frequent sipping of water are recommended for xero-stomia. Anti-retroviral therapy may be administered for the management of HIV. Rarely radiotherapy and parotidectomy may be beneficial in advanced condition.

Iodine 131 indiced sialadenitis: Aetiology-high dose of oral Iodine 131 in treatment of thyroid carcinomas can adversely affect the salivary glands leading to sialadenitis. The incidence of acute salivary gland inflammation range from 24%-67% and that of chronic salivary gland inflammation range from 11%-43%[29-31]. Clinical feature: The patients present with pain and swelling of the salivary glands with or without dry mouth condition[32]. Treatment- Administration of oral sialagogues/oral hydration, serotonin receptor blocker and dexamethasone are recommended.

Sialadenosis: Sialadenosis also known as sialosis is an enlargement of salivary glands which is non-inflammatory and non-neoplastic more commonly affecting the parotid

salivary glands.

Etiology: This condition can be associated with: Endocrine disorders: (1) Diabetes mellitus and insipidus; (2) Accromegaly; (3) Hypothyroidism; and (4) Pregnancy. Nutritional status: (1) Anorexia nervosa; (2) Bulimia; (3) Chronic alchoholism; and (4) General malnutrition. Medication induced sialadenosis[33]: (1) Psychotropic medications; (2) Antihypertensive drugs; and (3) Sympathomimetic drugs. Clinical features- Patient presents with a slowly progressing bilateral (rarely unilateral) swelling of parotid salivary glands which may be asymptomatic[34]. Rarely patients may complain of reduced salivary flow. Treatment-Management of underlying systemic condition may help in reversing the sialdenosis.

Traumatic: Mucocele

Aetiology-They is caused due to rupture of a salivary gland duct mostly due to trauma resulting in spillage of mucin into the surrounding tissues. Clinical features: Clinically a mucocele appear as bluish thin walled lesion which is fluctuant, and the most common site of occurrence is on the lower lip. Ranula: is a special type of mucocele which grows in the floor of the mouth, usually unilateral and is called due to its similar appearance to enlarged abdomen region of a frog. Treatment- in case of superficial recurrent or deep mucoceles, surgical intervention is indicated while large ranulas are treated by marsupialization. A study by Wilcox *et al*[6] recommends intra lesional corticosteroids administration before surgery.

Nicotinic stomatitis: Aetiology-The long standing habits of tobacco and or alcohol/hot liquid consumption. Clinical feature: Exhibits whitened areas of the hard palate due to hyperkeratosis caused by the thermal irritation. This irritation also causes inflammation and dilatation of the duct openings of the minor salivary glands of the palate manifesting as red patches or spots on a white background[1]. Treatment- discontinuation of the habits reverses the condition back to normal.

Autoimmune: Sarcoidosis

Aetiology-is an autoimmune chronic granulomatous inflammatory condition which causes destruction of the tissue by T lymphocytic, mononuclear phagocytic infiltration and granuloma formation[6]. The parotid salivary glands are affected in 10%-30% of cases. Clinical feature: The patient presents with a hard, bilateral enlargement of the parotid gland usually asymptomatic in nature. Sarcoidosis of parotid glands along with uveitis and facial nerve paralysis is termed as Heerfordt's syndrome or uveo parotid fever[35]. The patient may complain of dry mouth and minor salivary gland biopsy confirms the diagnosis. Treatment- palliative treatment primarily relieving of the symptoms of salivary component of sarcoidosis is advised.

Corticosteroid or with Chloroquine has been recommended. Immunosuppressive and immune modulatory medications are administered in patients who do not respond the corticosteroids.

Sjogrens syndrome: Aetiology-is an autoimmune disorder associated with HLA-DR3 AND HLA-B8. The disease was described by Henric Sjogren in 1933. Clinical feature: The primary Sjogren syndrome/sicca complex exhibit dry eyes and mouth. The secondary Sjogren syndrome develops SLE, polyarteritis nodosa, polymyositis, rheumatoid arthritis and in scleroderma.

This condition is most commonly seen in women over 40 years with male: female in the ratio of 1: 10.

Sjogrens syndrome case definition[6,34] requires at least 2 out of the following 3 criteria as mentioned in Table 2. Laboratory findings: Anti salivary duct antibodies, anti-nuclear antibodies, rheumatoid factor increased ESR, Lip biopsy-lymphocytes around salivary glands. The other tests are Schirmer test, Rose Bengal dye test, Sialography and sialochemistry[6]. Treatment- to limit the harmful effects of the disease especially the ocular and oral conditions, symptomatic relief of administration of artificial tears, saliva substitutes, fluoride applications and oral hygiene measures are suggested[6,34].

Mikulicz's disease: Aetiology-Mikulicz's disease of unknown aetiology was first reported by Johann von Mikulicz-Radecki in 1888. However, it has been demonstrated that autoimmune, viral, and genetic factors may contribute to the pathogenesis of the disease. Clinical feature: Patients suffering from Mikulicz's disease present with asymptomatic, bilateral swelling of the parotid, and submandibular salivary glands along with lacrimal glands. This disease closely resembles Sjogren's syndrome. However the lacrimal and salivary secretion depletion is very minimal in Mikulicz's disease. Histologically the disease resembles Sjogren's syndrome, but lacks the characteristic anti-SS-A and anti-SS-B antibodies of Sjogren's syndrome. Studies have found increased levels of IgG4 antibodies in the serum of patients with Mikulicz's disease. Treatment- Mikulicz's disease is very much responsive for steroid therapy particularly to[35] methylprednisolone.

Neurological

Frey's syndrome also known as Auriculo temporal syndrome which is characterized by sweating in the pre auricular and temporal areas after gustatory stimulation.

Aetiology-the condition most commonly caused due to faulty regeneration of sympathetic and parasympathetic nerve fibres which were injured during parotid tumor surgery or ramus resection. Clinical feature: Post-surgery the parasympathetic fibres start innervating the sweat glands and vasculature of the skin around the parotid area. The symptoms usually appear within few minutes of the start of mastication or during stimulation of saliva and

Table 2 American College of Rheumatology Classification Criteria for Sjögren's Syndrome: Sjogren's syndrome case definition requires at least 2 out of the following 3

Positive serum anti-SSA and/or anti-SSB or (positive rheumatoid factor and ANA ⩾ 1:320)
Ocular staining score ⩾ 3
Presence of focal lymphocytic sialadenitis with focus score ⩾ 1 focus/4 mm^2 in labial salivary gland biopsies

may remain up to 30 min after discontinuing mastication. The diagnosis of the syndrome can be confirmed by starch iodine test[36]. Treatment- Reassurance to the patient is advocated in most of the cases. Intra cutaneous injection of botulin toxin is found to be effective in severe condition and Tympanotomy[23] may be the treatment of choice with severe symptoms.

Degenerative

Sialolithiasis-is a condition of unknown aetiology. However, there could be several coexisting causes leading to the salivary stone formation. Some of these cofactors may be related to disturbed pH of saliva, abnormalities in the sphincter mechanism related to salivary duct opening and abnormal calcium metabolism[5,6]. Clinical Feature: This condition most often will not produce any signs and symptoms. Rarely, it may cause complete ductal obstruction, pain and swelling of the salivary glands. Treatment- Large salivary stone are managed by extracorporeal or intracorporal lithotripsy[6,17] procedure.

Non inflammatory non neoplastic

Sialadenosis is a non-infectious, non-inflammatory gland enlargement usually affecting the parotid bilaterally. This condition is most often seen in women causing salivary hypo salivation which can occur due to systemic disorders[6,12].

Vascular

Necrotizing sialometaplasia: Aetiology-The probable cause could be due to vascular infarction of the salivary gland lobules and is often mistaken for oral cancer[37]. Vascular compression is caused by a necrotic myocutaneous reconstruction of the flap used in palatal surgeries and embolization from carotid endarterectomises, Berger' s disease, Raynaud's phenomenon. Predisposing factors are dental injections, ill-fitting denture, traumatic injury, previous surgery and upper respiratory tract infections Clinical feature: appears as a non-neoplastic lesion that usually arises from a minor salivary gland in the lips, posterior part of the palate, and retro molar regions. Treatment: The condition is self-limiting and the healing of the lesion normally takes about 6-8 wk.

Neoplastic

Benign: Pleomorphic Adenoma, Papillary Cystadenoma Lymphomatosum (warthins tumor), Basal Cell Adenomas, Oncocytoma,Canalicular Adenoma, Myoepithelioma, Sebaceous Adenoma, Ductal Papilloma.

Malignant: Adenoid Cystic Carcinoma, Hyalinising Clear Cell Carcinoma, Mucoepidermoid Carcinoma, Acinic Cell Carcinoma, Adeno carcinoma, Pleomorphic Adenoma, Lymphoma[5,6,12].

Oral diagnostic approaches to the patients with salivary gland disorders: (1) Imaging of salivary glands: Salivary gland is one of the main soft tissue structures in the maxillofacial area. Imaging is useful in identifying the masses of salivary glands and also in differentiating them from the masses/pathologies of adjacent cervical spaces, especially para pharyngeal, masticator, submental spaces and mandibular lesions. Conventional radiography has a very limited role in the diagnosis of salivary gland pathology which includes plain radiography. It aids in identifying mainly salivary stones and calcifications. Gland plain radiography like in postero anterior skull projection with cheeks blown out to delineate the parotid duct and submandibular gland radiography includes lateral oblique radiograph with mouth wide open; and (2) Sialography was used as the sole imaging technique before the advent of advanced imaging techniques which include ultrasonography, elastography, computed tomography, scintigraphy, and magnetic resonance imaging. Sialography, an imaging technique of salivary gland, uses contrast medium to delineate the ductal system of salivary glands. Due to use of contrast medium this technique is not suitable and is contraindicated in acute conditions of salivary glands[38]. However sialography is found to be useful in assessment of salivary gland dysfunction secondary to obstructive disorders of the gland[39].

Studies have suggested other various diagnostic methods-magnetic resonance (MR) sialography is a non-invasive technique useful in evaluating the hypo functioning of salivary glands. Sialo endoscopy assist in detecting ductal anomalies that may not be[40] possible to detect by means of either traditional or new imaging techniques.

Sialography, Sialoendoscopy, and MR Sialography are indicated for evaluation of the ductal system of the salivary glands.

Ultrasonography, computed tomography, magnetic resonance imaging is helpful in assessment of the parenchyma of the salivary glands[41]. However; all these diagnostic aids have their own limitations in the diagnosis of salivary gland lesions.

Ultrasonography: Ultrasound examination of salivary glands with a high resolution transducer is found to be a highly sensitive, a non-invasive method for salivary gland evaluation[42]. It is a cost effective imaging tool which displays high definition images useful in evaluating the superficial structures particularly the peripheral areas of the affected salivary gland. High frequency linear probes of 7.5-12 MHz are used in imaging of salivary glands[43]. In acute conditions such as acute radiation

induced sialadenitis, the gland appears swollen and show anoechic appearance on ultrasonography[44]. In a recent clinical study ultrasonography was found useful in diagnosing lymph node and salivary gland enlargement in submandibular region and suggested that it also helps in identifying the salivary glandular tissue in accessory salivary gland and salivary calculi[45].

Shock-wave lithotripsy: Shock-wave lithotripsy is a non-invasive diagnostic tool suggested for the management of sialolithiasis. Iro *et al*[46] in 1989 introduced the application of extracorporeal shock-wave lithotripsy (ESWL) in the management of salivary gland. Sialolithotripsy helps in removing salivary stones into smaller particles and thereby removal by flushing action is possible from the salivary duct system or after salivation induced by citric acid or other sialagogues. The shock-waves are generated extra-corporeally by using Piezoelectric and electromagnetic techniques or intra-corporeally using electro-hydraulic, pneumatic or laser endoscopic devices[46].

Sonoelastography: Elastography is an ultrasonography technique which measures the tissue elasticity *in vivo.* This imaging technique measures the elasticity of the glandular parenchyma and is useful in evaluating the hypo function of saliva especially in post radiation hypo function of salivary glands[41].

Computed tomography: Computed tomography (CT) scans of the salivary glands are useful in delineate the extent of the lesion and its relation to adjacent structures[47]. Multi detector CT scans help in characterizing tumours of salivary glands like Warthin tumor which demonstrates peak enhancement of signals after administration of contrast agents which is not found in other tumors of salivary glands. However CT scans perform poorly in characterizing the histopathologic nature of the tumors. CT scans help in differentiating the benign and malignant neoplasms of salivary glands. The irregular tumor margin and surrounding tissue infiltration is the characteristic feature of malignancy[48]. However studies have found overlap of CT scan characteristics between malignant and benign lesions. Apart from tumor identification CT scan also aids to view dystrophic calcifications in salivary glands.

CT sailography: Interpretation of sailography findings depend on the imaging technique used to acquire sailography images. Traditionally plain radiographs were used for assessment of salivary glands after injection of the contrast medium. Introduction of CT and MRI scans in maxillofacial imaging have shifted the focus from plain radiography to these advanced imaging techniques. However CT sialography may have limited applications due to the accessibility and cost factors. Moreover the prolonged image acquisition time of CT scans may jeopardize the viewing of CT contrast medium uptake[49]; (3) Special imaging; cone beam computed tomography (CBCT): some of the limitations of CT sialography have been addressed by use of CBCT technology with sialography. A study reported the usefulness of CBCT in demonstrating the secondary structures of submandibular salivary glands in comparison with plain radiography coupled with sialography. The same study reported that the effective dose from CBCT scans were comparable to that of plain radiography when a smaller field of view (FOV) was used[50].

Magnetic imaging resonance (MRI) scans are useful in assessment of salivary glands. The wide variety of soft tissue signals differences and multi planar image acquisition have made MRI an effective imaging modality for assessment of salivary gland tumors. This imaging modality is helpful in assessment of tumors affecting the deep lobes of parotid glands, skull base invasion of the tumours of salivary glands, evaluation of recurrent pleomorphic adenomas and much more[51]. Also high resolution MRI scans delineate the intra parotid course of facial nerve which is an important landmark for surgeons operating on parotid glands[52].

Magnetic resonance sialography-Major limitations of conventional sialography include use of iodine based contrast agents and inability of the contrast agent in overcoming the strictures within the ductal system of the salivary gland which in turn prevent the visualization. These limitations can be overcome by switching on to MR sialography which uses patients own saliva as a contrast medium. MR sialography also demonstrates the actual ductal diameter due to non-use of contrast agents[53].

Scintigraphy-Salivary gland scintigraphy uses Tc-99m pertechnetate which helps in assessment of salivary gland dysfunction in disorders like Sjogrens syndrome. This technique is valuable in assessment of xerostomia[54].

The minimally invasive techniques for preserving the glandular tissue which are currently being used in the management of obstructive salivary disease are sialoendoscopy, shockwave lithotripsy, interventional radiology, endoscopically video-assisted trans-oral and surgical retrieval of stones, and botulinum toxin therapy. Three dimensional reconstruction imaging (MR sialographic) and MR virtual endoscopy have recently been suggested for salivary gland ducts studies on par with their applications in medical field[55].

Emerging imaging based diagnostics: Positron emission tomography (PET) scan: A PET scan focuses for areas of high cellular activity suggesting a sign of cancer growth. It also helps to diagnosed cancer, and to assess its spread to lymph nodes or any other parts of the body. This test requires an injection of a very small quantity of radioactive substance usually a type of sugar known as FDG, which will be excreted by the body later in a day. As cancer cells growth is faster in the body, they absorb more of the radioactive sugar. After about an hour, the patient is moved onto a table and made to lie for about 30 min. Meanwhile a special camera

captures a picture of areas of radioactivity in provide helpful information about whole body. It is also possible to take a PET and CT scan at the same time (PET/CT scan). This enables the doctor compare areas of higher radioactivity on the PET scan with the more detailed picture of that particular area on the CT scan[56]; (4) Sialochemistry and Sialometry: Sialochemistry deals with chemical analysis of saliva whereas Sialometry is concerned with measuring salivary flow rates and these two measurements of saliva helps in assessment of functioning of salivary glands. The normal volume of the saliva produced by both the major and minor salivary glands constitutes around 600 to 1000 mL per day[57]. This volume varies in different individuals and it may alter in different systemic conditions.

Sialometry can be in relation to whole saliva or gland specific saliva. Whole saliva is a mixture of salivary gland secretions, non salivary secretions including serum transudates, gingival crevicular fluid, food debris and oral microbes[58]. Most often clinicians assess the salivary gland functions through collection of whole saliva. This method is easy to perform and does not require any special equipment. However, whole saliva analysis is of limited value due to its low sensitivity in detecting gland specific dysfunction and gland specific changes in salivary chemical composition[59]; and (5) Salivary gland biopsy or fine needle aspiration (FNA) helps to determine whether the tumor is benign or malignant. In some cases this type of biopsy can help a clinician to avoid unnecessary surgery. Incisional biopsy; is a type of biopsy sometimes preferred if the FNA biopsy does not get a large enough sample to examine. For salivary gland tumors these types of biopsies are not done often. Surgery can both provide enough of a sample for a diagnosis and treat the tumor at the same time[6,17].

WHO classification of salivary glands *neoplasm is listed in Table 1*

Salivary gland neoplasm-Salivary gland cancers include tumors of different patho histologic characteristics and biological behaviour. The most prevalent salivary gland tumors[6,60-62] are: (1) Benign Condition: Pleomorphic Adenoma, Papillary Cystadenoma Lymphomatosum (Warthins Tumor), Basal Cell Adenomas, Oncocytoma; and (2) Malignant tumors: Muco Epidermoid Carcinoma, Adenoid Cystic Carcinoma. Salivary gland neoplasms according to study report represent less than 3% of all tumors.

Prevalence: The tumors can arise in about 80% in parotid gland, 15% in submandibular gland and 5% in the sublingual and minor salivary gland. 65% of submandibular, 50% of minor salivary gland and 20% of sublingual gland tumors are benign[6,12,60]. Aetiology: of the salivary gland neoplasm is not known. However, certain environmental factors and abnormalities are implicated. Environmental factors such as radiation, viruses, extensive use of tobacco and their products, molecular changes and genetic factors are considered as the causative factors. Clinical features: Subjects with benign tumor of parotid gland present with a unilateral, asymptomatic swelling of the involved gland and rarely suffer from pain, difficulty in swallowing and extrusion of fluid from the ears. The benign tumor of other types of salivary glands also present as asymptomatic mass of the affected gland without compromising the functions of the individual.

Malignant tumor of the salivary glands may also present as asymptomatic mass and in advanced stages may cause pain and mucosal/skin ulceration. One third of patients with parotid gland malignancy most often present with facial nerve paralysis[60]. The signs of malignancy in a previous benign tumor of parotid gland can be a sudden increase in the size of the mass, with facial nerve paralysis and shows ulceration of the skin overlying the parotid mass[61].

Pleomorphic adenoma: this tumor has many names-Mixed tumor, Endothelioma, *etc*. which was termed by Willis. In 90% of the cases the tumors affects the parotid gland, most often present in lower pole of superficial lobe of the gland. It occurs more frequently in females than in males between 4-6 decade with average of 43 years. Clinical features: The lesion presents as small, painless quiescent nodules which slowly begin to increase in size, sometimes showing intermittent growth. Surgical excision is the treatment of choice. Treatment: Based on factors like the high recurrence rate, the patient's age, and extensiveness of resection, XRT may be a useful therapy for this type of tumor.

Papillary cystadenoma lymphomatosum (warthins tumor): is the most common tumor in salivary glands first recognised by Albrecht in 1910 and later in 1929 it was described by Warthins. Clinical features: The tumor occurs mainly in parotid, seen over 60 years of age with the sex prediction is male to female 5:1 ratio. Clinically seen bilateral in 6%-12% of patients as painless lesion unless it is secondarily affected[6,12]. Treatment is mostly by surgical excision.

Basal cell adenomas: **Clinical features:** a benign salivary gland adenoma constitute to about 1%-2% of the salivary adenomas occurring mostly in the parotid gland and upper lip of the minor salivary gland. The other types of fewer occurrences of benign salivary adenomas are Canalicular Adenoma, Myo Epithelioma, Ductal Papilloma, and Sebaceous Adenomas. Oncocytoma is another benign tumor particularly affecting the parotid bilaterally seen in both men and women[6,61-63]. Treatment is by conservative surgical excision.

Muco epidermoid carcinoma is the most common malignant tumor of the salivary gland mostly affecting the parotid gland and these accounts for 5% of salivary

gland tumor. Clinical features: This tumor also affects minor salivary gland in 15% of these cases. They are seen in the age group of 40-50 years with female predilection. The tumor is classified as low grade or high grade depending on the ratio of epidermal cells to mucous cells. In this type of tumor the most common cytogenic abnormality is the recurrent translocation between chromosomes 11 and 19 to form CRTCI-MAML2 fusion protein[34,61]. Treatment is the surgical excision of the tumor with post-operative radiotherapy[61].

Adenoid cystic carcinoma accounts for 30% of tumors in minor salivary glands and 6% affecting the parotid gland. Clinical features: It occurs in the middle and older individuals. The tumor has the ability to infiltrate the nervous tissue and spread along the nerve pathways. Biomarkers of epithelial to mesenchymal transition (EMT) such as Snail and Slug appear to be helpful in the diagnosis of adenoid cystic carcinoma[62,64-68].

Treatment is the radical surgical excision followed by Photon beam radiotherapy has shown to be effective.

Adenocarcinoma is the tumor which takes its origin from epithelium of the salivary duct[62]. This group of salivary gland tumors includes specific lesions, like polymorphous low grade adenomacarcinoma, salivary duct carcinoma, Cribiform adenoma carcinoma, *etc.* These tumors present a painful swelling of the affected gland and are very rare in occurrence. Management of these tumors depends on the histologic type of the tumors.

Cribriform adenocarcinoma of the tongue and minor salivary glands (CATMSG) is a low grade salivary gland tumor affecting the minor salivary glands of the oral cavity. This tumor was earlier described by Michal *et al*[64] in 1999 under the name Cribriform Adenocarcinoma of the tongue. In later years studies suggested its origin to be renamed and considered as Cribriform Adenocarcinoma of minor salivary glands a distinct neoplasm[64].

Hyalinizing clear cell carcinoma (HCCC) is a rare, unique low grade tumor affecting minor salivary gland. Milchgrub *et al*[65] in 1994 first described this tumor which exhibited nests, cords, trabeculae and eosinophilic cells in a hyalinised stroma. Clinical features: It primarily arises in the oral cavity but has been described at all salivary gland and seromucous gland sites. Dardick extensively studied under electron microscope, and after re-examination the features of HCCC it was confirmed that it is a squamous lesion[65]. Treatment: the various salivary gland tumors exhibit different histo biological features. In benign tumors no other treatment is usually needed. But when the lesion spreads beyond, treatment of malignant salivary neoplasms depends on the appropriate diagnosis, histologic findings, the clinical stage/condition at presentation and the more recent is the considerations of genetic factor[66,67]. At present the treatment approach is towards conservative elective surgical procedures, combined with the application of postoperative irradiation and chemotherapy[6,17,48,60]. A clinician should have a thorough knowledge of the subject, also be aware of their recent advancements, and work with the group of associated specialists in the management of salivary gland disorders. By following the required, appropriate, systematic diagnostic procedures it helps the clinician to establish a definitive diagnosis and finally assesses the potential for treatment.

Metastatic malignant salivary gland neoplasms: Studies suggests that polymorphous low grade adenocarcinoma, adenoid cystic carcinoma and muco epidermoid carcinoma of the salivary glands[67] are found to have increased metastatic potential. Adenoid cystic carcinoma[68] has been found to metastasize to lungs, bones, skeletal muscles and skin.

Advances in radiotherapy for head and neck cancer sparing the salivary glands

Intensity modulated radiotherapy (IMRT) for head and neck cancer has partial parotid sparing effect which reduces the intensity of post radiotherapy xerostomia[66].

Exposure of salivary glands to Ionizing radiation cause damage to the secretory apparatus of the glands causing xerostomia which could be avoided by the use of any one of the presently available[67] techniques: (1) Shielding of one or more salivary glands form radiation-During radiotherapy for the tumors of parotid gland and areas outside the oral cavity, radio protecting shield can be used to protect the major salivary glands. Shielding may not be feasible in radiotherapy for midline lesions, cancer of oropharynx and larynx due to the position of the cancer and the alignment of the radiotherapy port. Use of conformational dose delivery techniques-The 3 dimensional imaging techniques like CT scans provide for accurate and precise delivery of radiation to the affected tissues with no or minimal damage to the surrounding normal structures. These radiotherapy techniques helps in minimizing the radiation induced xerostomia; (2) Stimulation of acinar cells prior to Radiotherapy-Administration of salivary stimulants like Pilocarpine before each radiotherapy session is found to reduce the complication of diminished salivary flow. However in radiation dose above 50 Gy this beneficial effect is reduced; (3) Use of salivary sparing agents during radiotherapy-Use of agents like Amifostine and heat shock proteins during radiotherapy for head and neck cancer helps in protecting the salivary glands against radiation induced damage; (4) Transplantation of the salivary gland away from the radiation filed-A few studies have reported the beneficial effects of transplanting the major salivary gland away from the radiation filed with maintenance of the ductal connection; and (5) Advanced methods like gene therapy for repairing the damaged acinar cells, injecting the stored pre radiotherapy salivary cells after the completion of radiotherapy, inducing the hematopoietic stem cells to differentiate into salivary acinar cells and thereby replacing the damaged cells

and fabricate artificial salivary tissues from donor tissues and introducing them in place of damaged glands using tissue engineering techniques help in restoring the functions of salivary glands and reduce the complications of reduced salivary flow[68-75].

Emerging salivary diagnostics: Molecular and protein markers of oral diseases

In the oral cavity the presence of multifarious microbial flora exhibits more than many hundreds of microbial species which have been identified so far. Advance microbial research has thrown open to much more new insights and saliva has become the major source to a library of information, and the biomarkers represent the disease and health status of the oral cavity[69-72].

Saliva is a fluid that can be easily collected and contains locally and systemically derived markers of oral disease[68]. The term "salivaomics" was coined in 2008 to reflect the rapid development of knowledge about the various "omics" constituents of saliva. Salivaomics includes five diagnostic alphabets proteins, mRNAs, miRNAs, metabolic compounds, and microbes offers substantial advantages because disease states may be accompanied by detectable changes in one, but not all, dimensions[69]. Human salivary proteome analysis is important for understanding oral health and disease pathogenesis.

Metabolomics is the global assessment and validation of endogenous small-molecule metabolites within a biologic system that has gained increasing popularity and significance in life sciences[70]. Analysis of these key metabolites in body fluids has become an important role to monitor the state of biological organisms and is a widely used diagnostic tool for disease. Metabolomics provides potential advantages that classical diagnostic approaches do not, based on the discovery of clinically relevant biomarkers that are affected by the disease[71-80].

Increase in the incidence of oral cancer has prompted research in salivary biomarkers for oral cancer. More than 100 different salivary biomarkers for oral cancer have been identified. A review on salivary biomarkers for oral cancer categorized this vast variety of salivary biomarkers under different groups which include: (1) Non-organic compound biomarkers, *e.g.*, sodium, calcium, magnesium; (2) Peptide or protein biomarkers, *e.g.*, P53 autoantibody, alpha amylase, *etc.*; (3) DNA, RNA and microRNA biomarkers, *e.g.*, P53 gene codon 63, IL 8, miR-125a, *etc.*; (4) Metabolomic biomarkers, *e.g.*, Valine, lactic acid, *etc.*; and (5) Miscellaneous biomarkers, *e.g.,* Telomerase activity[63,81-95].

Salivary biomarkers are also used for assessment of caries risk. DNA based methods like DNA hybridization, mono clonal antibody (MAb) technique, 16S rRNA/ rDNA, gene cloning and genomic sequencing or T-RFLP methods of analysis help in identification and cariogenic microbial taxonomy using saliva without the need for culture methods[62,73,77,80].

PCR based identification techniques allow for accurate measurement of cariogenic microbiota. Salivary diagnostics suggests a new diagnostic tool for the detection and quantification of oral pathogens directly from its liquid state without the need for isolation of bacterial cells. In children low salivary levels of alpha defensins HNP1-3 may represent biological factor that contributes to caries susceptibility while salivary IgA antibody responses to streptococci mutants can be observed in early childhood[73].

Salivary epithelial cells are found to secrete proteins into blood stream which has led to research on the duacrine function of salivary glands. This function of salivary epithelial cells is being researched as a potential target site for *in situ* gene transfer producing proteins for treating several systemic disorders[74,82].

Saliva, the fluid bathing the oral cavity, is one of the important secretions in the human body. One of the main functions of saliva is digestion of complex carbohydrates and lipids. Technological advancements in the field of diagnostics have opened new avenues to understand the other important and far reaching functions of saliva. The constituents of saliva, also known as biomarkers, act as an index for underlying systemic disease ranging from infections to malignancies.

Salivary glands are surrounded by a rich network of vasculature allowing the biomarker constituents of blood to enter salivary acinus and finally into the salivary secretions. Biomarker is defined as an objectively measured and evaluated indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.

Biomarkers can be in the form of proteins, carbohydrates, lipids or microorganisms. Change in the constitution of these biological molecules may reflect the status of underlying disease processes and can aid in diagnosis, management, evaluating the prognosis and monitoring the outcome of the condition[75]. Biomarkers in saliva have the potential to be used for screening purposes in epidemiological studies.

Matias I, Gatta-Cherifi B, in their study were able to quantify endocannabinoids in human saliva as potential and useful biomarker of obesity[76,96]. Two major forms of Ghrelin (GAH) a recently identified peptide hormone in saliva shows their decrease levels in salivary samples in obese type 2 diabetic patients.

The levels of mRNAs regulating the metabolism of endocannabinoids, N-acylethanolamines and of cannabinoid type 1 [CB (1)] receptor, were assessed in human salivary glands. The study helps in further understanding of the physiopathological mechanisms leading to type 2 diabetes and obesity.

There are numerous investigative tools to identify and quantify the type and load of microbes in the oral cavity. Most of these tools are based on microbial culture methods for identifying disease specific pathogens. Biomolecular microarray based diagnostics (quantitative 16S rRNA gene sequencing, terminal restriction

fragment length polymorphism analysis, *etc.*) are advancements over the conventional culture methods. These methods, when combined with microbial culture techniques, help in enhancing the chances of accurate identification of pathogens[77]. Salivary fluid can also be used for detection of systemic infections. Saliva based enzyme-linked immunosorbent assay (ELISA) has shown promising results in detection of HIV pathogens with 99.3% sensitivity and 99.8% specificity. However positive test results are to be confirmed with western blot analysis. Other systemic infections which can be detected by salivary analysis include hepatitis A, B, C infections, malaria, Ebola, Dengue, CMV, EBV[77,92,93,97] and human herpes virus (HHV) infections. These infections are identified by assessing the viral load, viral antibodies and viral antigens in saliva. These diagnostic parameters are found to correlate well with their corresponding levels in serum. Leptin, is a cytokine identified in human saliva play a protective role in bacterial P. gingivalis infection[9,86] induced inflammatory responses. Another salivary component Ghrelin is found to have a countering effect on P. gingivalis induced impairment of mucin synthesis which plays a role in periodontal infections[61].

Molecular analysis of saliva employing next generation sequencing and human microbe identification micro array techniques have enabled the clinician to identify and characterize a large number of oral microbiota in diseases including Crohn's disease, pancreatic cancer, oral cancer and obesity. In children suffering from Crohn's disease, there is an overall decrease in diversity of oral microorganisms as compared to healthy children. Studies employing the advance microarray techniques report suggests overall significant reduction in Neisseria elongate and Streptococcus mitis species count in the saliva of patients with pancreatic cancer as compared to normal subjects[61,75].

Future research direction: Advances in the management of salivary gland tumours studies stress the need towards molecular targeted therapy of the unusual subpopulation of tumorigenic cancer cells which could arrest the recurrence and metastasis of the tumor. In this direction the cancer stem cell research needs to be further explored in the salivary gland tumors[78,98,99].

Recently a non-invasive, academic prototype chair side cancer diagnostic kit (GC America Inc.) has been devised by Wong DT for the early detection of cancer[79]. Newer field like Proteomics helps in the analysis of the salivary proteins which is extensively used in identification of a specific protein biomarker in saliva for diseases including AIDS, oral cancer, diabetes, periodontal disease and mammary gland carcinoma. The transudate of oral mucosa contains secretory immunoglobulin IgG, IgM and IgA, which serve as a valuable source for immunodiagnostic-based procedures. Using Point-of-care salivary diagnostic screening tests kit[79,92] it is possible to detect viruses in viral infectious diseases such as human papillomavirus (HPV), HCV and HIV.

Advanced Molecular Salivary tests for caries susceptibility may further aids in motivation and patient's education, evidence based dentistry and also in determining effectiveness of anti-caries therapy or caries-control measures including community based services and caries vaccine[73,79]. Further advancements are now being focused at "Omic technologies", which include genomics, proteomics, transcriptomics, and metabolomics have already set their mark in life science research studies[69,77,79]. These emerging technologies have shown to offer highly sensitive, specific, quick and affordable diagnostic test kits in future. Local drug delivery system is another interesting area with the advent of Nano medicine being used in pharmaceuticals industry and biomedical engineering field have shown promising results in future therapeutics. In cancer therapeutics, Nano particles, such as, semiconductor quantum dots, biodegradable micelles, iron oxide nano-crystals[78,81,82,94], are linked with bio targeting ligands, to aim at specific sites in malignant tumors, helpful in cancer therapeutics. Endothelin-1 is one of the probable salivary biomarkers for oral cancer has been reported[63,82,95] for early cancer detection. Dependability of saliva for early diagnosis of dengue disease especially useful in dengue endemic countries is awaited[96]. Salivary ghrelin plays an important protective role in chronic periodontitis and needs further research[86,97,98]. Salivaomics, the future of saliva-based techniques for early diagnosis of dental diseases, is promising. However, further long term studies are needed before these newer methods are adapted to routine clinical practice.

Conclusion: Saliva reflects the physiologic state of the body. Salivary gland diseases may be inflammatory, non-inflammatory, non-neoplastic or neoplastic lesions. Only when a definitive diagnosis is established, treatment depends upon the lesion size, cause, severity, extent and other clinical considerations of the disease. However, a thorough knowledge of the subject including their recent advancements together with a team of associated medical and dental specialists, it is possible to detect the diseases of salivary glands in their early stage and manage them more efficiently. Salivaomics, the future of saliva-based techniques for early diagnosis of dental diseases is promising. Saliva being readily available can be used as a diagnostic tool to help the clinicians for early detection of oral diseases like caries, periodontal disease, oral cancer, salivary gland disorders and non-oral diseases by adapting the advance non-invasive technique and technologies.

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REVIEW

Diagnostic imaging: Morphological and eruptive disturbances in the permanent teeth

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Abstract

This paper reviewed the literature on newer three-dimensional imaging techniques and their applications in diagnosis and treatment planning of various dental anomalies. Developmental anomalies can occur during any of the developmental stages and are manifested clinically after the tooth is fully formed. These dental anomalies may involve a single tooth, a group of teeth, or the entire dentition. Two-dimensional diagnostic imaging, including periapical, occlusal, panoramic, or cephalometric radiographs are essential in localization and management of morphological and eruptive disorders. However, due to their inherent limitations such as insufficient precision because of unusual projection errors and lack of information about spatial relationships, these methods are considered unreliable. Thus, the use of newer image acquisition techniques that allow comprehensive three dimensional imaging and visualization of dental abnormalities is highly recommended for making a confirmatory diagnosis. The significance of accurate endodontic, surgical and orthodontic treatment planning in dental abnormalities cannot be overstated as it pertains to critical anatomic landmarks such as proximity to adjacent teeth or the mandibular canal. The precise information on spatial relationships provided by multiplanar imaging helps the dental surgeon to establish more accurate diagnosis, management strategies and also increases the patient safety. This review highlights the use of high-end diagnostic imaging modalities in diagnosis of the various morphologic and eruptive dental abnormalities.

Key words: Three-dimensional imaging; Spiral computed tomography; Magnetic resonance imaging; Eruptive malformations; Morphological disturbances; Conebeam computed tomography

Core tip: The advent of cone beam computed tomography, Spiral Computerized Tomography and Magnetic resonance imaging in the field of dental radiology has greatly facilitated access to the internal morphology of soft tissue and dental hard tissue structures. These techniques are beneficial in viewing spatial relationship of the suspected anomalous tooth and surrounding structures. Multiplanar imaging resolves the ambiguity of conventional two-dimensional radiographs by allowing the rotation of images at arbitrary angles without image magnifications and distortions.

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INTRODUCTION

Dental anomalies are expressed as morphological and eruption disorders. Morphological dental abnormalities occur due to disturbances during odontogenesis and it includes abnormality in number (supernumerary teeth), abnormality of tooth shape and size (dens in dente, dilaceration, fusion, root dwarfing)[1]. Eruption disturbances are mainly divided as disturbances related to time (premature eruption, delayed eruption or impactions) and disturbances related to position (ectopic eruption and transpositions).

A number of factors are responsible for eruptive and morphological dental abnormalities including genetic and congenital anomalies, metabolic disturbances, post operative complications of head and neck radiation therapy, use of chemotherapeutic agents, traumatic injuries to the primary dentition affecting the permanent tooth formation, *etc*[1,2]. The embryologic tooth development of teeth starts around one and half month of intrauterine life and is characterized by distinct odontogenic stages including initiation, proliferation, histodifferentiation, morphodifferentiation, apposition, and calcification. Each stage has its own significance and disturbance at any stage may result into the development of an anomaly. The development of primary dentition occurs in a protected prenatal environment and duration of deciduous teeth development is small, therefore, they are less prone to dental abnormalities than the permanent teeth.

Radiographic method is considered to be advantageous for the preoperative determination of position and nature of these dental anomalies. Conventional plain film radiographic methods such as periapical, occlusal, panoramic, or cephalometric radiographs are a de-facto standard for diagnosis and treatment planning. However, due to their inherent limitations such as insufficient precision because of unusual projection errors and lack of information about spatial relationships, these methods are considered unreliable[3,4]. Earlier, computed tomography (CT) has been used widely in maxillofacial imaging to provide detailed 3-dimensional (3D) information. It forms an integral part in diagnosis of oral and maxillofacial regions as it facilitates access to the internal morphology of dentofacial structures. Its benefits of viewing spatial relationship of the anomalous tooth with surrounding structure by the rotation of images at arbitrary angles and absence of image magnification and distortions are well recognized and widely accepted in the literature. It is considered as more desirable morphometric tool than conventional plain film radiography[5-7]. However, it has certain limitations reducing its diagnostic reliability in dentistry including relatively low between-slice accuracy, no interslice gaps, artifacts, edge gradient effects and high radiation dose[8]. Recently, a series of cone beam computed tomography (CBCT) scans for three dimensional imaging of dentomaxillofacial region have been developed[8-13]. The aim of the study is to present a systematic review of the literature on the diagnostic imaging of the morphological and eruptive disturbances in the permanent teeth.

DIAGNOSTIC IMAGING TECHNIQUES FOR ASSESSMENT OF ERUPTIVE AND MORPHOLOGICAL DENTAL ANOMALIES

CBCT is relatively newer image acquisition techniques that allow comprehensive multiplanar imaging and visualization of dental abnormalities in which resolution is measured in voxels[13,14]. It uses cone-shaped X-ray beam centered on a two-dimensional (2D) detector. The source-detector system performs one rotation around the object producing a series of 2D images. The 3D images are obtained by reformatting the 2D data in volume using a modification of the original cone-beam algorithm developed by Feldkamp *et al*[15] in 1984. CBCT scanners for the dentofacial region were originally developed in the late 1990s independently by Arai *et al*[16] in Japan and Mozzo *et al*[17] in Italy. CBCT has little projection effect and no magnification errors because X-ray beams are orthogonal, resulting in undistorted 1:1 measurements. It provides information for the entire craniofacial region[18,19] (Table 1).

IMPACTED TEETH

Impaction is defined as eruption failure of a tooth to its normal site in occlusion during its normal growth period because of malposition, lack of space, abnormal habit or mechanical obstruction in its eruption trajectory. The precise localization and diagnosis of an impacted tooth is required for proper surgical access and treatment planning. Nakajima *et al*[20] demonstrated the importance of CBCT image acquisition technique in cases of delayed eruption of the maxillary left second premolars and severe impaction of a maxillary second bicuspid. CT images provided more accurate information for orthodontic diagnosis and management strategies than conventional radiographic images such as precise observation of tooth morphology, root condition, and superimposition of bone.

3D imaging in impacted permanent incisors

Traumatic injuries to primary dentition cause eruptive malformations in underlying permanent tooth germs as they are in a close contact with their primary predecessors[21]. It may hinder the eruption pathway of the permanent tooth germ leading to delayed or failure of

Table 1 Role of conebeam computed tomography for assessment of eruptive and morphological disturbances

Ref.	Year	Eruptive or morphological disturbances	Tooth involved
Nakajima *et al*[20]	2005	Impaction	Maxillary second bicuspid
Walker *et al*[12]	2005	Impaction	Canine
Siraci *et al*[45]	2006	Talon cusp	Facial and lingual surfaces of a supernumerary primary tooth
Maverna *et al*[34]	2007	Impaction	Maxillary canine
Andrade *et al*[49]	2007	Root dilaceration	Maxillary right central incisor
Liu *et al*[14]	2007	Supernumerary teeth	Complete dentition
Liu *et al*[32]	2008	Impaction	Maxillary canines
Haney *et al*[18]	2010	Impaction	Maxillary canine
Patel *et al*[59]	2010	Dens invaginatus	Mandibular lateral incisor
Song *et al*[73]	2010	Fusion	Right maxillary first molar and supernumerary tooth
Gurge *et al*[24]	2011	Impaction	Upper lateral incisor
Alqerban *et al*[33]	2011	Impaction	Maxillary canine
Kaneko *et al*[60]	2011	Dens invaginatus	Maxillary lateral incisor
Narayana *et al*[61]	2012	Dens invaginatus	Maxillary right lateral incisor
Vier-Pelisser *et al*[62]	2012	Dens invaginatus	Maxillary left lateral incisor
Kfir *et al*[63]	2013	Dens invaginatus	Right maxillary central incisor
Kato[64]	2013	Dens invaginatus	Maxillary lateral incisor
Cantin *et al*[65]	2013	Impaction	Mesiodens
Oenning *et al*[30]	2014	Impaction	Third molar
Mahesh *et al*[50]	2014	Root dilaceration	Permanent maxillary right central incisor

eruption[22]. The effect on the underlying tooth bud is related to its stage of odontogenesis, type and direction of impact[23].

Gurge *et al*[24] carried out CBCT imaging for comprehensive multiplanar evaluation, exact localization and conservative management of non erupted permanent upper lateral incisor with a previous history of a trauma through the primary predecessor in a 9-year-old patient. They highlighted the need for CBCT image acquisition techniques in the cases of impacted teeth where 3D visualization is necessary.

Impacted 3rd molars

Third molars are the most frequently impacted teeth. The dental literature has revealed these teeth are usually associated with pericoronitis, cheek biting, cysts, odontogenic tumors, and external root resorption of proximal teeth[25-29]. CBCT has proven to be an efficient method for evaluation of spatial relationship in different planes before deciding on management of impacted third molars.

Oenning *et al*[30] assessed external root resorption of second permanent molars associated with impacted third molars by conventional radiography and CBCT imaging. They observed significantly higher number of cases of ERR with CBCT imaging technique and highly recommended its use in cases of impaction specially mesioangular or horizontal impactions.

Impacted canines

Maxillary canines are the 2nd most commonly impacted teeth. Earlier tube shift technique was used to reveal the position of unerupted canine but the exact extent of displacement cannot be determined. CBCT imaging is beneficial in providing the accurate labial/palatal position and angulation of the impacted canine[3,31].

Walker *et al*[12] assessed the spatial relationship of impacted canines with adjacent structures and root resorption of incisor with the aid of 3D images produced from NewTom QR-DVT 9000. 3D volumetric imaging of impacted canines depicted the size of the follicle, inclination of the long axis of the tooth, relative buccal and palatal positions, bone covering the tooth, 3D proximity and resorption of roots of proximal teeth, local anatomic considerations and stage of dental development.

Liu *et al*[32] determined the position of 210 impacted maxillary canines and resorption of adjacent incisors with CBCT images. The angular and linear measurements depicted the spatial variations of the impacted canines which provided the picture for three dimensional relationships of the impactions relative to the adjacent dental arch.

Haney *et al*[18] compared differences in the diagnosis and treatment planning of impacted maxillary canines with traditional 2D imaging techniques and 3D CBCT volumetric images. The results yielded that the clinicians' confidence in the accuracy of diagnosis and treatment plan was statistically greater for CBCT images ($P < 0.001$).

Alqerban *et al*[33] compared two CBCT systems *vs* traditional 2D imaging for assessing the location of impacted maxillary canine and identification of root resorption and observed that CBCT was more sensitive than panoramic radiography.

Maverna *et al*[34] evaluated different radiographs for the localization of impacted maxillary canines [orthopantomography (OPT), laterolateral and postero-anteriorteleradiography, parallax method, laterolateral, occlusal radiography, computerized axial tomography, cone beam CT]. They concluded that CBCT provided elements which escaped during traditional radiographic analysis and

is therefore indicated in case of impacted teeth or craniofacial structural anomalies.

ECTOPIC ERUPTIONS

Transposition is a rare type of ectopic eruption where a permanent tooth erupts in the position normally occupied by another permanent tooth[35-39]. Transposition are more frequently observed in upper arch (68.5%-76%) than in lower arch[39-42]. Maxillary canine and the first premolars are most commonly transposed[40]. The ultimate success of the treatment plan is based upon accurate assessment and precise localization of the transposed teeth. Ericson and Kurol[43] reported that, in a sample of Swedish children, conventional periapical radiography successfully localized only 80% of ectopic canines. Rest 20% required tomography for exact localization especially in cases with overlapping lateral incisor (Table 1).

TALON CUSP

Talon cuspis an unusual morphological dental anomaly that is most commonly seen in the form of an accessory cusp-like structure projecting from the lingual or facial surface of anterior teeth[44]. A talon cusp is morphologically well-delineated. The appearance of projection is conical and resembles an eagle's talon. Talon cusp may occur in both primary and permanent dentitions. It can occur in maxillary or mandibular anterior teeth. It is seen in both sexes[45].

Siraci *et al*[46] demonstrated unusual presentation of a talon cusp, occurring on both the facial and lingual surfaces of a supernumerary primary tooth. Existence of pulpal extensions was investigated using cone beam X-ray CT. It revealed distinct existence of pulpal extensions within the facial and palatal talon cusps. According to Mader and Kellogg[47], it was very difficult to distinguish the existence of a pulpal extension, which was confirmed in this case. Other two radiographs were taken from different angles but the interpretation of accessory pulp horns was uncertain which necessitated the utilization of a cone-beam CT for a correct diagnosis.

ROOT DILACERATION

Dilaceration is a developmental anomaly which occurs as a result of an abrupt change in the axial inclination between the crown and the root of a tooth. But the criteria in the literature for recognizing root dilaceration vary. Two possible causes of dilacerations are trauma and developmental disturbances. It has also been proposed that it might be associated with some developmental syndromes. Dilaceration is seen in both the permanent and deciduous dentitions, and it is more commonly found in posterior teeth and in the maxilla. Periapical radiographs are commonly used to diagnose the presence of root dilacerations[48].

Andrade *et al*[49] evaluated tooth displacement and root dilaceration after trauma to primary predecessor by CT. The tomograms were analyzed using a dental computed tomography software program in order to evaluate the root formation of the upper right permanent central incisor and its position in the anterior alveolar process.

Mahesh *et al*[50] described the use of CBCT for the 10-year-old patient with the complaint of non-eruption of the permanent maxillary right central and lateral incisors. A cone-beam CT scan was performed to assess the extent of dilaceration, if any, and to aid in the creation of a suitable treatment plan. It revealed palatal displacement of the crown and a gradual curvature in the apical 1/3rd of the root of right central incisor.

SUPERNUMERARY TEETH

Supernumerary teeth are a relatively frequent disorder of odontogenesis characterized by an excess number of teeth. It can be found in any region of the dental arch both in the primary and permanent dentition. Associated complications are failure of adjacent teeth to erupt, displacement and crowding of the adjacent teeth, abnormal diastema, root resorption.

Liu *et al*[14] used CBCT for evaluation of 626 supernumerary teeth in 487 patients. The ability of CBCT to visualize dental and skeletal structures relative to supernumerary teeth was also evaluated. A new system was proposed to classify the complex spatial location of supernumeraries in the maxillary anterior arch based on evaluation with CBCT. Type Ⅰ, type Ⅱ, and type Ⅵ were located palatal to the neighboring incisors in a variant craniocaudal position. Type Ⅲ and type Ⅳ were seen within the dental arch, oriented normally, inverted, or in cross section. Type Ⅴ was the supernumerary teeth located labially and superior to the incisor root and is rarest in occurrence. This classification system may yield an accurate picture for the 3D relationship of the supernumeraries relative to the adjacent dental structures, which is important during surgical or orthodontic evaluation. CBCT imaging yields accurate 3D pictures of local dental and bony structures, which is helpful for pretreatment evaluation of supernumerary teeth.

Supernumerary premolars are a rare anomaly in the maxillofacial complex. Its rarity and complex characteristics often makes it difficult to treat. CBCT plays an important role in assessment of both the location and the typing of supernumerary teeth[14,51,52]. Thus, CBCT is crucial for exact localization which assists in proper treatment planning, and for the surgical approach in cases of multiple supernumerary teeth[53]. The benefits of CBCT imaging being low radiation dose and accurate diagnosis of the complex pathology in case of supernumerary teeth[54]. Odontomas are odontogenic tumours, resulting from epithelial growth and differentiated mesenchymal cells, clinically asymptomatic, and often associated with changes to the eruption of the permanent dentition. In recent years,

CBCT has been used in the diagnosis and treatment planning of this condition[55].

DENS INVAGINATUS

Dens invaginatus (DI) is a dental developmental anomaly that results from invagination of the enamel organ into the dental papilla prior to the mineralization phase[56]. The cavity that forms in the case of dens invaginatus may serve as an external route of communication with the pulp or periapical tissues through the foramen caecum. The complexity of the internal anatomy in the case of dens invaginatus creates clinical challenges. Conventional periapical radiographs provide limited information regarding the anatomical configuration. The 3D imaging (CBCT) helps in identifying the morphology of the individual dens so that appropriate treatment planning and treatment options can be selected[57,58].

Patel *et al*[59] reported the use of CBCT in the assessment of chronic periradicular periodontitis associated with an infected invagination in an immature mandibular lateral incisor tooth. A CBCT scan revealed that there was no communication between the invagination and the main root canal. Endodontic treatment was carried out on the invagination. It was observed that that the true nature of dens invaginatus cannot be estimated from conventional radiographs accurately. Cone beam computed tomography is a useful diagnostic tool in the management of dens invaginatus.

Kaneko *et al*[60] described the use of CBCT to diagnose Oehlers' type Ⅲ dens invaginatus in a maxillary lateral incisor. The CBCT scans demonstrated inaccessible and unfilled canal and invagination areas because of complex internal morphology. It was characterized by C-shaped cross-sectional canal configuration with constrictions at different points in different root levels and a prominent intraradicular cavity that was communicated with the enamel-lined invagination and opened into the apical periodontium. It was however judged that further endodontic treatment of the same was not feasible. CBCT helped in the diagnosis thus decision of avoiding further intervention was made that could have been difficult to negotiate.

Narayana *et al*[61] used CBCT to aid in the diagnosis and treatment-planning phase in 11-year-old male who reported for the treatment of maxillary right lateral incisor. 3D imaging helped in identifying the morphology of the individual dens which further guided the selection of the treatment provided. The morphology of the dens invaginatus was identified, and a periapical radiolucent area was detected that was not visible on a standard periapical radiograph.

Vier-Pelisser *et al*[62] presented the case of a maxillary left lateral incisor with Oehlers' type Ⅲ dens invaginatus in which CBCT was used as an adjunctive resource in the diagnosis and in the planning and 2-year follow-up of the nonsurgical/surgical treatment. The CBCT scans revealed that the periapical radiolucency was significantly larger than seen radiographically and the increased thickness of the buccal cortical plate was also seen.

Kfir *et al*[63] investigated the use of 3D plastic models, printed CBCT data, for accurate diagnosis and conservative treatment of a complex case of dens invaginatus. The CBCT scan provided with the information about the true nature of invagination and its relationship to the main canal. It was useful for demonstrating how the invagination had compressed the pulp space of the main canal at different levels which led to irregular main canal with a cross-section resembling a thin crescent encircling the invagination. It was also seen that there was no communication between the invagination and the pulp space.

Kato[64] described a case of surgical and non-surgical endodontic therapy for a maxillary lateral incisor with type Ⅲ dens invaginatus with necrotic pulp and an associated large periradicular lesion. CBCT was used for three-dimensional observation of the morphological details of this area. It was observed that even complicated cases of dens invaginatus can be diagnosed and treated using non-surgical root canal management with the help of CBCT.

Dens invaginatus can also be associated with other abnormalities such as dysmorphic mesiodens. Though this condition can be detected by chance on the conventional radiography, the three-dimensional nature and the exact morphological patterns of DI can be determined by CBCT. Cantin *et al*[65] presented a morphological study of impacted mesiodens in a 9-year-old girl in whom the three coronal invaginations were detected only by CBCT.

The presence of double dens invaginatus is extremely rare. Understanding the type, extension, and complex morphology of dens invaginatus is essential for the proper treatment planning. Advanced imaging techniques, such as CBCT are very helpful in diagnosis of these complex anatomic variations as they give the 3 dimensional images unlike the conventional radiographic methods[66].

FUSION

Fusion also known as synodontia or false germination; occurs due to the union of 2 or more separately developing tooth germs at the dentinal level, presenting a single large tooth during odontogenesis, when the mineralization of crown is yet mineralized[67]. The prevalence of tooth fusion is estimated to be 0.5%–2.5% in primary dentition[68], whereas it is lower in permanent dentition. When fusion takes place between a normal tooth and a supernumerary tooth, the fused teeth shows an anomalous broad crown[69]. The pulp chambers and root canals can be joined or separated, depending on the stage of development when fusion took place[70]. Radiographic examination of fused teeth is important for management of endodontic problems. But the conventional intraoral periapical views produced only a 2D image which resulted in the superimposition

Table 2 Role of spiral computerized tomography in diagnosis of morphological disturbances of teeth

Ref.	Morphological disturbance	Tooth involved
Gopikrishna *et al*[79]	Unusual morphology of a single root and a single canal	Maxillary first molar
Ballal *et al*[77]	Fusion	Mandibular second molar with a paramolar
Metgud *et al*[78]	Single conical non bifurcated posterior root forms, taurodontism, dens invaginatus, labial lobes of the canines, pyramidal cusps of the premolars, dens evaginatii of the molar crowns, and localized reduction in tooth size	Entire dentition

of structures[71]. Nowadays a new diagnostic tool, CBCT, is being used in endodontics. Matherne *et al*[72] reported that CBCT imaging is useful in identifying root canal systems.

Song *et al*[73] discussed the endodontic management of a supernumerary tooth fused with a right maxillary first molar. They used CBCT for proper imaging of the same. Proper diagnosis and treatment planning can be done with the use of CBCT that further ensures predictable and successful results.

Ferreira-Junior *et al*[74] reported a case of fusion between an impacted third molar and a supernumerary tooth. The surgical intervention was carried out, with the objective of eliminating the dental elements. Proximity to the mandibular ramus made the final diagnosis difficult with panoramic radiography. Thus, CBCT was used to determinate the final diagnosis and also to help in the further surgical planning. It was observed that cone-beam computed tomography resulted in precise 3D information which was not possible with conventional radiography (Table 2).

Recently, a new CT technique, *i.e.,* spiral computed tomography (SCT) also called volume acquisition CT, has been developed which has a significant advantage. It uses simultaneous patient translation through the X-ray source with continuous rotation of the source-detector assembly[75]. SCT acquires raw projection data with a spiral-sampling locus in a relatively short period[76]. The data can be viewed as conventional transaxial images without any additional scanning time. This technique makes reconstruction of overlapping structures at arbitrary intervals possible. Thus the ability to resolve small subjects is increased.

The unique arrangement of the gantry and rotating X-ray source assembly reduces scan times. With standard incremental CT, small objects can be missed or their detection compromised if the patient's degree of inspiration and expiration varies from scan to scan. Moreover, multiplanar and 3D image reconstructions of structures from standard incremental CT data are degraded by motion-induced misregistration artifacts[77].

Ballal *et al*[77] reported a rare case of successful endodontic management of unilateral fused mandibular second molar with a paramolar. The rarity and complexity of the entity makes it difficult to diagnose and treat. The use of diagnostic imaging modalities such as spiral SCT helps in forming a confirmatory diagnosis and treatment plan.

Metgud *et al*[78] reported a unique case with a unusual combination of morphological dental anomalies, including single conical non bifurcated posterior root forms, taurodontism, dens invaginatus, labial lobes of the canines, pyramidal cusps of the premolars, dens evaginatii of the molar crowns, and localized reduction in tooth size involving the entire dentition. It was not related with any other apparent systemic complication. The accurate assessment of the presence of single conical non bifurcated posterior root forms was done with the help of spiral computerized tomography.

Gopikrishna *et al*[79] reported a maxillary first molar with an unusual morphology of a single root and a single canal. An accurate assessment of this unusual morphology was made with the help of a Spiral computed tomography which was then endodontically managed.

Both case reports highlighted the use of high-end diagnostic imaging modalities such as spiral computerized tomography in diagnosis of the various morphologic abnormalities (Table 3).

3D imaging techniques are being utilized in dentistry beyond maxillofacial surgical planning. Dental magnetic resonance imaging (MRI) is technique that adds a third dimension to treatment planning other then on CT and digital volume tomography.

MRI, is quite new in the field of dental radiology. The technique does not use ionizing radiation and is safe when no contraindications are present such as (cardiac pacemakers, implanted cardiac defibrillators, aneurysm clips, neurostimulators, metallic foreign bodies in the eyes, *etc.*). It has no limitations in the frequency of examinations. MRI is based on the nuclear magnetic resonance phenomenon, which takes place when nuclei of certain atoms (usually hydrogen in medicine) are placed in a strong static magnetic field and absorb energy of an alternating magnetic field of a specific, resonant frequency. The use of spatially varying magnetic fields makes it possible to spatially encode the nuclei and perform tomographic imaging.

The signal measured on MRI usually originates from soft tissues and liquids in the human body. The teeth and jawbone appear black in images taken by MRI due to low water content and short relaxation constants of hydrogen atoms. The dental pulp, jawbone marrow, mandibular canal containing the mandibular artery and vein and the alveolar nerve, saliva, gingiva, facial soft tissues, tongue, and palate produce a signal on clinical MRI. But there is no measurable signal from dental enamel, dentin, cortical bone, and air. The structures that do not produce any sign can also be measured indirectly from the contrast

Table 3 Magnetic Resonance Imaging in diagnosis of eruptive and morphological dental anomalies of permanent teeth

Ref.	Morphological disturbance	Tooth involved
Tymofiyeva *et al*[81]	Impaction	Entire dentition
Tymofiyeva *et al*[83]	Mesiodens, gemination, dilacerations, transmigration, transposition	Entire dentition

with adjacent signal-emitting structures.

MRI enables 3D measurement of the mandible because of the contrast between the cortical bone and the surrounding soft tissue[80]. Impacted teeth can be visualized and their positions in all 3 dimensions can be assessed. As there is difference in contrast between the teeth and the surrounding tissue, such as the gingiva, tongue, cheek, saliva, and jaw bone marrow[81].The most significant advantage of dental MRI is the complete absence of ionizing radiation when compared to other 3D imaging techniques.

Tymofiyeva *et al*[81] discussed economic aspects and technical properties of MRI compared with cone-beam CT in diagnosing impacted teeth. Gaudino *et al*[82] reported that MRI shows a comparable accuracy and better visibility in the detection of teeth and periodontal anatomy compared with cone-beam CT.

Tymofiyeva *et al*[83] assessed the feasibility of MRI of dental abnormalities in 16 patients (mean age, 10.8 years). The selected patients included 3 with a mesiodens, 9 with supernumerary teeth other than a mesiodens, 1 with gemination, 1 with dilacerations, 1 with transmigration, and 1 with transposition. MRI was found to be a suitable imaging modality for the diagnosis of dental abnormalities in children and for orthodontic treatment and surgical planning. MRI had various advantages when compared with conventional radiographic methods such a 3 dimensionality and complete elimination of ionizing radiation, which is relevant for repeated examinations in children.

CONCLUSION

In conclusion, the results of this review showed that 3D imaging techniques are crucial for exact localization and provides precise three dimensional information on spatial relationships by depicting the size of the follicle, inclination of the long axis of the tooth, relative buccal and palatal positions, bone covering the tooth, proximity and resorption of roots of proximal teeth, local anatomic considerations and stage of dental development. Thus, the use of newer image acquisition techniques such as Cone Beam Computed Tomography, Spiral Computerized Tomography and Magnetic Resonance Imaging are advocated for final diagnosis and precise treatment planning of eruptive and morphological dental anomalies.

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Klippel-feil: A syndrome in the occipital-cervical spine field and its dentofacial manifestations

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Abstract

Klippel-Feil syndrome (KFS) is defined by congenital cervical vertebral spine fusion and is seen with a wide spectrum of dental manifestations and craniofacial profiles. Previous studies on lateral cephalograms have documented an association between fusion of the cervical vertebrae and deviations in the craniofacial profile in non-syndromic patients with severe malocclusion. To our knowledge, no previous studies have described the craniofacial profile including the cranial base of KFS patients on lateral cephalograms. Therefore KFS and its craniofacial and dental manifestations were described according to existing literature and additionally the craniofacial profile and cranial base was analysed on lateral cephalograms of two patients with KFS. According to the literature the dental manifestations of KFS-patients included oligodontia, overjet, cross bite, open bite and deep bite. The craniofacial profile was clinically described as reduced lower facial height, midfacial hypoplasia, and mandibular prognathia. The analyses of the two lateral cephalograms showed increased mandibular inclination, increased vertical jaw-relationship, increased jaw angle and maxillary retrognathia. The cranial base was normal in both cases. The sagittal jaw relationship and mandibular prognathia varied between the two cases. The literature review and the analyses of the two lateral cephalograms have shown that deviations in the occipital and cervical spine field as KFS were associated with deviations in the teeth and craniofacial profile.

Key words: Occipital and cervical spine field; Klippel-Feil syndrome; Notochord; Embryology; Cervical column morphology; Malocclusion

Core tip: Klippel-Feil syndrome (KFS) is defined by congenital cervical vertebral spine fusion and is seen with a wide spectrum of dental manifestations and craniofacial profiles. According to the literature dental manifestations of KFS-patients included oligodontia, horizontal maxillary overjet, cross bite, open bite and deep bite. The craniofacial profile was clinically described as reduced lower facial height, midfacial hypoplasia, and mandibular prognathia. Furthermore, two cases showed

increased mandibular inclination, increased vertical jaw-relationship, increased jaw angle and maxillary retrognathia. The literature review and case analyses showed that deviations in the occipital and cervical spine field as KFS were associated with dentofacial deviations.

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INTRODUCTION

Spranger *et al*[1] define syndromes accordingly: "A syndrome is a pattern of multiple anomalies thought to be pathogenically related and not known to represent a single sequence or a polytopic field defect". "Sequence" is defined as "A pattern of multiple anomalies derived from a single known or presumed prior anomaly or mechanical factor" and polytopic field defect is defined as "A pattern of anomalies derived from the disturbance of a single developmental field"[1]. Dorland's Illustrated Medical Dictionary defines syndromes as: "A set of symptoms that occur together; the sum of signs of any morbid state; a symptom complex. In genetics, a pattern of multiple malformations thought to be pathogenically related"[2].

In Gorlin's Syndromes of the Head and Neck[3] states that: "Congenital malformations of the head and neck are common and most resolve spontaneously within the first few days of postnatal life". In some cases these "congenital malformations" turns out to be a part of a syndrome.

There are many different syndromes of the head- and neck-region and these syndromes are characterized by various clinical manifestations[3]. The manifestations can be specific for one particular syndrome, but different syndromes can have common manifestations[3]. Clinical manifestations can be revealed as skeletal deviations, soft-tissue-deviations or a combination of both[3].

Klippel-Feil syndrome (KFS) is a syndrome of the head- and neck-region. The syndrome has originally been described in the 16th Century, but was not named until 1912 by Hennekam *et al*[3]. KFS is defined by faulty segmentation of two or more cervical vertebrae[3] resulting in the occurrence of fusion of two or more cervical vertebrae on head film radiographs as lateral cephalograms. KFS is characterized by a triad of clinical symptoms such as short neck, limitation of head movement, and low posterior hairline. Furthermore, there are many associated anomalies in the craniofacial field in patients with KFS[3].

KFS is located in the occipital and cervical spine developmental field. The occipital and cervical spine field consists of structures of common embryological origin initiated by notochordal induction of the sclerotome formation in the somites, which develop into the cervical spine and the osseous structures in the occipital region[4-9]. The developmental field is funnel-shaped and limited anteriorly by the vertebral bodies, the basilar part of the occipital bone, and the postsphenoid bone. Posteriorly, the developmental field is limited by the cartilaginous part of the occipital bone and the vertebral arches[5,6].

A series of recent studies of non-syndromic patients with severe skeletal malocclusion traits have described the occurrence of cervical vertebral column fusion anomalies and analyzed the association between fusion anomalies and the craniofacial profile on lateral cephalograms[10-13]. These studies have documented significant associations between fusion and a large cranial base angle, between fusion and retrognathia of the jaws, and between fusion and inclination of the jaws. These findings indicate an association between fusion of the cervical vertebral column and the craniofacial profile including the cranial base in non-syndromic patients with severe skeletal malocclusion traits[10-14].

To our knowledge no previous studies have described the craniofacial profile including the cranial base of KFS patients on lateral cephalograms.

Therefore, the aim of the present study was to describe KFS and its craniofacial and dental manifestations according to previous literature. Additionally, the aim was to describe the craniofacial profile including the cranial base on lateral cephalograms of two patients with KFS.

RESEARCH

A literature review was performed in order to describe KFS and the dentofacial manifestation. Furthermore, lateral cephalograms of two patients with KFS with no other known symptoms (one boy, 8 years old and one girl, 15 years old; Figures 1 and 2) were included in the study. The two KFS patients comprise all KFS patients with no other known symptoms from Professor Sven Kreiborg's archive, Department of Odontology, Copenhagen University. The craniofacial profile including the cranial base were measured by points and lines according to Solow and Tallgren[9]; illustrated in Figure 3. The landmarks used in the present study were marked on acetate sheets fixed to the radiograph. The variables were measured using a protractor and are shown in Table 1. The variables were compared to normal values of the craniofacial profile according to Björk *et al*[15] (Table 1). As the present study was a literature review and description of two lateral cephalograms no statistical analyses have been applied.

LITERATURE REVIEW

Definition, prevalence and diagnosis

KFS is a rare, congenital, skeletal malformation. It is defined by failure of normal segmentation of any two of the seven cervical vertebrae[16,17]. KFS may

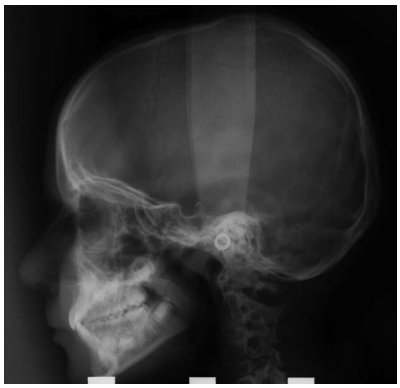

Figure 1 Lateral cephalogram of an 8-year-old boy with Klippel-Feil syndrome with no other symptoms.

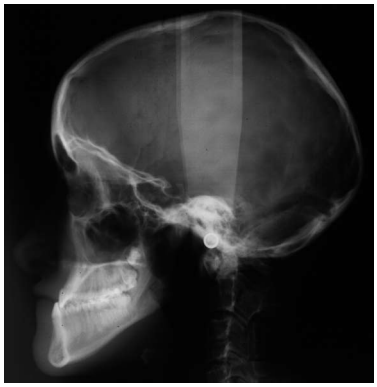

Figure 2 Lateral cephalogram of a 15-year-old girl with Klippel-Feil syndrome with no other symptoms.

include fusion caudally of the cervical region. However, fusion in the lower spine, in the absence of cervical vertebral fusion, is not classified as KFS[18]. Generally, the second and the third vertebrae (C2-3)[18-20] and the fifth and sixth vertebrae (C5-6)[19] interspaces are most commonly fused. The C2-3 interspace fusion is thought to be an autosomal dominant inheritance, while C5-6 interspace fusion is considered to be autosomal recessive[19].

The absence of population screening studies has made it impossible to define the exact incidence and prevalence of KFS, but it has been estimated that it occurs in approximately 1:40.000-42.000 births[21,22]. Other studies have suggested, that KFS has an incidence of up to 0.5% of live births[18]. The incidence was none significantly slightly higher in females[19,21,22].

Although affected patients have cervical anomalies at birth, KFS is usually diagnosed at a later age[22]. It has been suggested that the fusion process in KFS patients is not fully present at birth and could be ongoing until skeletal maturity[20]. The disorder is often discovered incidentally when radiographs have been taken for other reasons[22]. The prognosis for most individuals diagnosed with KFS is good if the disorder is diagnosed early. But diagnostics of KFS is often complicated because the presence of cervical fusion

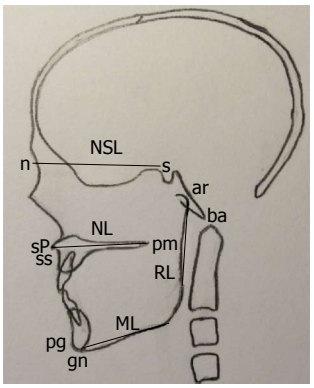

Figure 3 Illustration of reference points and lines according to Solow and Tallgreen[9], 1976 on lateral cephalogram. NSL: Nasion-Sella line; NL: Nasal line; ML: Mandibular line; RL: Ramus line.

cannot be determined in children younger than 8 years due to the development and ossification of the cervical vertebrae[17,22].

Occipitalization is also seen in KFS[20], and patients with atlantoaxial fusions are often diagnosed with KFS at younger ages than patients with more caudal fusions[22].

Etiology, pathogenesis and classification

KFS is both affected by genetic and environmental factors and is morphologically and etiologically heterogeneous[3,23]. The heterogeneity of patients with KFS has made the diagnosis and classification difficult and has complicated elucidation of the genetic etiology of the syndrome[22]. Mutations in Pax1 have been found in patients with KFS, but the significance remains uncertain[3].

The earliest classification of KFS, by Feil, was based only on the anatomic distribution of the fused segments. Patients with KFS were assigned to one of three types[19]: Type Ⅰ applied to patients with extensive cervical and upper thoracic fusion. Type Ⅱ defined patients with one or two cervical interspace fusions and are often associated with hemivertebrae and occipitoatlantal fusion. Type Ⅲ classified individuals with both cervical and lower thoracic or lumbar fusion[19].

Whereas Feils classification was based on the extent of vertebral fusion, a classification made by Clarke *et al*[18] focused on the etiology and genetic origins of the syndrome. Clarke *et al*[18] used three families, all affected with KFS, as a model for a new and comprehensive classification consisting of four different classes of KFS (KF1-4). Their study showed that an association exists between the position of the most rostral fusion, mode of inheritance, and some specific KFS associated anomalies[18].

Craniofacial and dental symptoms

Fusion of the cervical vertebrae can be symptomatic or asymptomatic. Some studies have found that up to 68% of KFS patients reported symptoms related to their syndrome[20]. The classic triad of KFS included low

Table 1 Measurements of the craniofacial profile in two Klippel-Feil syndrome patients (case Ⅰ and Ⅱ) and normal values

	Boy 8 yr (Figure 1) Case Ⅰ	Girl 15 yr (Figure 2) Case Ⅱ	Normal values according to Björk *et al*[15]
Sagittal dimensions			
s-n-ss	78.5°	76°	82° (SD 3.5°)
s-n-pg	75°	86°	80° (SD 3.5°)
ss-n-pg	3.5°	-10°	2° (SD 2.5°)
Vertical dimensions			
NSL/NL	9.5°	8°	8° (SD 3°)
NSL/ML	39.5°	37°	33° (SD 6°)
NL/ML	30°	29°	25° (SD 6°)
Cranial base			
n-s-ba	132°	134°	131° (SD 4.5°)
Jaw angle			
ML/RL	142°	131°	126° (SD 6°)

Normal values according to Björk *et al*[15]. SD: Standard deviations; NSL: Nasion-sella line; NL: Nasal line; ML: Mandibular line; RL: Ramus line.

posterior hairline, short neck, and limitation of the neck movement[17,19]. However, the triad is present in only 50% of the patients[16,19,22]. Several other anomalies have been associated with KFS in varying degrees[18]. The anomalies included both systemic manifestations and craniofacial manifestations[24].

The most common manifestations in the craniofacial field are cleft palate[19,24], bifid uvula, and facial asymmetry[24]. Less frequently reported is the incidence of craniosynostosis and facial appearance. Facial appearance includes: reduced lower facial height, midfacial hypoplasia, mandibular malformation, and hypoplasia[19,24] and mandibular prognathia[25].

Moreover, KFS-patients show jaw anomalies, *e.g.*, multiple jaw cysts, abnormal bony masses, duplication of the rami of the mandibular, and pseudoankylosis of the TMJ[24]. The documentation of the manifestations was based on clinical examinations and visual assessment of lateral cephalograms without any linear or angular measurements reported to describe the craniofacial profile.

The described dental manifestations included: oligodontia[17,24,26], horizontal maxillary overjet, cross bite, anterior open bite[24], and deep bite[17]. A case report has shown persistent primary teeth due to late eruption of the permanent dentition. Furthermore, the report showed velopharyngeal insufficiency causing difficulty in chewing and talking[17].

It has not been determined whether the craniofacial and dental findings were of random association or if they were truly related by any malformation mechanism of KFS[17].

ANALYSIS OF THE TWO CASES

The results of the analyses of the craniofacial profile on lateral cephalograms and the normal values according to Björk *et al*[15] are shown in Table 1.

Regarding the vertical dimensions of the craniofacial profile the KFS patients showed an increased mandibular inclination (NSL/ML), increased vertical jaw-relationship (NL/ML) and an increased jaw angle (ML/RL) compared to normal values (Table 1, Figures 1 and 2). In the sagittal plane the two cases showed retrognathia of the maxilla (s-n-ss) compared to the normal values whereas the prognathia of the mandible (s-n-pg) was larger in case Ⅱ and smaller in case Ⅰ. Furthermore, the sagittal jaw relationship (ss-n-pg) was larger in case Ⅰ and smaller in case Ⅱ compared to normal values (Table 1, Figures 1 and 2). The inclination of the maxilla (NSL/NL) and the cranial base angle (n-s-ba) was comparable to normal values.

DISCUSSION

KFS is a rare, congenital malformation defined by faulty segmentation of two or more cervical vertebrae[3]. Therefore, KFS is located in the occipital and cervical spine field[4-9]. The syndrome is morphologically and etiologically heterogeneous and within the group of KFS patients several anomalies have been reported[22]. In the literature the dental manifestations in KFS-patients were reported as oligodontia, horizontal maxillary overjet, cross bite, anterior open bite, and deep bite[24]. The craniofacial profile was clinically described as reduced lower face height, midface hypoplasia, and mandibular prognathia[24].

When comparing the clinical reports in the literature on the craniofacial profile with the two cases analyzed on lateral cephalograms in the present study some similarities are evident. The midface hypoplasia is described in the literature[24] and is also found in the two cases as retrognathia of the maxilla. Only in one case (case Ⅱ) was mandibular prognathia seen in agreement with the clinical reports in the literature[24]. The increased inclination of the mandible, the increased vertical jaw-relationship, and the increased jaw angle indicating an increased lower face height in both cases in the present study was in disagreement with previous clinical reports in the literature[24]. The agreement and disagreements between the literature and the cases in the present study may reflect the morphologically and etiologically heterogeneous within the group of KFS patients[22] which complicates the understanding of this developmental syndrome[18].

Recently, a series of studies have shown significant associations between fusion of the cervical vertebrae and retrognathia of the jaws, between fusion and inclination of the jaws, and between fusion and a large cranial base angle in non-syndromic patients with severe skeletal malocclusion traits[10-12,14]. The measurements of the two KFS-cases showed both similar but also deviant patterns compared to those documented in the non-syndromic patients. Retrognathia of the maxilla was in agreement with previous findings in non-syndromic

patients with fusion of the cervical vertebrae as well as inclination of the mandible[10-12,14]. On the other hand, retrognathia of the mandible was only seen in one case (case Ⅰ) whereas mandibular prognathia was found in case Ⅱ. Surprisingly, none of the cases showed a large cranial base angle, which was expected according to the literature[10-12,14].

An explanation for the association between the cervical spine and the craniofacial profile including the cranial base found in KFS and non-syndromic patients with fusion of the cervical vertebrae could be the notochord in the early embryogenesis[4]. The notochord develops in the human germ disc and determines the development of the cervical vertebrae, especially the vertebral bodies and the basilar part of the occipital bone in the cranial base (the posterior part of the cranial base angle)[4-9]. The para-axial mesoderm forming the vertebral arches and the remaining parts of the occipital bone are also formed from notochordal inductions[4-9]. The notochord is, by direct or indirect signaling, responsible for the formation of the structures in the occipital and cervical spine field in the early embryogenesis[4,5]. Therefore, a deviation in the development of the notochord may influence the surrounding bone tissue in the spine as well as in the posterior part of the cranial base to which the jaws are attached[10-14,27]. Furthermore, the jaws, including the condylar cartilage, develop from tissue that derives from the neural crest. The neural crest cells migrate to the craniofacial area before the notochord is surrounded by bone tissue and disappears[4]. In the first branchial arch, the neural crest cells migrate from the neural crest towards the mandible, followed by the cells to the maxilla, and lastly by the cells to the nasofrontal region[4] before the notochord is surrounded by bone tissue[28]. Therefore, it is understandable that a disturbance in the amount of migrating maxillary and mandibular cells or timing of the migration of the maxillary and mandibular cells may influence both the sagittal development (retrognathia of the jaws) and vertical development (inclination of the jaws)[11-13]. How the migration of the neural crest cells are influenced by signals from the notochord is still unclear, but signaling during early embryogenesis between the notochord, para-axial mesoderm, the neural tube, and the neural crest, as described above, is believed to be important for the associations between malformation of the craniofacial structures and the cervical vertebrae[14,27].

The associations found in KFS and in non-syndromic patients with severe malocclusion traits between fusion of the cervical vertebrae and deviations in the craniofacial profile may lead to considerations regarding etiology and classification of KFS.

CONCLUSION

KFS is defined by congenital vertebral fusion of the cervical spine and is seen with a wide spectrum of dental manifestations and craniofacial profile. According to the literature the dental manifestations of KFS-patients include oligodontia, horizontal maxillary overjet, cross bite, open bite and deep bite. The craniofacial profile is clinically described as reduced lower facial height, midfacial hypoplasia, and mandibular prognathia.

The analyses of the two lateral cephalograms showed increased mandibular inclination, increased vertical jaw-relationship, increased jaw angle and maxillary retrognathia. The cranial base was normal in both cases. The sagittal jaw relationship and mandibular prognathia varied between the two cases.

The literature review and the analyses of the two lateral cephalograms have shown that deviations in the occipital and cervical spine field as KFS were associated with deviations in the teeth and craniofacial profile.

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Treatment of mouth and jaw diseases with intralesional steroid injection

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Abstract

Many lesions of the oral region are treated with surgical methods such as curettage and resection. Chemotherapy and radiation therapy with or without surgical intervention can be used as an adjunct in some cases. Intralesional steroid injection is a conservative procedure which is already used in various regions of the body and joints. This technique is used also for a number of mouth and jaw lesions. Localized langerhans cell histiocytosis, central giant cell granuloma, oral submucous fibrosis, oral lichen planus, lichen sclerosus of the oral mucosa, lymphatic malformations and orofacial granulomatosis can be considered among these diseases. The purpose of this review is to investigate the effects of intralesional steroid injections in the treatment of oral diseases.

Key words: Intralesional injections; Steroids; Langerhans Cell histiocytosis; Giant cell granuloma; Oral submucous fibrosis; Oral lichen planus; Orofacial granulomatosis

Core tip: Intralesional steroid injections are often used in the lesion occurred in oral and maxillofacial region in recent years. Especially in large lesions, it can be applied as an alternative or adjunct to surgical procedures. It is an effective treatment method, because, without the need for major surgical procedures and providing patient comfort.

Esen A, Işık K, Dolanmaz D. Treatment of mouth and jaw diseases with intralesional steroid injection. *World J Stomatol* 2015; 4(2): 87-95 Available from: URL: http://www.wjgnet.com/2218-6263/full/v4/i2/87.htm DOI: http://dx.doi.org/10.5321/wjs.v4.i2.87

INTRODUCTION

Corticosteroids are one of the most widely used drugs due to their anti-inflammatory, anti-allergic and immunosuppressive effects. Today they are used as systemic, topical, intra-articular and intralesional in the clinic. They were first used systemically in a patient with severe rheumatoid arthritis in 1948 by Hench *et al*[1].

Consequently a further 15 patients were successfully treated. In 1950, their discovery of the effect of cortisone brought Hench, Edward and Reichstein the Nobel Prize in Medicine and Physiology[2]. Beneficial effects of intra-articular corticosteroid (hydrocortisone acetate) injection was first published in 1951[3]. In 1956, prednisolone was introduced by Rothermich and Phillips[4] as an satisfactory and more potent alternative for intra-articular injections. Boland and Liddle[5] compared methylprednisolone with prednisolone and found them equally effective. Triamcinolone acetonide was applied in the treatment of dermatoses by Robinson[6] in 1958. Later, triamcinolone hexacetonide was reported to be a potent synthetic corticosteroid for intra-articular usage[7]. In the 1970s, corticosteroids were administered in intra-osseous lesions such as bone cysts[8-10]. Intralesional steroid injection (ISI) has been performed in both of bone and mucosal lesions of oral and maxillofacial region since 1980. Currently, this method is widely accepted as an alternative or aid to surgical treatment especially in large reactive lesions.

In this review, we review the hard and soft tissue lesions of oral region that can be treated with intralesional steroid injections. Under the each disease's title, we also discuss the action mechanism of steroids.

DISEASES THAT CAN BE TREATED WITH ISI

Bone lesions

Localized langerhans cell histiocytosis (eosinophilic granuloma) and central giant cell granuloma.

Soft tissue lesions

Soft tissue lesions include oral submucous fibrosis; oral lichen planus; oral lichen sclerosis; lymphatic malformations; and orofacial granulomatosis.

LOCALIZED LANGERHANS CELL HISTIOCYTOSIS (EOSINOPHILIC GRANULOMA)

Langerhans' cell histiocytosis (LCH), formerly known as histiocytosis-X, is a disease characterized by cell proliferation exhibiting phenotypic characteristics of Langerhans cells[11]. There are three clinical forms of the disease: Letterer-Siwe disease, Hand-Schüller-Christian syndrome and Localized Langerhans Cell Histiocytosis (LLCH) or eosinophilic granuloma[12].

Letterer-Siwe disease is an acute disseminated form of LCH and characterized by hepatosplenomegaly, lymphadenopathy, anemia, skin rash, and bone lesions with dissemination. It usually affects young children and follows an acute or sub-acute course.

Chronic disseminated form of the LCH is called Hand-Schüller-Christian syndrome and it is usually associated with a triad of exophthalmos, diabetes insipidus and punched-out bone lesions. This form of the disease typically affects the patients in the second and third decades or older age[13].

LLCH, a localized disease. It accounts for 60%-70% of LCH cases and it can be found as solitary or multifocal bone defects. While mandible, skull and ribs are most often affected in children, long bones and vertebrae are more frequently involved in adults. The disease peaks in the first three decades and males are affected twice as females[14]. Possible symptoms are swelling, pain and tenderness over the lesion's site. General malaise, fever, headache, toothache, bleeding, loose teeth and sensory disturbances may accompany as well. It is also possible not to see any symptoms[15]. The lesions appear as radiolucent areas with well-demarcated borders in radiographic views. Pathologic fractures may arise due to resorption of the overlying cortical bone[16]. Treatment options of LLCH include resection or curettage, chemotherapy, radiotherapy or a combination of them[17]. Spontaneous healing has been reported, too[18].

Treatment of the LLCH with ISI

Currently there is not a clear treatment protocol for the ISI in the LLCH. Previously a few studies have been reported. In 1980, Cohen *et al*[19] first employed this technique in eosinophilic granulomas of the bones. While they did a single dose of methylprednisolone directly into the lesions in various parts of the body, in mandibular lesions they did a second injection. Jones *et al*[16] applied single intralesional dose of 165 mg methylprednisolone to a mandibular LCH and reported complete resolution of the lesion after 8 mo. Others[20] have described a case with multifocal LCHs in the mandible, who failed to respond to radiotherapy and systemic therapy with prednisone and etoposide. On a weekly basis, the authors injected 2 mL of 25 mg/mL triamcinolone into the lesion for six times. Complete remission was reached by the 15 mo. Putters *et al*[21] treated three LLCH cases of the mandible in a one-stage procedure. They performed intralesional injections of 80, 40, and 80 mg of methylprednisolone succinate, respectively. The lesions showed radiologically and clinically complete remission after 6 mo. In another case[22], 200 mg of intralesional methylprednisolone injection was used in a mandibular lesion and complete resolution had been achieved after 17 mo. Esen *et al*[23] reported a case of LLCH of the mandible, which also caused a non-displaced pathologic fracture. They started repeated ISIs and the fracture line disappeared within 14 mo without using any reduction or fixation methods. By the end of the 36-mo follow-up, the lesion was entirely healed. In a later paper[24], two patients were treated in one-stage procedures with intralesional methylprednisolone injections. The lesions healed clinically and radiologically 35 and 15 mo after treatment.

Action mechanism of steroids

Many questions remain to be clarified to understand the therapeutic effects of corticosteroids in LLCH. It is unknown whether they suppress T lymphocytes, the Langerhans cells, eosinophils or stimulate osteogenesis. It has been suggested[8,25] that corticosteroid micr-ocrystals can break the connective tissue of the cystic wall and allow secondary osteogenic repair or IL-1 is inhibited by steroids. Even though these hypotheses may explain the improvement in bone cysts, it does not apply to LLCH, because there is no membrane covering the lesion.

As evident from the literature, ISI is a successful method in cases of LLCH. It is an effective treatment method, because, without the need for major surgical procedures and providing patient comfort.

CENTRAL GIANT CELL GRANULOMA

The central giant cell granuloma (CGCG) was first described by Jaffe[26] in 1953. CGCG occurs almost solely within the jaws and it is a benign proliferation of fibroblasts and multinucleated giant cells. It typically presents as a solitary radiolucent lesion of the mandible or maxilla. The lesions occur twice as often in the mandible than in the maxilla. It is predominantly found in young adults before the age 30 with a female preponderance[27]. Based on its clinical behavior, CGCG has been classified as non-aggressive and aggressive lesion. Non-aggressive lesions tend to grow slowly and do not perforate the cortical bone. Recurrence usually is not seen after treatment. Aggressive lesions are characterized by rapid growth, pain, expansion or perforation of the cortical bone, root resorption, and a high recurrence tendency[28]. The traditional treatment of CGCG is curettage or resection depending on the lesion's behavior, size, location, and radiographic appearance. Non-surgical treatment methods are systemic administration of calcitonin, intralesional injection of corticosteroids and administration of α-interferon[29-32].

Treatment of the CGCG with ISI

The treatment of CGCG with corticosteroids was first reported by Jacoway *et al*[33] in 1988. They suggested is a 50/50 mixture of 2% lidocaine with 1:100000 epinephrine and triamcinolone acetonide (TA) to inject 2 mL/1 cm of lesion as seen on a panoramic radiography and to repeat this six times at weekly intervals. Later, Terry and Jacoway[34] presented four patients treated with steroids in 1994. A weekly done ISI during six weeks resulted in a complete resolution in three patients, while one patient needed additional surgery. Kermer *et al*[35] published another case of CGCG treated with corticosteroids in the same year. Rajeevan and Soumithran[36] reported that intralesional triamcinolone acetonide was administered to a 17-year-old girl who had CGCG in 1998. They indicated that almost healing the lesion of the left mandible was observed after the sixth month. In 2000, Khafif *et al*[37] applied the same protocol to a 36-year-old female patient who had a CGCG of maxilla and they reported that a complete remission was seen after two years. Kurtz *et al*[38] also used ISI to a 10-year-old CGCG patient in 2001. They reported that the proper healing was seen after 5 years. In 2002, Carlos *et al*[39] added four new cases to the literature. They reported that the lesions showed clinically and radiologically recovery approximately 6-7 years after treatment except for one case in which partial remission was observed. Abdo *et al*[40] reported that a recurrent CGCG in a 14-year-old girl in the anterior region of the mandible was treated successfully by ISI in 2005. Sezer *et al*[41] also reported that an 11-year-old boy with a CGCG is successfully treated with intralesional corticosteroid injections after 3 years follow-up in the same year. Comert *et al*[42] preferred to use prednisolone in their patient who had CGCG of the maxilla. They reported that a partial recovery was achieved and a limited surgery could be performed. Wendt *et al*[43] employed ISI for a maxillary CGCG with a 1:1 triamcinolone acetonide (10 mg/mL) and 0.5% bupivacaine. The solution was injected into the lesion for a period of 11 wk. After 6-years follow-up, the treatment was found to be successful clinically and radiographically. Mohanty and Jhamb[44] performed same protocol and reported that two patients were successfully treated with triamcinolone acetonide injections. Nogueira *et al*[45] contributed to literature with 21 new cases in 2010 using ISI with triamcinolone hexacetonide. Two patients did not responded to the treatment and surgical resection was needed; a moderate improvement noted in four patients (curettage in two patients) and 15 of the cases showed good response (curettage in one patient). Shirani *et al*[46] performed ISI in an aggressive and extensive case and they could not get the answer to the treatment. Ferretti *et al*[47] applied the same protocol to 16-year-old female patient who had CGCG and they reported that a complete remission was seen after 4 years follow up. Rachmiel *et al*[48] performed combination therapy consisting of ISI, calcitonin nasal spray and curettage in a 24-year-old female patient and found that no recurrence after 5-year follow-up. da Silva *et al*[49] treated a 36-year-old male with a CGCG crossing the midline of mandible with ISI combined with alendronate sodium for the control of systemic bone resorption. They reported that no recurrence or side effects at the end of four years. Finally, Fonseca *et al*[50] reported that intralesional triamcinolone acetonide was administered to a 15-year-old boy who had CGCG. They indicated that partial resolution of the lesion was observed after the sixth month.

Action mechanism of steroids

There are several theories about the action mechanism of ISI in the CGCG. Osteoclasts achieve bone resorption by secreting lysosomal proteases. These agents mediate the process by creating an extracellular medium. It

has been showed[51,52] that 17β-estradiol (E2) could directly inhibit osteoclastic bone resorption. Moreover, at concentrations effective for inhibiting bone resorption, E2 could also induces osteoclast apoptosis.

On the basis of the aforementioned experimental evidence the mechanism of corticosteroids in the treatment of these lesions is suggested as inhibition of the extracellular production of lysosomal proteases and steroidal apoptotic action on osteoclast-like cells. These two mechanisms could cause cessation of bone resorption[39].

According to literature, ISI is an effective method in patients with CGCG. However, it is not always possible to obtain a positive response to the treatment in the multilocular or aggressive lesions. Hence, in such cases, it is necessary to apply surgical or combined treatment methods. In addition, serum calcium, phosphorus and parathyroid hormone levels should be examined on suspicion of hyperparathyroidism after definitive diagnosis result of the incisional biopsy. It should be noted that the images of brown tumor and CGCG can't be distinguished histologically. And before starting the ISIs, possible diabetes mellitus and the presence of peptic ulcers or any infection should be questioned.

ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis (OSF) is a chronic disease of the oral mucosa. It affects the pharynx, oral cavity, upper third of the esophagus and it is characterized by inflammation and a progressive fibrosis of sub-epithelial tissues[53]. Connective tissue fibers of the lamina propria and deeper parts change, which in turn lead to mucosal stiffness and limitation in mouth opening[54]. OSF is considered as high-risk precancerous disease[53-55]. Several factors contributing to OSF include general nutritional or vitamin deficiencies and hypersensitivity to various dietary constituents. The primary factor appears to be chewing of the areca (betel) nut. Genetic factors are thought to be involved in the etiology. It has been reported that a polymorphism of the promoter region of the matrix metalloproteinase 3 gene is common in OSF and may contribute to development of the disease[27]. The potential morbidities of OSF are restriction of mouth opening, difficulty with swallowing, mastication, speech, and a burning sensation as well. It has a mortality potential because of the possibility of transformation into squamous cell carcinoma[55]. Both nonsurgical and surgical treatment options have been suggested. Nonsurgical options are ISIs, hyaluronidase and interferon gamma. Surgery primarily targets to improve the mouth opening and comprises the excision of the fibrous bands, skin grafts and splitting of the temporalis tendon.

Treatment of the OSF with ISI

Treatment is generally intended to increase the mouth opening and to decrease the burning sensation. For early-stage submucous fibrosis cases, the results are better with non-surgical methods. In intralesional applications, the triamcinolone acetonide is the most preferred agent but different substances are also applied such as salvianolic acid B (SA-B) and lycopene. As far as we know, Gupta and Sharma[56] were the first who successfully treated the OSF with local injections of chymotrypsin, hyaluronidase, and dexamethasone. Later, sub-mucosal steroid injection and hyaluronidase or topical vitamin A, topical steroid application and oral iron preparations were applied by Borle and Borle[57] in 326 patients with oral submucous fibrosis. Khanna *et al*[58] presented 100 patients in their clinical study in which the author implemented intralesional injection of triamcinolone acetonide in patients with very early and early-stage of OSF cases while they performed surgical intervention in advanced cases. Satisfactory results were reported in long-term follow up. Kumar *et al*[59] applied the lycopene and lycopene combined with ISI of betamethasone in OSF patients. They reported that positive clinical response was obtained in both study groups when compared with placebo. Singh *et al*[60] compared the efficacy of hydrocortisone acetate and hyaluronidase at weekly interval *vs* triamcinolone acetonide and hyaluronidase at 15 d interval. They notified no significant differences in symptom or sign scores and any histopathological improvement between the groups. The authors conclude that treatment regimen of triamcinolone acetonide and hyaluronidase was more convenient to the patients because of less number of visits required and of cost efficiency. No side effects were seen[60]. Rao[61] treated the patients with OSF using alpha lipoic acid in addition to the ISI of betamethasone and hyaluronidase. He reported that the alpha lipoic acid group exhibited better relief of symptoms as compared to the controls and he concluded that the use of an antioxidant, alpha lipoic acid, along with conventional therapy of ISI is effective in the management of OSF. Jiang *et al*[55] investigated that the effectiveness of triamcinolone acetonide and (SA-B) intralesional combined injection in the treatment of oral submucous fibrosis (OSF) and they concluded that the triamcinolone acetonide + SA-B intralesional injections improved mouth open and burning sensation in these OSF patients. Shetty *et al*[62] examined the efficacy of spirulina as an antioxidant adjuvant to corticosteroid injection in management of oral submucous fibrosis. They treated the OSF patients in a group with spirulina+ betamethasone and placebo capsules + betamethasone in other group. They reported that the mouth opening and burning sensation was found to be statistically very highly significant in favor of the spirulina group.

Some complications can be seen after the procedure in the OSF cases. Chen *et al*[63] observed facial candida albicans cellulitis in a diabetes mellitus patient with oral submucous fibrosis after ISI treatment. Therefore, it should be noted that some complications can arise due

to the predisposing factors such as immunodeficiency (HIV), immune-suppression (systemic treatments with corticosteroids), chronic illness, obesity, diabetes, malnutrition, vitamin deficiency, alcohol misuse, tobacco smoking and intravenous drugs abuse.

Action mechanism of steroids

According to the hypothesis of Tsai *et al*[64], some alkaloids (arecoline, arecaidine) inhibit fibroblast phagocytosis and this contributes for the development of OSF. ISIs could cause an enhancement of fibroblast collagen phagocytosis. Juxta-epithelial inflammatory cell infiltration and then progressive hyalinization of the lamina propria and deeper connective tissues are associated with early OSF[55,65,66]. Use of ISI have been directed to chronic juxta-epithelial inflammation[55-57,60]. The steroids can prevent or suppress inflammatory reactions, so they fight with fibrosis by decreasing fibroblastic proliferation and collagen deposition[55,65,66]. Therefore, it can be more successful when the steroid injections administered in the early stages of the disease. According to the literature, triamcinolone acetonide or betamethasone appears to be a suitable choice.

ORAL LICHEN PLANUS

Oral lichen planus (OLP) is a chronic mucocutaneous disease of unknown cause, with oral lesions occurring most commonly in women over 30 years of age. Incidence of OLP is between 0.2% and 2% in the population. Different types of OLP have been described as reticular, plaque form, erosive, atrophic, or bullous. Intraorally, the buccal mucosa, tongue and the gingiva are commonly involved although other sites may be rarely affected. Oral mucosal lesions present alone or with concomitant skin lesions. The most common type is the reticular form which is characterized by numerous interlacing white keratotic lines or striae that produce an annular or lacy pattern[27,67]. The plaque form of OLP tends to resemble leukoplakia clinically but has a multifocal distribution. In the erosive form, the central area of the lesion is ulcerated. A fibrinous plaque or pseudomembrane covers the ulcer. The erithematous or atrophic type appears as red patches with very fine white striae. It may be seen in conjunction with reticular or erosive variants. Patients complain of pain, burning, sensitivity and generalized discomfort in particularly erosive and atrophic types[27,67]. The risk of malignant transformation varies between 0.4% and 5% over periods of observation from 0.5 to 20 years[68]. A few studies have reported that the malignant potential of OLP and hepatitis C virus infection apparently increased the risk for oral squamous cell carcinoma[69-71]. Patients with reticular and other asymptomatic OLP lesions usually require no active treatment but symptomatic lesions may also need treatment. Nonsurgical treatments are systemic drug therapy, topical corticosteroids-calcineurin inhibitors - retinoids, injection of steroids and ultraviolet irradiation. The other methods are surgery, laser therapy and cryosurgery[67].

Treatment of the OLP with ISI

According to literature, intra- and sublesional treatment of OLP with triamcinolone acetonide was reported by Sleeper[72] for the first time in 1967. The author reported that after 72 h, examination of the lesions showed 45% to 50% involution with corresponding relief of symptoms. In three cases the entire lesion disappeared in two weeks. In the other four cases with larger lesions, approximately 10% to 15% of the lesion remained, but the patients were completely symptom free. In 1974, Randell and Cohen[73] applied dexamethasone in patients with OLP and they reported successful results. Then Zegarelli[74,75] performed ISI with triamcinolone acetonide and methylprednisolone in patients with erosive or ulcerative OLP. Xia *et al*[76] studied with 45 patients with clinical and histologically confirmed ulcerative OLP. Each participant received 0.5 mL intralesional triamcinolone acetonide injection (40 mg/mL) on one side and other side was left as control. The treated areas gave rapid relief of signs and symptoms, while the control areas showed minimal decrease. Thirty-eight (84.4%) patients demonstrated complete response in ulceration size. No complications were noted with triamcinolone acetonide injections. They concluded that intralesional triamcinolone acetonide injection in ulcerative OLP is effective and safe in achieving lesion and pain regression. Xiong *et al*[77] compared the intralesional polysaccharide nucleic acid fraction of bacillus Calmette-Guerin (BCG-PSN) and triamcinolone acetonide in patients with erosive OLP. They randomly assigned 56 OLP patients receive either intralesional injection of 0.5 mL BCG-PSN every other day (n = 31) or 10 mg triamcinolone acetonide (a positive-controlled group, n = 25) every week for 2 wk. After the cessation of treatment, patients were followed up for 3 mo. After 2-wk treatment, 27 of 31 BCG-PSN-treated patients (87.1%) and 22 of 25 TA-treated patients (88.0%) healed. There were no statistical differences between the two groups in erosive areas and pain scores. They concluded that topical intralesional BCG-PSN injection is as effective as triamcinolone acetonide for erosive OLP. Lee *et al*[78] investigated intralesional injection *vs* mouth rinse of triamcinolone acetonide in 40 patients with OLP in terms of pain and burning sensation. They concluded that the efficacies of both treatments were similar. The rate of adverse effects was significantly lower for intralesional injection of triamcinolone acetonide than mouth rinse of TA. In another clinical study, intralesional triamcinolone acetonide plus oral prednisolone was applied by Kuo *et al*[79] in 50 patients with erosive OLP. They reported that although the patients showed complete response in 90% of cases after three weeks, recurrence of erosive or ulcerative lesion was observed after 3-24 (mean 12) mo of follow-up in all of these cases. Liu *et al*[80] analyzed

the efficacy and safety of intralesional betamethasone in the treatment of erosive OLP. They implemented intralesional betamethasone 1.4 mg to the experimental group and 8 mg intralesional triamcinolone acetonide to the control group once a week for two weeks in 61 patients with erosive OLP. They found that 93.1% of participants were healed after two intralesional injections of 1.4 mg betamethasone, and 66.7% of participants were healed after two intralesional injections of 8 mg triamcinolone acetonide and authors concluded that intralesional betamethasone was a more effective way to treat erosive OLP.

According to the literature, triamcinolone acetonide is the most preferable agent as intralesional injection in patients with OLP. Recently, betamethasone seems to be also effective. General usage of triamcinolone acetonide is to dilute 10 to 20 mg in 0.5 mL saline or 2% lidocaine, then to inject into the lesion once 1 wk for 2 times[81]. Injections are administered into the connective tissue below the erosive lesion from the adjacent normal mucosa. The treatment is absolutely required in patients with erosive and erythematous types due to the daily life is affected by pain and burning sensation. Generally, patient comfort is provided and the lesions disappeared within one to two weeks after ISI. However, recurrence of the lesions may occur on the long-term follow-up. Disadvantages include mucosal atrophy, difficulty to deposit sufficient quantities into gingival lesions and painful injection[82].

Action mechanism of steroids

While the etiology of OLP is not clear, it has been suggested that it could be caused by a immune response with an inflammatory cell population composed of T lymphocytes[83,84]. When the steroids are injected directly into the connective tissue below the lesions, they can suppress T cells and show a strong anti-inflammatory and immunosuppressive effect[80].

OTHER DISEASES

Other oral diseases treated with ISI are very limited in the literature. Azevedo *et al*[85] used intralesional injection of triamcinolone acetonide in patients with oral lichen sclerosis. The authors reported that the patients showed improvement and elasticity of oral tissues enhanced. Luo and Gun[86] found that intralesional injection of pingyangmycin with triamcinolone acetonide was more effective than pingyangmycin alone for management of lymphatic malformations in oral and maxillofacial region. Anjomshoaa *et al*[87] performed intralesional injections of triamcinolone acetonide in a patient with follicular lymphoid hyperplasia. In addition, they reported that complete resolution of the lesion was obtained at 7-mo follow-up. Another disease in which ISI could be effective is orofacial granulomatosis (OFG). It is an uncommon disease, usually presents as recurrent or persistent swelling of the soft tissues in the orofacial region, predominantly on lips, causing significant cosmetic and functional problems[88,89]. The reason of this disease is unknown. OFG may also be part of the triad of Melkersson-Rosenthal syndrome (MRS) and some consider it a monosymptomatic form of MRS[90,91]. Sakuntabhai *et al*[92] used high-volume intralesional triamcinolone acetonide injections (3 to 10 mL of 10 mg/mL) and they reported that intralesional triamcinolone injections reduced lip swelling. However, Mignogna *et al*[91] performed small volume, high concentrate, delayed release, intralesional injection of triamcinolone acetonide in patients with OFG. They reported that all patients remained without recurrences or with cosmetically acceptable slight lip enlargement for a mean time of 19 mo and this method was very affective and it did not require nerve blockage. The same researchers investigated the long-term outcome in patients treated with intralesional triamcinolone acetonide injections and reported that complete clinical remission were obtained in all patients for a mean time of 56.3 ± 18.2 mo[93]. Several other clinical studies have reported that injections of intralesional steroids are clinically successful method in patients with OFG[88,89,94].

Exogenous corticosteroids are usually classified based on their relative glucocorticoid and mineralocorticoid potency as well as duration of their effects. The most potent glucocorticoids are also the most potent suppressors of the hypothalamic pituitary adrenal axis. While short-acting steroids (*e.g*., Cortisol) are effective for less than 12 h, intermediate-acting steroids (Prednisone, Prednisolone, Methylprednisolone and Triamcinolone) can stay active for 12-36 h and long-acting steroids (Betamethasone, Dexamethasone and Flumethasone) are effective for more than 36 h[95]. Most prominent properties of corticosteroids are their anti-inflammatory, anti-allergic and analgesic effects. Glucocorticoids help keeping normal vascular permeability and stabilize lysosomal and cellular membranes. On the other hand, in acute inflammation, they decrease vascular permeability and inhibit the migration of polymorphonuclear lymphocytes into tissues. They also induce apoptosis in normal lymphoid cells; inhibit the clonal expansion of T and B lymphocytes; and reduce the eosinophils, basophils, and monocytes in the circulation. Glucocorticoids have different effects on neutrophils. They hinder margination of neutrophils and increase the release of mature neutrophils from the bone marrow.

However, they may also decelerate wound healing[95]. Long-term use of corticosteroids can cause osteoporosis, hypertension, electrolyte imbalance, hyperglycemia, delayed wound healing, and a tendency for infections. There are some contraindications for steroids such as history of allergy, peptic ulcer, Cushing syndrome, uncontrolled diabetes, renal failure, anticoagulation usage, fungal diseases and varicella zoster infection[96]. Although intralesional injection can be performed easily, several precautions should be taken during the

processing. The injection must always be made using sterile procedures and anatomy of the area should be known. Adjacent nerves should be kept away and intravenous injections should be avoided because of the possibility of systemic effects such as adrenal suppression[96].

CONCLUSION

ISI is one of the most preferable non-surgical methods for the treatment of mucosal or bone reactive lesions occurred in oral and maxillofacial region. The accumulating evidence suggests that ISI is well tolerated by patients, the likelihood of postoperative complications is less than those of other methods and patient complaints diminish rapidly. Especially in large lesions, it can be applied as an alternative or adjunct to surgical procedures. This method is also minimally invasive and relatively inexpensive.

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MINIREVIEWS

Oral graft *vs* host disease: An immune system disorder in hematopoietic cell transplantation

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Abstract

Graft *vs* host disease (GVHD) is a complication of patients who are treated by hematopoietic cell transplantation. National Institutes of Health in 2005 by Working Group on Diagnosis and Staging Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD (cGVHD) established 2 principal categories of oral GVHD, acute and chronic. The oral mucosa may be the first site of manifestation of the disease. Clinical diagnosis needs to be confirmed by a biopsy of oral mucosa and minor salivary glands. Microscopic results have played a major role in the diagnosis and management of acute and chronic oral GVHD. Development of second malignancies is the greatest risk of oral cGVHD patients, mostly regarding squamous cell carcinoma. The focus of oral GVHD therapy is to improve symptoms and maintain oral function. The aim of this review article is to update the information on the oral GVHD in its clinical, microscopic features and their complications.

Key words: Stem cell transplantation; Graft *vs* host disease; Mouth mucosa; Diagnosis; Oral; Salivary glands

Core tip: Graft *vs* host disease (GVHD) patients are susceptible to recurrent and deadly infections due to immune system harm. Chemotherapy treatment may cause a range of complications, such as neuropathic pain resulting from vincristine adverse effects, overgrowth of gingival due to cyclosporine and effects on bones and teeth growth and development during childhood and youth. Oral GVHD patients must have follow-ups due to risks of oral infections, bleeding, and cancerous developments.

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INTRODUCTION

Graft *vs* host disease (GVHD) is a complication of patients who are treated by Hematopoietic Cell Transplantation (HCT). GVHD has immunoregulatory characteristics when donor T cells react against histocompatibility antigens of the host. National Institutes of Health (NIH) in 2005[1] by Working Group on Diagnosis and Staging Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD (cGVHD) established 2 principal categories of GVHD, acute and chronic, and each of the categories has 2 subcategories. The acute stage occurs with the absence of diagnostic or distinctive features of chronic GVHD. This stage also involves (1) classic acute GVHD that occurs within 100 d after transplantation; and (2) persistent, recurrent, or late acute GVHD (features of acute GVHD that occur beyond 100 d, often during withdrawal of immune suppression). The wide category is chronic GVHD, which involves (1) classic chronic GVHD (without features or characteristics of acute GVHD); and (2) an overlap syndrome, in which diagnostic or distinctive features of chronic GVHD and acute GVHD appear together[1,2].

Both forms, acute and chronic, may affect oral cavities and be highly morbid. Skin rash, mucosal ulcerations, elevated liver enzymes, and diarrhea indicate the acute form. Sjögren's syndrome, scleroderma, thickening and lichenoid lesions of the skin and mucosa, xerostomia, mucositis, and dysphagia indicate the chronic form[3,4].

GVHD patients are susceptible to recurrent and deadly infections due to immune system harm. Chemotherapy treatment may cause a range of complications, such as neuropathic pain resulting from vincristine adverse effects, overgrowth of gingival due to cyclosporine, and effects on bones and teeth growth and development during childhood and youth. Oral GVHD patients must have follow-ups due to risks of oral infections, bleeding, and cancerous developments.

INCIDENCE OF ORAL GVHD

Ion *et al*[5] in a retrospective study characterized a cohort of patients treated with HCT over 15 years (total 2578 patients). The study found that only 21 patients had developed acute GVHD (aGVHD), but 5 demonstrated only oral manifestations. Acute GVHD occurred in a median time of 22 d (8 to 154 d), and oral aGVHD occurred in a median time of 35 d (11 to 159 d). Oral features included an erythema and ulcerations of buccal mucosa (19 of 21; 90%), tongue (18 of 21; 86%; dorsum in 8), labial mucosa (16 of 21; 76%), palatal mucosa (15 of 21; 71%; hard palate in 7), and floor of mouth (7 of 21; 33%). Eight cases (38%) presented lip ulceration and crusting[5].

Some risk factors were tissue incompatibility (HLA and "minor" non-HLA antigens) between donor and recipient, advanced donor's age and patient's age, and the intensity of the conditioning therapy used for HCT preparations[6].

Chronic GVHD occurs more frequently (40% to 70%) in allogeneic bone marrow transplantation patients (allo-BMT), but is not necessarily related to a prior history of aGVHD manifestations[7]. Risk factors for cGVHD were related to donor, graft, and transplant-related older patient age, history of aGVHD, genders of donor and patient, certain underlying diagnoses (*e.g.*, chronic myelogenous leukemia or aplastic anemia), the use of mismatched or unrelated donors, infusion of donor lymphocytes, use of peripheral blood stem cells instead of bone marrow, and lack of T-cell depletion[6].

CLINICAL FEATURES

The oral mucosa may be the first site of the manifestation of the disease, which suggests that other organs should be investigated[8]. The signs and symptoms are divided by direct and indirect effects of cGVHD. The direct signs and symptoms are distributed in the areas[9,10]: (1) Mucosa - Lichenoid striation, plaque, papule, erythema, ulceration, atrophic glossitis (Figure 1); (2) Salivary glands - Dryness, mucocele (multiple); (3) Musculoskeletal - Limitation of mouth opening, limited tongue mobility; (4) Taste buds - Taste alteration; and (5) Gingiva - Desquamative gingivitis, lichenoid.

The indirect effects are dental decay, loss of attachment of periodontium, osteonecrosis of the jaw, candidiasis, and malignant transformation of oral mucosa and salivary glands[9,10].

The NIH Consensus Development Project defined 4 different types of manifestations of oral cGVHD that should be used to assess the severity of oral cGVHD (Figure 2). In the 0- to 15-point system, clinical evidences are assessed globally to reflect the severity and extent of oral involvement[11].

Clinical diagnosis needs to be confirmed by a biopsy of oral mucosa and minor salivary glands. The criteria for obtaining these specimens are an incisional biopsy of a nonulcerated site to include underlying gland lobules[12] (5 to 10 lobules is recommended) and the site of preference as the lower lip mucosa, which is the clinical manifestation in this area of the mouth. The orientation regarding characteristics of the biopsy and the sequence of observation for microscopic structures facilitate the process of the histological diagnosis[13].

MICROSCOPIC FEATURES

Chronic GVHD is a multifactorial disease with clinical and histopathologic features that can often confuse the pathologist. Horn *et al*[14] published the first study about the significance of oral mucosa and salivary glands after allogeneic HCT. It was based on the lichen planus-like lesions in association with xerostomia in

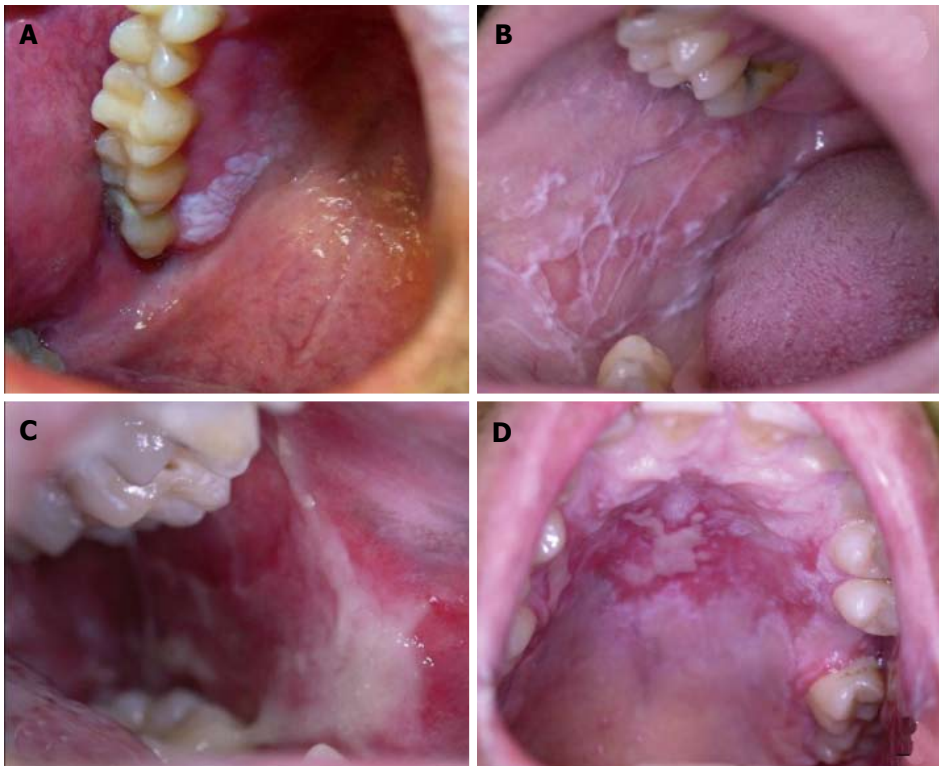
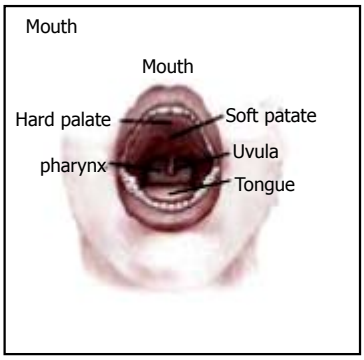


Figure 1 Clinical features of oral chronic graft *vs* host disease. A: Plaque; B: Lichenoid striation; C: Papule ulceration; D: Erythema ulceration.



Mucosal change	No evidence of cGVHD		Mild		Moderate		Severe	
Erythema	None	0	Mild erythema or moderate erythema (< 25%)	1	Moderate (≥ 25%) or severe erythema (< 25%)	2	Severe erythema (≥ 25%)	3
Lichenoid	None	0	Hyperkeratotic changes (< 25%)	1	Hyperkeratotic changes (25%-50%)	2	Hyperkeratotic changes (> 50%)	3
Ulcers	None	0	None	0	Ulcers involving (≤ 20%)	3	Severe ulcerations (> 20%)	6
Mucoceles[1]	None	0	1-5 mucoceles	1	6-10 scattered mucoceles	2	Over 10 mucoceles	3
			[1]Mucocales scored for lower labial and soft palate only				Total score for all mucosal changes	

Figure 2 NIH's oral chronic graft *vs* host disease clinical scoring instrument. Adapt from Treister *et al*[37].

59 transplanted patients[14]. The study established that the histopathological criteria for oral GVHD should include both oral mucosa and salivary glands features (Table 1)[14]. The histopathological features of oral cGVHD in epithelium and lamina propria included basal vacuolization, exocytosis, and interstitial inflammation (Figure 3). Salivary glands features included mild to severe destruction of ducts and acini (Figure 4)[14].

Histopathology has played a major role in the diagnosis and management of acute and chronic GVHD[12]. However, histological observations of cGVHD lesions are not specific. The changes may vary, depending on time between HCT and biopsy, biopsy size, number of serial sections, presence of ulceration area, insufficient depth, and the coexistence of other inflammatory processes at the site[15]. Histopathological changes can be more apparent after 60 d post-HCT and may be represented by nonspecific inflammation that leads to false-negative GHVD diagnosis[12,16]. Therefore, the NIH consensus of the Working Group highlighted the importance of considering the clinical features, such as lichen planus-like lesions and xerostomia, to define the diagnosis of oral cGVHD[12].

The NIH consensus presented the minimal criteria necessary to diagnose GVHD (whether acute or chronic) and the diagnostic features for chronic GVHD in each involved organ system (Tables 2 and 3). The minimal histological criteria for oral cGVHD follows basic criteria; this includes localized or generalized epithelial changes comprising lichenoid-like inflammation, exocytosis, apoptosis, the presence of intralobular, periductal lymphocytes with or without plasma cells, and exocytosis of lymphocytes into intralobular ducts and acini[13]. Apoptotic bodies in both epithelium and salivary glands can be seen in oral cGVHD[12].

The most frequent microscopic features in the epithelium include acanthosis, lymphocyte exocytosis, and the thickening of basal lamina. In the lamina propria, the most frequent features include interstitial

Table 1 Histological grading of oral mucosa and salivary glands according to Horn *et al*[14]

Grade	Definition
Grade Ⅰ	Mucosa: Vacuolization of basal cells, moderate lymphocytic infiltrate, moderate epithelial exocytosis Salivary glands: Mild interstitial inflammation
Grade Ⅱ	Mucosa: Epithelial cells with basal vacuolization and dyskeratotic, necrotic keratinocytes with satellitosis, moderate to heavy lymphocytic infiltrate in the submucosa and moderate epithelial exocytosis Salivary glands: Mild acinar destruction, ductal dilation, squamous metaplasia, mucous pooling, mild fibrosis, duct cell proliferation, periductal lymphocytic infiltrate
Grade Ⅲ	Mucosa: Focal cleavage between the epithelium and connective tissue, intense lymphocytic infiltrate in the connective tissue, dyskeratotic epithelial cells, lymphocyte exocytosis Salivary glands: Marked interstitial lymphocytic infiltrate. Diffuse destruction of ducts and acini
Grade Ⅳ	Mucosa: Separation of epithelium and the connective tissue Salivary glands: Nearly complete loss of acini, dilated ducts, interstitial fibrosis with or without inflammation

Table 2 Minimal criteria for diagnosis of oral chronic graft vs host disease and categories

Category	Definition
Not GVHD	No evidence for GVHD
Possible GVHD	Evidence of GVHD but other possible explanations (*e.g.*, Clinical features that suggest or favor a drug reaction)
Consistent with GVHD	Clear evidence of GVHD with mitigating factors (*e.g.*, Unequivocal evidence of CMV yet abundant apoptotic epithelial changes that are not associated with CMV- infected cells by immunostaining)
GVHD	Unequivocal evidence of GVHD and no further comment necessary (*e.g.*, Inflammation may be minimal despite extensive destruction of the targeted epithelia)

Adapt from Shulman *et al*[12]. GVHD: Graft *vs* host disease: CMV: Cytomegalovirus.

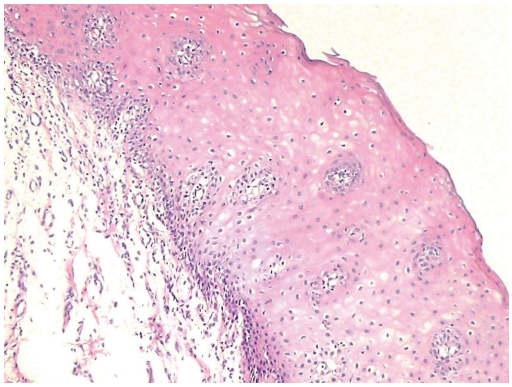


Figure 3 Epithelium and lamina propria showing basal vacuolization, exocytosis and interstitial inflammation.

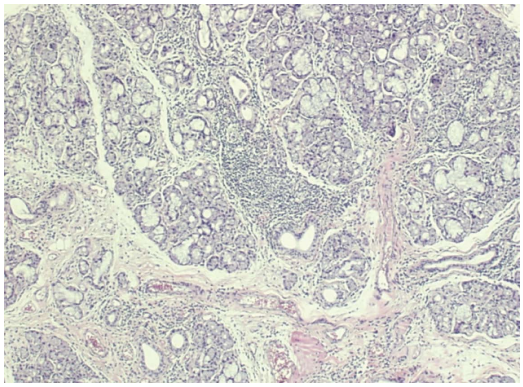


Figure 4 Salivary glands features included mild to severe destruction of ducts and acini.

lymphocytes infiltration. In minor salivary glands, they include periductal fibrosis and inflammatory infiltrate both in acini and periductal sites. Salivary glands analysis must be done carefully because they can be affected, even before the development of mucosal injury. Major salivary glands can reflect the same features of inflammatory infiltration and fibrosis[17].

Horn's criteria[14] and the NIH Consensus[12] are different in objectives and subjective features (Tables 1-3). In fact, any correlation between clinical and histopathological severity of oral GVHD leads to a nonsynchronous understanding of the epithelium and salivary gland disease. The absence of clinical and histopathological correlation does not diminish the importance of histological analysis of cGVHD. A comparison of the NIH Consensus[12] and the Horn criteria[14] for histopathological diagnosis of cGVHD shows that they are related in a certain way. This suggests that the use of the NIH Consensus[12] for oral mucosa and salivary glands may be better to characterize the extent of cGVHD[13]. Moreover, a differential diagnosis is possible with infectious lesions and drug reactions[13,18,19].

COMPLICATIONS OF THE ORAL CGVHD

Viral, fungal, and bacterial infections of the oral mucosa are frequently superimposed in patients with cGVHD. Mainly due to the dryness and immunosuppression[9].

Related to fungal infections acute pseudomembranous candidiasis is the most frequent presentation[20], but all clinical forms: erythematous, pseudomembranous,

Table 3 Shulman *et al*[12] chronic graft-*vs*-host disease histologic classification of oral mucosa and salivary glands, according to National Institutes of Health Consensus

Epithelium	Epithelial thickness (normal, atrophic, hyperkeratosis and acanthosis), presence of vacuolization, apoptosis, spongiosis, atypical keratinocytes, exocytosis of lymphocytes, presence of other inflammatory cells and thickening of basal lamina
Lamina propria	Predominant cell type in the inflammatory infiltrate and their distribution in relation to the salivary duct and epithelium
Salivary glands	Lymphocytes within the duct, periductal mixed infiltrate, presence of lymphocytes within the acini, apoptosis in the ducts and acini, periductal fibrosis, acinar cell degeneration, interstitial fibrosis, duct ectasia and loss of polarity of epithelial cells of the duct

hyperplastic, and angular cheilitis can be seen at some point in the course of the disease. To prevent and treat candidiasis nystatin and chlorhexidine mouth washes may be prescribed[20,21]. When multiple areas of the mouth are affected and there are risk of invasive candidiasis, the systemic fluconazole is indicated.

When patients with oral cGVHD are thrombocytopenic, there is a risk for bleeding mouth, gums and also primarily associated with ulcers of the mucosa[22]. In these cases, careful and effective oral hygiene in biofilm reduction are important.

SECOND MALIGNANCIES AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

The biggest concern regarding late complications, with patients who underwent HCT, is the development of second malignancies. These patients have a higher risk of developing hematological malignancies, lymphoproliferative disease, and solid tumors (lung, esophagus, skin, oral mucosa, colon, melanoma, glioblastoma, sarcoma, and other organs), owing to various risk factors, including total body irradiation, chemotherapy, and cGVHD[23,24].

Besides the cellular mechanisms that link to chronic inflammation of GVHD with malignant transformation of the affected sites, there are other possible mechanisms of malignant transformation that are related to the prolonged use of immunosuppressive therapy, performed for the treatment of chronic GVHD. Such suppression can facilitate infections with oncogenic viruses, such as Human Papiloma virus or Epstein-Barr virus, which would normally be controlled by the immune system[23,25,26]. In young patients with cGVHD reached the peak of development of oral squamous cell carcinoma (SCC) occurs 8 to 9 years after HCT[24].

Studies that evaluated the risk of cancer in patients after HCT showed an increased risk of developing secondary malignancies in comparison to the general population[25,27,28]. It is noteworthy that in patients with Fanconi anemia, the risk of developing a second tumor is even higher[29,30] being observed an increased risk of 10 to 15 times, a difference that may be related to chromosomal instability and deficiency in the repair process of the disease[26]. Chronic GVHD is associated with risks of developing oral SCC[26,28,31].

In a retrospective study, Atsuta *et al*[28] evaluated 17,545 patients who underwent HCT. The researchers concluded that in recipients of allogeneic HCT (myeloablative conditioning), extensive-type chronic GVHD was an important risk factor for the development of secondary solid cancers (RR = 1.9, $P < 0.001$); it was an independent risk factor for cancers in the oral cavity/pharynx and esophagus. In a cohort study, Majhail *et al*[32] evaluated 4318 patients who underwent HCT (Acute Myeloid Leukemia 1742, Chronic Myeloid Leukemia 2576) and found that out of cancer patients, 72% had a diagnosis of cGVHD. In this study, cGVHD was the only significant risk factor associated with oral cavity cancer. Chen *et al*[33] evaluated 170 patients who underwent allogeneic HCT over twenty years with a median follow-up of 14.1 years (range 5.1-23.3 years). Eight (4.7%) patients developed secondary carcinoma: 5 developed squamous cell carcinomas in the oral cavity, 1 in the esophagus, 1 ovarian adenocarcinoma, and 1 breast. In this group, 7 patients (87.5%) were subjected to treatment for cGVHD with a median time post-transplant diagnosis of 10 years. Patients who had cGVHD after HCT were at risk of developing secondary carcinomas (RR = 15.374; 95%CI: 2.168-59.875). In this study, before the development of oral squamous cell carcinoma, all 5 patients had signs and symptoms of recurrent oral ulcers, warts, and white lesions in the regions of developing cancer. It is important to note that HPV was not associated with carcinogenesis in these patients with oral SCC.

While oral cancer is represented mostly by squamous cell carcinoma and very aggressive-type behavior, it is important to emphasize that this particular type represents about 50% of all solid tumors in patients who undergo HCT[23,25,31-36]. Abdelsayed *et al*[34] mentioned that oral cancer in patients with GVHD might have more aggressive biologic potential with a higher tendency for recurrence and the development of new lesions. Mawardi *et al*[37] evaluated 26 post-HCT patients who had developed verrucous hyperplasic hyperplasia (12%), dysplasia (19%), and invasive carcinoma (69%). Twenty-four patients (96%) had cGVHD, and of these patients, 96% (23/24) presented oral features.

Due to the increased amount of patients who survive HCT and remain free of the original disease, attention should be paid to the presence of potentially cancerous lesions or tumors that already exist. Studies reported that after HCT, patients had an increased risk of developing secondary tumors in comparison to the general population[25,27]. Therefore, there is concern about the early detection of a second primary

tumor in these patients. Currently, the consensus on screening guidelines and long-term follow-ups of HCT complications is that oral mucosa and dental status should be examined during the annual examination of patients with GVHD and every 6 mo for patients with Fanconi's anemia[23,29,38].

TREATMENT

Oral cGVHD management focuses on ameliorating symptoms, maintaining oral function, and restoring mucosal integrity by treating symptomatic oral abnormalities and ulcerative lesions[9].

The first-line therapy for cGVHD in other areas beside oral mucosa, involves systemic corticosteroids. When the oral cavity is the only site involved, the topical management of oral cGVHD may be indicated[10]. Therapy is indicated based on corticosteroid with presentations on solutions[10] such as Dexamethasone, Budesonide, Prednisolone, Triamcinolone, and Betamethasone[37]. The corticosteroids with presentation gel, creams, and ointments are Fluocinonide, Clobetasol, Betamethasone, and Triamcinolone. The nonsteroidal immunosuppressive solution and ointment is Tacrolimus[10].

CONCLUSION

GVHD is a common sequela of patients who are treated by HCT. Diagnosing oral complications and manifestations of GVHD disease is fundamental for dental management during medical therapy.

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Oral lichenoid lesion: A review of the literature

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Abstract

The oral lichenoid lesion (OLL) is response that occurs on the oral mucosa. The OLL include allergic response to the dental materials, drugs, and on graft-*vs*-host disease (GVHD). OLL to dental material happen when restorative materials, most commonly amalgam, are in direct contact with the mucosa in sensitized individuals. Medications that produce OLL are oral hypoglycemic agents, angiotensin-converting enzyme inhibitors, and nonsteroidal anti-inflammatory agents. GVHD is a complication in bone marrow transplantation and OLL is a common lesion observed in this disease especially in chronic GVHD. The clinical and histological aspects of OLL are similar to oral lichen planus and turn it difficult to make a differential diagnosis. The purpose of this paper is review about OLL related to the dental materials, drug use and GVHD.

Key words: Oral lichenoid lesion; Lichenoid contact reaction; Lichenoid drug reaction; Lichenoid related to graft-*vs*-host disease

Core tip: There are various oral manifestations like oral lichen planus. These lesions are related to local or systemic factors and are important in oral diagnosis and patient's management. Considering the increased of number of these lesion in current moment, we investigated previous publications and aim to present a literature review.

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INTRODUCTION

Oral lichenoid lesion (OLL) is a chronic inflammatory lesion of the oral mucosa that occurs as an allergic

response to dental materials, to use of certain medications, in patients with graft-*vs*-host disease (GVHD), in patients with systemic diseases, *e.g.*, chronic hepatitis C[1] and patients vaccinated against hepatitis B[2]. Various terminologies have been used to describe this condition, as oral lichenoid lesions, oral lichenoid reaction, oral lichenoid tissue reaction, lichenoid contact stomatitis or lichen-planus-like lesions, due the similar clinical and histological aspects of OLL and oral lichen planus[3].

The OLL is a frequent condition, with prevalence 2.4% in general population[4]. These lesions occurs generally in oral mucosa of adults[5], mostly in women with average age of 53 years old[6]. The lesions are mostly present in the buccal mucosa, lateral border of the tongue and oral mucosa of the lips, when associated with composite restorations. It is generally limited size and unilateral. This find can be important to distinguish OLL from the oral lichen planus (OLP) lesions, which occurred commonly bilateral in oral mucosa[2,3,6,7].

Clinically the lesions showed as white striations, plaques, erythema, ulcers or blisters, asymptomatic. The patients can complain of sensitivity to spicy foods or burning sensation. Histologically, observed a hyperkeratinization, liquefaction degeneration of the basal cell layer and band-like layer of lymphocytic infiltrate in the connective tissue[2,6-9]. OLP, OLL and GVHD could not be histopathologically discriminated[10,11], however some authors investigate possible histopathological aspects among the lesions[12]. Moreover, the clinical and histological aspects are not useful to distinguish OLL from OLP[13,14]. Some authors showed that the level of salivary IgA and IgG in OLP and OLL patients is higher than healthy controls, but they cannot be used as differential diagnosis of both alterations[15].

The purpose of this paper is review about OLL associated with dental materials, with medication use, with systemic diseases, in patients vaccinated against hepatitis B and in patients with GVHD. The methodology was a search of the literature, from 1966 through December 2014, about OLL related to the dental materials, drug use, GVHD listed on PubMed. The search was conducted in both English and Portuguese, and the keywords used were "oral lichenoid lesion", "oral lichenoid lesion and dental materials", "oral lichenoid lesion and drug" and "oral lichenoid lesion and GVHD". Additional studies were found in the reference lists of the selected articles.

ORAL LICHENOID LESION TO THE DENTAL MATERIALS

Resin-based composite, gold and the amalgam and its components can cause hypersensitivity reactions on the oral mucosa[6,13,16-19]. According to Lygre *et al*[20] the principal cause of OLL was associated with adverse reaction to dental materials, being amalgam fillings as responsible for 84% of the cases. The OLL associated with amalgam restorations can be observed in about 2% of population[7,20-22]. Although uncommon, the composite resin also can be associated with OLL[23].

The induction of OLL to the dental materials is probably the long-term. The contact of oral mucosa with dental material develops hypersensitivity reaction over a period of days and the clinical manifestations may present many years after initial contact with the dental material[7]. In case of the amalgam, reaction can occur the release of corrosion products from the restoration surface, and may result in lymphocyte activation and induction of a cell-mediated autoimmune response directed at basal keratinocytes[13]. The cell mediated type Ⅳ hypersensitivity response of amalgam restoration can result in immune-mediated damage of the basal keratinocytes[7,17,21,22]. In most cases of OLL the hypersensitivity is the mercury, however other components of amalgam fillings as copper, tin or zinc can be associated with the reaction[6,16,18]. Some authors suggested that this reaction occurs in susceptible individuals for long time of exposure[21,13], since OLR does not develop in all individuals with amalgam alloys in contact with oral mucosa. However, the levels of IL-6 and IL-8 in saliva of patients underwent to amalgam filling replacement showed significant reduction[8].

Although the use of patch test for OLL to dental material is controversial, showing limited value[16,24,25], Thornhill *et al*[13] showed that the combination of a positive patch test and the presence of oral lesions together to amalgam restoration were an important predictor of lesion improvement. An interesting observation made by these authors was that the desquamative gingivitis clinical aspect was not observed in any of the patch test positive patients or patients with a strong clinical association between the lesions and their fillings. Moreover, these patients did not demonstrated history of skin lesions.

Conflicted points related to skin patch test can be described: amalgam components to use in the test; distinguish sensitivity from irritant responses; how long the material should remain in contact with the skin; and the value of skin patch testing in identifying true oral lichenoid lesions. Furthermore, there is debate about the validity of extrapolating skin reactions to mucosal responses[17]. Nevertheless, the patch test may be helpful to determinate an alternative material to use when replacing amalgam[17,25].

The final diagnosis of OLL to dental material is confirmed by clinical and histological aspects associated with the resolution of the lesions after replacement of the restoration[6,16]. Most of the OLR associated to amalgam disappear in 3 to 15 mo after that the restoration was changed[6,8,13,17,18,24].

The study by Thornhill *et al*[14] confirmed the difficulty of histological distinction between OLL and OLP, showing that five oral pathologists were able to differentiate both conditions in just one-third of the cases. According to the authors, some features may be present in OLL and absent in OLP: an inflammatory infiltrate located

Table 1 Medications related to the induce oral lichenoid lesion described in literature

Type of medication	Example
Antibiotics and chemotherapeutic agents	Penicillin, tetracycline, streptomycin, pyrazinamide, sulfadoxin, ketoconazole, pyrimethamine, demeclocycline
Antidiabetic agents	Chlorpropamide, tolbutamide
Antiepileptic agents	Carbamazepine
Antihypertensive agents	Methyldopa, labetalol, propanolol, captopril
Antimalarials	Chloroquine, quinacrine
Antimaniac drugs	Lithium salts
Antiplatelet agent	Clopidogrel
Antirheumatic agents	Gold Salts
Antiulcer medication	Bismuth
Benzodiazepines	Lorazepam
Nonsteroidal anti-inflammatory drugs	Salicylates, indometacin, fenelofenac, isoxicam, piroxicam

Adapt from Guijarro Guijarro and López Sánchez[27].

deep to superficial infiltrate in some or all areas; focal perivascular infiltrate; plasma cells and neutrophils in the connective tissue. Juneja *et al*[3] found increased epithelial thickness in OLL compared to OLP, probably due to the release of inflammatory mediators from the cellular infiltrate, inducing the proliferation of basal keratinocytes. However, the number of mast cells, neutrophils and macrophages is significantly higher in OLP than in OLL, besides a continuous thin, linear band of basement membrane and numerous strands extending into the irregularity connective tissue. Thus, these parameters can be considered useful to differential diagnosis between OLP and OLL[3]. However, it is necessary to emphasize the importance of excluding the presence of Candida infection, which it is common in association with OLLs[26], principally in areas of ulceration since in both of them may result in accumulations of neutrophils and plasma cells[14].

ORAL LICHENOID LESION TO THE DRUG

Drugs are identified as inducers of oral lichenoid lesion (OLL-d), principally associated with prolongation use of the drugs[17]. When a drug is suspected to cause the OLL-d, the change of them should be considered[21]. In contrast to cutaneous lichenoid lesion to the drugs, the OLL-d is uncommon[17]. The Table 1 presented drugs that can induce OLL-d[27].

The final diagnosis of OLL-d is difficult, and the readministration of the medication can help to establish if the oral lesions are drug-induced, though this can be dangerous for the patient[17,28]. Generally the lesion disappears after suspension of the drug[3,17,28]. However, the complete resolution of the lesion can hold out several months.

In some cases, the medication is potentially indispensable to survival of the patients; thus its suspension or replace is not possible[17,28]. In these cases, the lesions must be treated conventionally as OLP. According some authors the patients with OLL-d related with drugs to cardiovascular diseases, have reported to decrease unstimulated whole saliva secretion[29], suggesting the hyposativation as a trigger to OLL-d in these patients[19].

Other medications have been related to OLL-d and OLP[1]. It has been extensively demonstrated that IFN may induce or worsen immunological diseases. With the advent of pegylated IFN-α, a causal link among the treatment of chronic hepatitis C with combination of pegylated IFN plus ribavirin and several autoimmune events have been suggested. The development or exacerbation of OLP has also been reported after the introduction of IFN-α to treat hepatitis C[2,3,9,28,30-32], and also contribute to the development of new lesions as OLL. It is quite plausible that IFN-α may induce or worsen previous lesions due to its interference with the cytokine cascade[31]. Grossmann *et al*[33] described three cases of exacerbation of OLP during the treatment of chronic hepatitis C with pegylated IFN plus ribavirin. However, it is difficult establish if the lesions were exacerbation of previous lesions of OLP or new lesions of OLL-d.

McCartan and Lamey[30] investigated the use of a lichen planus-specific antigen as a marker to distinguish idiopathic OLP from OLL-d and demonstrate that it is not a useful marker.

ORAL LICHENOID LESION ON GVHD

GVHD is a very frequent complication of allogeneic bone marrow transplantation (BMT), and it is associated with morbidity and mortality. It is characterized by dermatological, gastrointestinal and hepatic lesions[31,34,35].

GVHD occurs when the donor immune system recognizes the host tissue as foreign and attacks its cellular constituents. Donor's T-lymphocytes reacts against recipient of antigens[17,31,32,34-36]. Three conditions can be observed in patients with GVHD[37]: the graft must contain immunologically competent cells; the recipient must express tissue antigens that are sufficiently different from those of the donor; or the recipient must be incapable of rejecting the graft because of either tolerance, lack of recognition, or immunosuppression.

There are acute and chronic GVHD: acute GVHD (aGVHD) occurs within the first hundred days after

transplant, while chronic GVHD (cGVHD) in more than 100 d after BMT[17,34,31,35], and systemic organs and oral mucosa are involved[17].

The aGVHD presents as painful desquamative and ulcerative lesions in oral cavity. Clinical manifestations of cGVHD appear very similar to those of autoimmune connective tissue diseases: white papular eruptions or reticular lesions with areas of erythema, erosion, or ulceration. Generally are symmetrically distributed and the areas of involvement include the tongue, buccal and labial mucosa[31,32,35-36]. It is commonly seen to arise or worsen after an infectious insult or when immunosuppression is reduced[17,31], and they can influence quality of life of patients[36].

Erythema, mucosal atrophy, and lichenoid changes are common oral findings in patients with cGVHD, with lichenoid reactions having the highest positive predictive value[31]. According to a study of Nakamura *et al*[35], OLL were the only clinical sign that had a statistically significant relationship to the diagnosis of cGVHD[25]. Clinically the OLL on cGVHD appears as lacy white striations similar to the striae of Wickham in the OLP[31]. Histologically these reactions consist of a degeneration of the basal cell layer and lymphocytic infiltration in the sub-mucosa. In some cases intracellular edema of epithelial cells can be observed[35].

The OLL can be controlled when treating the systemic GVHD with immunosuppressive therapy. If the oral lesions persist or represent an isolated feature of GVHD, the management with potent topical corticosteroids is generally indicated[32]. Some medications used for the treatment of OLL associated with GVHD are diphenhydramine with kaolin and pectin or clobetasol gargles, topic fluocinonide, oral prednisone (20 to 50 mg/d) or thalidomide (50 to 200 mg/d)[34].

CONCLUSION

In summary, the OLL are a group of intriguing lesion principally when investigates their causal relationship. Moreover, determinate the differential diagnosis of OLL and OLP is important, considering that the different management of this lesions. Thus, epidemiological and laboratorial investigations including a larger number of patients are warranted to elucidate important aspects of OLL until obscures.

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SYSTEMATIC REVIEWS

Effects of different root canal preparation methods on root fracture resistance: A systematic review of the literature

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Author contributions: Li XG and Wang Q contributed equally to this work; Li XG and Wang Q designed the research; Li XG performed the research, analyzed the data and wrote the paper; Wang Q modified the paper.

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Data sharing: Technical appendix and dataset available from the corresponding author at wangqing@sdu.edu.cn. Participants gave informed consent for data sharing.

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Abstract

AIM: To study the root fracture resistance after root canal preparation with Ni-Ti rotary instruments and stainless hand instruments by means of meta-analysis.

METHODS: Literature was researched in CNKI and CBMDisc, PubMed, CALIS, Proquest, Web of Science and 11 kinds of Chinese or English dentistry journals. Retrieval time on Internet was in all years and hand retrieval time was from January 2013 to October 2013. The literatures were selected through reading abstracts and full texts by two reviewers independently and Revman 5 software was used to analysize the literature.

RESULTS: Six articles met the inclusion criteria. According to Meta-analysis of tooth root bending properties, total standardized mean difference (SMD) was 0.63 (95%CI: -0.24-1.50, $P > 0.05$). That indicated there was no statistically significant between the two groups. Subgroup analysis was carried out. SMD were 2.22 (95%CI: 0.23-4.20, $P < 0.05$) and -0.61 (95%CI: -1.05- -0.17, $P < 0.05$) when the premolar teeth with a single canal or the mesiobuccal roots of molars were used as the materials for tests to compare the effects of different root canal preparation methods on root fracture resistance. That only indicated that there were statistically significant in two subgroups.

CONCLUSION: *In vitro* experiments, the effects on the fracture resistance of root had no statistical difference with Ni-Ti rotary instruments and stainless steel hand instruments in root canal preparation.

Key words: Root canal therapy; Root canal preparation; Root fracture; Meta-analysis

Core tip: There were different opinions on the effect of root fracture resistance using nickel-titanium rotary instrument. The present study carried out Meta-analysis on the references related to the influences on root fracture resistance of two different root canal preparation methods. The result can provide evidence for clinical therapy.The present study confirms that the effects of root canal preparation by Ni-Ti rotary instruments or manual stainless steel instruments on root fracture

resistance are not statistically significant. These two preparation methods are both safe and effective if dentin is not excessively cut under normal chewing conditions.

Li XG, Wang Q. Effects of different root canal preparation methods on root fracture resistance: A systematic review of the literature. *World J Stomatol* 2015; 4(2): 108-114 Available from: URL: http://www.wjgnet.com/2218-6263/full/v4/i2/108.htm DOI: http://dx.doi.org/10.5321/wjs.v4.i2.108

INTRODUCTION

So far, root canal therapy is the most thorough and perfect method for endodontic and periapical disease, and root canal preparation is the key procedure for root canal therapy. However, more and more studies showed that the teeth after root canal therapy may have an inclination towards longitudinal fissure in tooth root[1,2]. Sclerotic tissues of teeth lost nutrients due to removing endodontium in root canal preparation, and dentin became dehydrated and embrittled[3]. The studies demonstrated that there was a close relationship between tooth resistance and root canal cavity size, lumen wall thickness and cavity shape. Wilcow *et al*[4] studied 34 maxillary anterior teeth *in vitro* and indicated that subclinical crack was detected in almost 30% of the tooth roots under the same burden on root canal wall when the root canals were enlarged to 20%-30% of the diameters of the root canals. When the root canals were enlarged to 40% of the diameters of the root canals, longitudinal fissure in tooth roots were detected. Sathorn *et al*[5] prepared root canals and enlarged the middle diameter of root canals progressively in ten mandibular incisors *in vitro*. The results of finite element analysis showed that the root fracture resistance successively decreased for the root canals with diameters at 0.5, 1.0, 1.5 and 2.0 mm[5]. There was a close correlation between the degree of root canal preparation and tooth resistance. The larger cavity size lead to the less tooth resistance. Thus, the remaining dentine thickness was an important factor affecting root fracture resistance of teeth.

Nickel-titanium rotary instruments have an excellent shaping ability due to ideal remotion ability and flexibility, which ensures a perfect root canal preparation combined with crown-down technique[6]. Therefore, nickel-titanium rotary instruments have became the main instrument for root canal preparation. However, there were different opinions on the effect of root fracture resistance using nickel-titanium rotary instruments. The present study carried out meta-analysis on the references related to the influence on root fracture resistance of two different root canal preparation methods. The result can provide evidence for clinical therapy.

Table 1 PubMed search strategy

No.	Search history	Limits	Results
I	(Root canal therapy or root canal preparation) and root fracture	Human Chinese or English All years	1014
II	(Nickel-titanium rotary instrument or manual stainless steel instrument) and root fracture	Human Chinese or English All years	27
III	(Hand instruments or rotary instruments) and root fracture	Human Chinese or English All years	32
Total			1073

MATERIALS AND METHODS

Literature retrieval

Using three groups of research terms: "(root canal therapy or root canal preparation) and root fracture", "(nickel-titanium rotary instrument or manual stainless steel instrument) and root fracture", "(hand instruments or rotary instruments) and root fracture". The published literature were assessed in CNKI, CBMdisc, China Academic Library and Information System (CALIS), PubMed (Table 1), Proquest, Web of Science and in 11 Chinese or English dentistry journals by hand. Retrieval time on Internet was in all years and hand retrieval time was from January 2013 to October 2013. The literature was selected through reading abstracts and full texts.

Literature inclusion and exclusion

Literature inclusion criteria: Literature inclusion criteria were made according to Meta rules and then the literature were selected for second time. Literature inclusion criteria were: (1) All experiments were randomized controlled trials and the tested teeth were randomly divided into hand instruments preparation group and nickel-titanium rotary instrument preparation group; (2) The teeth *in vitro* were selected as materials. There were no significant defect and abnormal shape. In every included experiments, the differences in lengths of teeth, buccolingual diameters, mesiodistal dimensions and root tip curvatures should not be statistically significant; (3) Root canal preparation method was the only variable in every experiment; (4) Teeth were similarly handled before testing. Step-back technique was used to prepare root canal in hand instruments group, and crown-down technique was used in nickel-titanium rotary instrument group; (5) The universal loading machine of general international standards was used for the instrument for tests, and 1 mm/min was used as the loading rate; (6) The pressure values when root fracture appeared were recorded; and (7) All data were analyzed by statistic software.

Research data

There were six publications meeting the inclusion

Table 2 The information statistics of included literature

Ref.	Dental notation	Hand preparation numbers	Hand preparation mean	Hand preparation SD	Ni-Ti preparation numbers	Ni-Ti preparation mean	Ni-Ti preparation SD	Ni-Ti instruments taper
Lan *et al*[7] I	Molar	13	10.2	4.4	13	15.7	9.1	0.06
Lan *et al*[7] II	Molar	13	10.2	4.4	13	13.2	6.1	0.08
Ren *et al*[8]	Pre-molar	10	308	8.69	10	228	10.19	0.06
Sathorn *et al*[9]	Incisor	25	113.5	20.2	25	114.9	37.1	0.04
Shi[10] I	Pre-molar	8	459.5	163.4	8	436.75	146.58	0.02
Shi[10] II	Pre-molar	8	459.5	163.4	8	474.25	101.44	0.04
Shi[10] III	Pre-molar	8	459.5	163.4	8	431.38	90.67	0.06
Singla *et al*[11]	Pre-molar	10	482.78	19.33	10	399.07	13.279	0.12
Zare Jahromi *et al*[12]	Molar	16	50.33	19.1	16	63.1	25.46	0.06

citeria[7-12]. In Lam *et al*[7] study, there were two experimental groups in different Ni-Ti rotary instruments taper. So there were two randomized controlled trials among two experimental groups and a control group. They were marked as Lam Ⅰ and Lam Ⅱ in Table 2. In Shi[10] study, three experimental groups B1, B2 and B3 and a control group A took root canal preparation method as the only variable. So there were three randomized controlled trials among them. They were marked as Shi Ⅰ, Shi Ⅱ and Shi Ⅲ in Table 2. There were four groups in Singla *et al*[11] study, but only one randomized controlled trials between hand instruments group and Protaper rotary instruments group fit inclusion criterias[11].

Essential characteristics of all groups were showed in Table 2.

Meta analysis of inclusive literature

Revman 5 software was used for meta-analysis. Since the measuring units for the included references were not consistent, so standardized mean difference (SMD) analysis was carried out and represented by 95%CI. χ^2 test was carried out to test the heterogeneity among the references. Randomized effect model and fixed effect model were performed respectively in meta analysis when there was statistic significance heterogeneity or not ($P < 0.05$, $I^2 > 50\%$ or $P > 0.05$, $I^2 < 50\%$). Then the forest map was plotted.

Statistical analysis

This document certifies that the statistics in the above manuscript was reviewed and edited by the subject experts (Professional statistician and PhD-level American expert) at 4UPUB to ensure the statistics method, spelling, grammar, and word flow adhere to the standards of professional and academic journals.

RESULTS

Meta analysis of the included literature

In six included literature, nine groups of clinical trials, there was statistical heterogeneity ($P < 0.05$, $I^2 > 50\%$), so the random effect model of meta analysis was used. The results were showed in Figure 1.

SMD in Figure 1 was 0.63. This indicated that the teeth prepared by manual stainless steel instruments had more root fracture resistance. But the effect quantity was no statistically significant (95%CI: -0.24-1.50, $P > 0.05$).

Subgroup analysis was carried out according to the difference of the tooth positions in each experiment: (1) The premolar teeth with a single canal were used to compare the effects of different root canal preparation methods on root fracture resistance, and the results were shown in Figure 2. The figure indicated that this subgroup of references had statistical heterogeneity ($P < 0.05$, $I^2 > 50\%$). Thus the SMD analysis under the random effect model was used. SMD was 2.22 (95%CI: 0.23-4.20, $P < 0.05$), indicating that the preparation by using Ni-Ti rotary instruments was liable to cause root canal fracture; and (2) The mesiobuccal roots of molars were used to compare the effects of different root canal preparation methods on root fracture resistance, and the results were shown in Figure 3.

The figure mentioned above indicated that this subgroup of references had no statistical heterogeneity ($P > 0.05$, $I^2 = 0$). Thus the SMD analysis under the fixed effect model was used. SMD was -0.61 (95%CI: -1.05- -0.17, $P < 0.05$), indicating that the preparation by using manual stainless steel instruments was liable to cause root canal fracture.

Heterogeneity analysis

As shown in Figure 1, there was statistical heterogeneity among seven included references ($P < 0.05$, $I^2 > 50\%$). By reading literature, significant heterogeneity was found in the references of Ren *et al*[8] and Singla *et al*[11], and heterogeneity analysis should be carried out.

The pressure loading direction utilized in the reference of Ren *et al*[8] had an angle of 15 degrees to the long axis of teeth, namely it simulated the situation for root fracture under lateral pressure load, while the

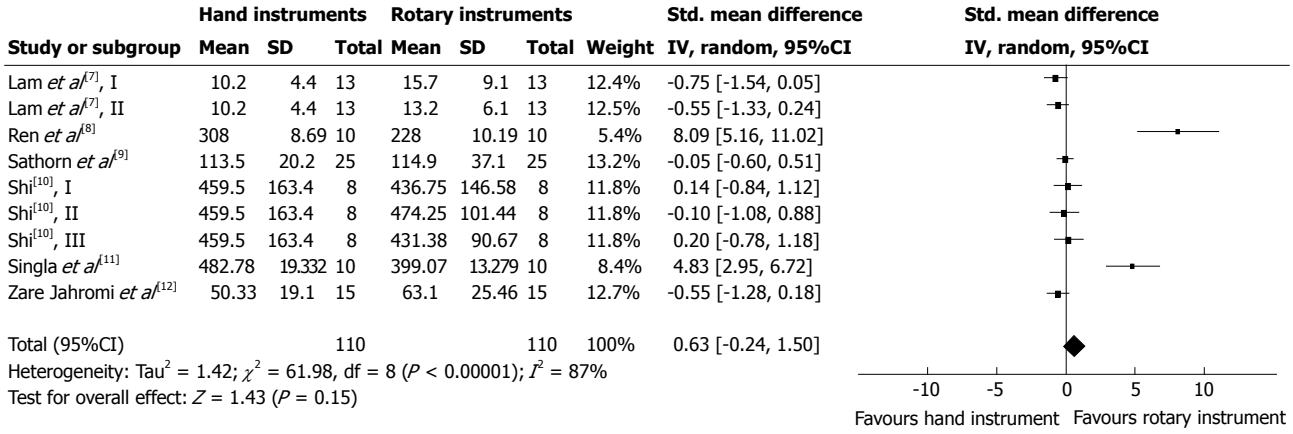

Figure 1 Forest plot of comparison of effects of root fracture resistance.

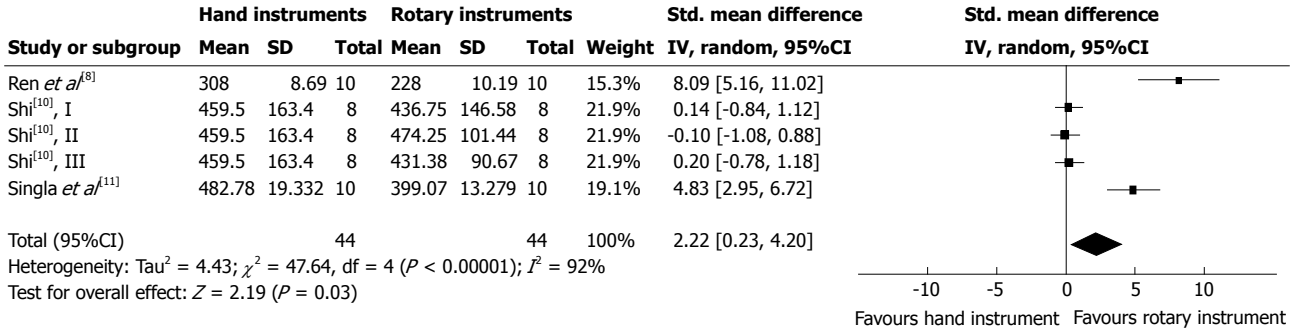

Figure 2 Forest plot of subgroup (premolar).

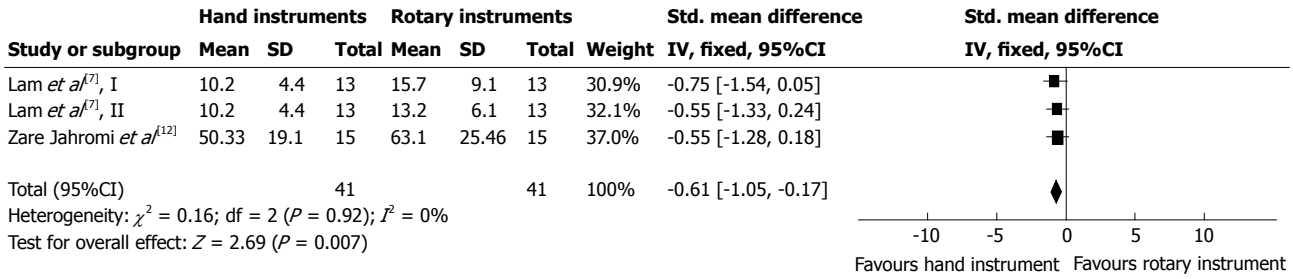

Figure 3 Forest plot of subgroup (molar).

pressure loading directions utilized in other references were paralleled to the long axis of teeth, namely they simulated the situation for root fracture under vertical pressure load. The research showed that the maximal VON MISES stress, the maximal tensile stress and the maximal compressive stress of tooth at lateral loading should be significantly higher than those at vertical loading under the same loading conditions, and they should be located at the middle parts of roots[13]. The studies also confirmed that the diameter of the middle part in the root canal after preparation with Ni-Ti rotary instruments was significantly bigger than that in the root canal after preparation with manual stainless steel instruments[14,15]. And the strength of root canals was closely related to the thickness of root canals, so the above factors may contribute to the heterogeneity.

The teeth roots were used in the loading tests after preparation but not filling up in the reference of Singla[11]. Studies have shown that the fracture resistance of root canal only after preparation was significantly lower than that after perfect root canal treatment[16,17]. Some scholars also found that filling sealer could significantly enhance the strength of the prepared root canal[18,19]. So the literature had large heterogeneity.

DISCUSSION

Root canal preparation is an important procedure for root canal therapy, and excellent root canal preparation is the prerequisite for successful root canal therapy. With the development of dentistry, various kinds of Ni-Ti

rotary instruments appear, which have good flexibilities and toughnesses. Using them can decrease root canal perforation and displacement apparently and shorten handling time. Increase in the taper of the instruments can effectively clean and prepare root canals, but it may damage more dentin and thus decrease the strength of tooth root. Many studies demonstrated that there was a positive relationship between the quantity of dentin and intensity of tooth root. There was a lower resistance in teeth after root canal preparation than in teeth without root canal preparation according to all inclusive literature. The statistic difference was significant ($P < 0.05$), which confirmed that root canal preparation decreases intensity of tooth root as well.

Dentin cutting was required for root canal preparation, while the remaining dentin thickness was the most important factor influencing the strength of teeth roots[20]. At 0.5 mm away from apical stop, same amount of dentin was cut by using manual stainless steel instruments and Greater Taper (GT) rotary instruments, while only 0.25 mm more dentin was cut by using Lightspeed (LS) rotary instruments. But at 4.5 mm away from apical stop, a little more dentin was cut by using GT rotary instruments than using other two kinds of instruments[7]. Certainly, more dentin is cut by using Ni-Ti rotary instruments than by using manual instruments, but the cross sections are arc-shaped triangles, which decreases the contacting area between rotary instruments and dentin in root canal walls. In addition, since the root canal compactibility was satisfactory, so the root canal taper after preparation was continuous and uniform and the stress could be effectively scattered, which could not only improve the cutting efficiency, but also decrease the stress on root surface during root canal preparation[21]. So this method can offset the deficiency that cuts more dentin in a certain degree. At the same time, the experiment *in vitro* have established that masticatory force could disperse quickly along the long axis of teeth *via* dental crown under vertical pressure load. Even root canal preparation and filling in large taper were also relatively safe[22].

The clinical trials confirmed that the stress concentration area in the tooth root was almost consistent with the direction of root fracture. And the stress concentration was closely related to root fracture[23]. Manual stainless steel instruments cut less dentine, but repeated lifting and dragging was required for preparing ideal root canal morphology, which lead to over-straightening of root canal. The weak parts were liable to fracture because of uneven forces on different parts of root canal wall. Moreover, relatively big stress would be produced on root canal wall and decreased the strength of tooth root during repeated lifting, dragging, enlarging and scraping[24]. From the point of view of mechanics, structure defects, crack or improper root canal preparation, which would produce multiplied stress, were likely to be the key factors influencing the root canal strength[25]. Other studies considered that the rigider root canal file caused stronger stress concentration, which increased the risk of dentin defect and lead to root fracture[26].

Meanwhile, with the development of digital modeling technique, some scholars analysed the stress of root canal preparation by tooth three-dimension finite element model. The studies showed that the tendency of whole stress distribution on prepared teeth was similar, and the stress at the root canal orifice in the stainless steel instrument group was the highest, but the differences in the stress on root canal wall between root tips and middle parts of root canals were not statistically significant[27]. Furthermore, the ultimate compressive strength of dentine is 232-305 MPa and the tensile strength is 48-105.5 MPa. Therefore, different root canal preparation methods are safe and reliable without other effective factor. In general, the preparation methods by using Ni-Ti instruments and manual stainless steel instruments may decrease the strength of tooth roots, but the mechanisms are different and the effects on root fracture resistance have not been well defined.

Previous studies have shown that premolar and mesiodistal root canals of mandibular first molar after preparation were liable to fracture, thus most of the researches utilized them as the teeth for tests[28]. However, premolar and the root of mandibular first molar are different in their morphology, and subgroups (1) and (2) indicated that the test results were slightly different. The root of premolar is straighter and thicker than the first molar. The two kinds of preparation methods can easily produce smooth and continuous cone-shaped root canals, and stress concentration is not easily produced. Therefore, the amount of dentin cutting becomes a major factor affecting root fracture resistance, so manual stainless steel instruments are more advantageous. In contrast, mesiodistal root canals of molars are relatively thin and curved in root tips, and the flexibility of manual stainless steel instruments is relatively poor, so weak parts and stress concentration areas are liable to be produced during root canal preparation. Manual stainless steel instruments may be liable for root fracture in comparison to Ni-Ti rotary instruments.

All references included in the present study utilized *in vitro* loading tests. The methods were simple, and the experiments can be easily repeated, thus they have become the main method for investigating the effects on the root strength of preparation by using different root canal preparation instruments. However, *in vitro* loading tests also have their limitations: the direction of forces in loading tests is single, the maximal load exceeds the physiological chewing force and it could not mimic the physiological force loading of teeth in oral cavity. However, the direction of forces on teeth is one of the important influencing factors for root fracture resistance and changes in loading direction may change the research results. Among all of the references included in the present study, the maximal loads of tooth roots

all exceeded the physiological chewing force in oral cavity. Direction[29] and positions[30] of forces may lead to significant effects on the test results. Lateral forces may be liable to cause stress concentration in teeth roots in comparison to vertical forces under the same loading conditions. Therefore, the seen root fracture in clinical practices is always induced by lateral stress. Meanwhile, high frequency of load may be produced when teeth play the role in chewing under physiological conditions, so the fatigue resistance of tooth also affect fracture resistance. The effects of different root canal preparation methods on root fracture resistance require more tests on lateral forces and fatigue tests.

The present study confirms that the effects of root canal preparation by Ni-Ti rotary instruments or manual stainless steel instruments on root fracture resistance are not statistically significant. These two preparation methods are both safe and effective if dentine is not excessively cut under normal chewing conditions.

COMMENTS

Background

Root canal therapy is a perfect treatment method for pulposis and periapical disease. And root canal preparation is one of the key procedures for root canal therapy, which includes traditional hand stainless steel instruments preparation method and rotary nickel-titanium instruments preparation method. Rotary nickel-titanium instruments, which have excellent root shaping ability, ideal remotion ability and flexibility and high preparation efficiency are research hotspots. But many studies have shown that the teeth prepared by rotary nickel-titanium instruments may show an inclination towards longitudinal fissure, because more dentin is cut off.

Research frontiers

Many researches have carried out to study the different effects of two root canal preparation methods on root fracture resistance, but different results were obtained, which have led to puzzles in clinical operations for dentists.

Innovations and breakthroughs

Meta-analysis was done for the effect of root fracture of Ni-Ti rotary instruments and stainless steel hand instruments preparation methods. Meta-analysis provides a high quality system review.

Applications

The result can provide evidence for clinical therapy. Dentists can choose different root canal preparation methods according to the tooth type or tooth position and root curvatu.

Terminology

Meta-analysis is a quantitative statistical analysis of several separate but similar experiments or studies in order to test the pooled data for statistical significance.

Peer-review

This paper is potentially valuable.

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