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FROM EDITORS DESK

Dear Esteemed Contributors, Reviewers, and Readers,

We hope this message finds you in good health and high spirits. As the editorial team at the Karnataka State Dental Journal, we wanted to take a moment to share some exciting updates and important announcements with you.

We are excited to announce that we are planning to bring out editions of the Karnataka State Dental Journal, focusing on the latest advancements and research in the field of various fields of dentistry. We invite all researchers, academicians, and experts in the respective field to contribute their valuable insights and research findings for these edition. Your contributions will undoubtedly enrich the knowledge base of our esteemed journal. We encourage diverse topics within the realm of dentistry and oral health to foster a comprehensive understanding of the field. We would like to extend our heartfelt appreciation to our dedicated pool of reviewers who play an indispensable role in ensuring the quality and rigor of the articles we publish. Your insightful feedback and expertise contribute significantly to the growth of our journal, and we are immensely grateful for your continued support. In our pursuit of promoting knowledge dissemination, we are actively exploring opportunities to provide wider access to our content. We believe that open access initiatives align with our mission to contribute to the advancement of dental science and education.

We look forward support, contributions, and readership from the esteemed office bearers of Karnataka State branch and local branches. Together, we can elevate the Karnataka State Dental Journal to new heights and foster a vibrant community of dental professionals and researchers.

For any inquiries, submissions, or suggestions, please feel free to reach out to us at editorksdj2023@gmail.com



Dr. Mohan Kumar K. P
Hon. Editor

PRESIDENT MESSAGE

Dear members,

I extend my heartfelt gratitude for the opportunity to address you in this esteemed Karnataka State Dental journal. As we come together to explore the frontiers of dentistry, it is evident that oral health holds a significant place in our society's overall well-being.

In today's ever-evolving world, advancements in dental science are shaping the landscape of healthcare, bringing hope and improved quality of life to countless individuals. With each passing day, our understanding of oral health's intricate connection to systemic health deepens, emphasizing the importance of collaboration among dental professionals, researchers, and healthcare stakeholders.

Through shared knowledge and research, we can pave the way for innovative treatments, preventive measures, and patient-centered care. By embracing cutting-edge technologies and evidence-based practices, we empower ourselves to meet the evolving needs of our patients and communities.

Moreover, let us not forget the power of education and awareness. As we disseminate vital information about oral health, we empower individuals to take charge of their well-being, promoting a culture of proactive and preventive dental care.

In this pursuit, we must also acknowledge the challenges that lie ahead. From disparities in access to dental care to the environmental impact of oral health practices, we must remain vigilant in addressing these issues collectively, fostering a more equitable and sustainable dental healthcare landscape.

As the leaders of tomorrow, our dedication to excellence and continuous improvement knows no bounds. Let us uphold the highest standards of professionalism, ethics, and compassion in our daily practice, ensuring that patient-centric care remains at the core of everything we do.

Together, we can make a significant impact on the oral health of our communities and inspire positive change in dental healthcare globally. I urge each of you to continue your invaluable contributions to this noble field and join hands in shaping a brighter, healthier future for all.



Best wishes and regards
Dr Ramamurthy T K
President
IDA Karnataka State
Branch

SECRETARYMESSAGE

It is learnt that the Indian Dental Association, Karnataka state branch is going to publish the First edition of the IDA Karnataka State Dental Journal for the year 2023.

I appreciate the efforts made by the editorial team under the leadership of Dr Mohan Kumar in bringing up this scientific journal.

I convey my warm regards to all the IDA members and wish good luck for the editorial team for their future endeavors.



Best wishes
Dr Mahesh Chandra K
Secretary
IDA KARNATAKA STATE

INDIAN DENTAL ASSOCIATION KARNATAKA STATE BRANCH

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ENDODONTIC MICROBIOTA

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ABSTRACT:

Root canal is the soft tissue component of tooth that occupies the internal cavity of root of the tooth. It may get infect from many number of microorganisms to which they are exposed. There are many ways by which microorganisms reach the pulp and infect the root canal. There are various routes through which microorganisms reach the root canal namely – after a carious lesion or during dental procedure through dentinal tubules, direct pulp exposure of traumatic origin, through periodontal membrane, faulty restorations etc.

Around 700 microbes present in the oral cavity. Endodontic infection are poly microbial in nature, with obligate anaerobic bacteria conspicuously dominating the microbiota in primary infection. This paper emphasises on various microbiota in root canal infection.

KEY WORDS: Root canal, microorganisms, carious lesion.

INTRODUCTION:

Microorganisms play an important role in the root canal infection. Since the root canal is enclosed and surrounded by hard tissue all around which is favourable condition for the growth of microorganisms inside the root canal. Endodontic infections occur and progress when the root canal system gets exposed to the oral environment by one reason or the other and simultaneously when there is fall in the body's immune response.¹

Primary infection of root canal occurs mainly through carious lesion or during dental procedure through dentinal tubules, direct pulp exposure of traumatic origin, through

periodontal membrane, faulty restorations etc.². Secondary infection in the root canal occurs due to the failure of endodontic treatment and presence of bacterial infection in the root canal.³

PATHOGENESIS OF ENDODONTIC INFECTION:

The microorganisms in the root canal has the ability to initiate periapical inflammation through combination of species. Prevotella and Porphyromonas resist abscess resolution by increasing the accumulation of leukocytes. Since endodontic infection is polymicrobial in nature, phagocytosis, intracellular and growth factors might protect the host to certain extent.

But in mixed cultures, obligate anaerobes can interfere with this defense mechanism of facultative anaerobic. In primary infection of root canal the predominating bacteria that are commonly present are Firmicutes, Actinobacteria, Synergistes, Spirochaetes, Fusobacteria, proteobacteria, TM 7, SR1, Bacteroidetes, black pigmented bacteria such as saccharolytic species – prevotella and saccharolytic species – porphyromonas, spirochetes, gram positive anaerobic rods and gram positive cocci and campylobacter species.⁵

Secondary infections are produced by microorganisms resistant to chemical – mechanical procedures or as a result of bacterial invasion from microleakage of coronal restorations.⁶ This usually happens due to introduction of microbes into the root canal system during endodontic therapy especially in cases where the tooth is left open for one reason or the other, leakage from temporary fillings during inter appointment periods, coronal leakage from defective permanent restoration etc. ⁵

The major bacteria that are responsible for secondary infections are Gram negative anaerobic rods namely fusobacterium nucleatum, prevotella species, campylobacter rectus and other gram positive bacteria are Streptococcus gordonii, Streptococcus mitis, Streptococcus anginosus, Streptococcus oralis, Lactobacillus paracasei, Lactobacillus acidophilus, Staphylococci, E. faecalis, Olsenella uli, Parvimonas micra, Pseudoramibacter alactolyticus Propionibacterium spp, Actinomyces spp. Etc Other than bacteria there are many other microbes that will play an important role in root canal infections such as Archaea, viruses, and fungi. Archaea are a group of prokaryotes which are different from bacteria and are characterized by a special feature of being able to survive in extreme environments and hence they are referred to as Extremophiles.

Viruses that are associated with root canal system are Human immunodeficiency virus and Herpes viruses. Among Herpes viruses human cytomegalovirus and Epstein Barr virus have pathogenesis in apical periodontitis.⁷ According to Grossman, 17% of infected root canals may contain candida species, candida albicans are most commonly involved.⁸

CONCLUSION:

Successful endodontic treatment is mainly dependent on the thorough disinfection which renders microbial free root canal prior to obturation. For this a thorough knowledge and understanding of microbes that are responsible for endodontic infection is a must.

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CARIES EXCAVATION EMYSTIFIED – A NARRATIVE

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ABSTRACT

The techniques used in carious dentin removal have been developed by GV Black which includes the operative treatment of carious lesions. He proposed the removal of sound tooth structure and anatomical form at sites that might otherwise encourage plaque retention.

From this, it has evolved to the concept of minimally invasive dentistry. Various caries removal strategies from complete removal to partial removal to no removal have been advised. There are numerous caries excavation methods that claim to remove only the infected carious part of the tooth structure. Another milestone is the shift from surgical model to medical model in caries management. This article tries to demystify the entire concept of caries excavation.

Introduction

From 'Drill and Fill' to 'Clean and Fill'

Understanding about carious process has changed drastically in the last 30 years. From GV Black's 'Extension for prevention', the concept has changed to 'Minimal invasive dentistry'. So according to Charles Wakefield, it is 'Restriction with conviction instead of 'extension for prevention'. 1

When the first operative dentistry was established, the term "caries excavation" was used as a synonym for "cavity preparation". Caries excavation procedures were considered as one of the many mandatory steps to prepare a tooth to receive the filling material. Furthermore, it has been emphasized that the carious lesion should be excavated "until a hard pulpal floor has

reached" and when the cavity has been cut to form, no carious dentin should remain.2

Hence, when non adhesive restorations were the only available option to directly restore decayed teeth, there was no distinct separation between caries removal and cavity preparation.

That means excavation of carious dentin was performed to remove necrotic, soft material in order to best accommodate the filling material. But the ability of restorations to bond to the tooth structure has downgraded the rules for cavity preparation. Still the question raised was Does carious dentin need to be removed prior to restoration placement. Clinical studies had shown that once the cavity

margins are laid in relatively sound tissue, adequate marginal sealing is guaranteed and hence further progression of the carious lesion can be arrested. Henceforth, well-sealed margins determine the long-term success of adhesive restorations, in particular with respect to arresting the caries progress.³

Next question raised was till where the caries has to be excavated. The “normal” hardness feeling of sound dentin when probed by hand instruments is considered as the endpoint of caries excavation. Further the concept of infected and affected dentin has laid down the foundation for a more rational approach for caries removal. Removal of the heavily infected dentin and preservation of the residual affected dentin were thus considered as prerequisites for effectively arresting the carious process. This has currently raised some discussion about moving towards more objective and hopefully more conservative approaches to selectively remove caries. Thus operative dentistry evolved from ‘Drill and Fill’ to ‘Clean and Fill’.

Caries management strategies

Current caries management strategies include;

Selective removal of carious tissue (to both soft and firm dentine)

1. Stepwise removal technique
2. Non selective removal to hard dentine
A comprehensive expansion of these caries removal techniques include;
 1. Atraumatic Restorative Treatment
 2. No removal (which includes fissure sealants)
 3. The Hall Technique
 4. Non -restorative cavity control
 5. Selective removal to soft dentine
 6. Selective removal to firm dentine
 7. Stepwise removal
 8. Non-selective removal.

Caries excavation techniques

There are different techniques presently available for caries removal. It produces

residual dentin substrates of different natures and thus different receptiveness for adhesion.

CATEGORY	TECHNIQUE
Mechanical, rotary	Handpieces+ burs
Mechanical,non-rotary	Hand excavators, Air-abrasion,Air-polishing, Ultrasonics, Sono- abrasion
Chemo-mechanical	Caridex™, Carisolv™, Enzymes
Photo-ablation	Lasers

1. Conventional excavation with burs

Tungsten-carbide or carbon-steel burs in low-speed counter-angle handpieces are the most efficient method to excavate carious lesions in terms of time, and hence this is most widely used caries excavation method.⁶

- Polymeric bur
In the aim to selectively remove caries, a “plastic” bur was made of a polyamide/imide (PAI) polymer, was introduced, which possess slightly lower mechanical properties than sound dentin. It works in such a way that if the bur touches sound or caries-affected dentin, it suddenly becomes dull and produces undesirable vibration, making further cutting impossible. The blade design was developed so as to remove dentin by locally depressing the carious tissue and pushing it forward along the surface until it ruptures and is carried out of the cavity⁷.



Polymeric bur

- **Ceramic bur**

The Cera Burs are all-ceramic round burs made of alumina- yttria stabilized zirconia and are available in different diameter sizes. The manufacturer claims that it has got high cutting efficiency in infected, soft dentin, and it replaces both the explorer and the excavation spoon by simultaneously providing tactile sensation, hence reducing preparation time.⁶



CeraBurs6

2. Chemo-mechanical Excavation

- **Sodium hypochlorite-based agents**

Caridex (National Patent Medical Products; New Brunswick, NJ, USA), the new caries removing system contains sodium hypochlorite solution buffered with an amino-acid containing mixture of amino butyric acid, sodium chloride and sodium hydroxide. But it has the disadvantage of need of a specific apparatus so as to deliver the solution into the cavity, has short shelf- life, needs longer treatment time, and higher treatment cost. ⁸ To overcome these Carisolv was introduced which is sodium hypochlorite, and is available in the form of a gel (Carisolv, MediTeam Dental;



Savedalem, Sweden). However, histological evaluation using toluidine blue as a staining agent still had shown a higher percentage of



teeth presenting bacteria invading the dentinal tubules. A greater number of residual bacteria was also detected at the DEJ, where direct access of the gel and the hand instruments is difficult.⁹

3. Application of Carisolv¹⁰

- Pepsin-based caries excavation

The main advantage of this new enzyme-based solution is that it can be more specific by digesting only denatured collagen than the sodium hypochlorite-based agents. Some studies have found partially demineralized intertubular collagen fibrils and some tubule occlusion upon treatment of artificially-formed dentin caries with a pepsin-based caries-excitation agent.

4. Excavation by sono-abrasion

It is based on the use of cutting tips coupled to high-frequency, sonic, air-scaler handpieces under water cooling. The handpiece oscillates in the sonic region (< 6.5 kHz), while the tips of the system perform an elliptical motion. Cariex system was launched, including two sets of cutting tips: two diamond-coated tips with different diameters for enamel preparation and two tungsten-carbide tips with different diameters used for dentin excavation. The effectiveness and efficacy of these new tungsten carbide tips in removing carious dentin have not yet been found.⁶

Sonicsys micro diamond-coated hemispherical cutting tips¹¹

5. Air-abrasion Excavation

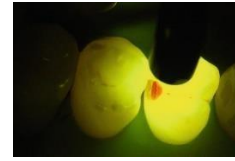
Air-abrasion systems use the kinetic energy of abrasive particles to cut tooth structure in a less invasive way, while rounding off internal and cavo surface angles so as to benefit subsequent adhesive restoration. Pure aluminum oxide particles (alumina) have been most frequently used as the abrading agent, and the advantages include their high cutting effectiveness, chemical stability, low cost, low affinity for water, and neutral color. The major disadvantage of air-abrasion excavation of carious dentin is that sound dentin is more efficiently removed than carious dentin.¹²

Air-abrasion excavation¹³



6. Fluorescence-aided Caries Excavation ("FACE")

FACE was developed as a direct method to clinically differentiate between infected and affected carious dentin. It is based on the fact that several oral microorganisms produce orange-red fluorophores as by-products of their metabolism (porphyrins), and hence infected carious tissue will fluoresce especially in the red fraction of the visible spectrum due to the presence of proto- and meso-porphyrins. In this way, continuous visual detection of orange-red fluorescence during caries excavation was found to be convenient for clinicians. The FACE method also proved to be very efficient, with less time needed to excavate caries, increased caries-removal efficacy which is not associated with an increased cavity size or risk of over excavation.¹⁴



Fluorescence-aided Caries Excavation (FACE)¹⁵

8. Laser Excavation

Several advantages have been related to the use of laser irradiation including more conservative cavity design, antibacterial activity, reduced enamel solubility and thus helps in prevention of recurrent caries.

Also, laser ablation provides more comfort to the patient due to the absence of vibration and lower pain sensation. Irrespective of the parameters used during laser irradiation, the effectiveness of carious dentin removal with erbium lasers has been doubtful. Laser-irradiated dentin has also been reported to change the composition and conformation of the organic matrix of dentin, which can impair adhesive penetration and facilitate collagen degradation.¹⁶

9. Enzymes

Goldberg and Keil in 1989 reported that they have successfully removed soft carious dentin using bacterial *Achromobacter* collagenase, and it did not affect the sound layers of dentin beneath the lesion. A study conducted by Norbo in 1996 has used the enzyme pronase, a non-specific proteolytic enzyme originating from *Streptomyces griseus*, to help remove carious dentine. More studies have to be conducted in this area to widen its implications.⁶

Changing concept from surgical model to medical model of caries management

Caries management has been shifted from surgical model to medical model. This paradigm shift aims to maintain a caries-free dentition and is anticipated to promote true oral health-related quality of life. The medical

model of caries management addresses the preventive aspect of the caries disease. Healthy dental tissues are protected against caries by fluoride application, fissure sealants and dietary alterations or improvements. It includes (1) control of bacterial infection; (2) reduction of risk levels; (3) remineralization of teeth; and (4) long-term follow-up¹⁷

Conclusion

Caries excavation techniques have evolved in past few years. All the techniques will remove carious dentine with differing levels of efficiency, but it is still unknown if these techniques will discriminate between the outer, soft, necrotic, highly infected zone which could be retained. It can be lead to overpreparation of cavities . Hence it is important to access the effects of these techniques for their efficiency and extent of removal of carious dentine.

A study conducted by Sree S et al¹⁸, to assess the knowledge of current trends in dental caries excavation among dental students in Chennai found that most of the participants are not aware of current methods in dental caries excavation in dentistry. Hence the study concludes that , the awareness and knowledge of the usage of current methods in dental caries excavation among dental students have to be improved. Furthermore, the students need to be trained on these grounds to help them treating their patients with current methods of dental caries excavation to consume time and for better treatment outcome.

Also the paradigm shift from a surgical to a medical model of caries management aims to pursue the ultimate intention of maintaining a caries-free dentition and also it anticipates to promote true oral health-related quality of life.

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Comparison Of B-Catenin Expression in Generalized Periodontitis & Healthy Gingiva: A RT-PCR Study

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ABSTRACT

Background

Periodontitis is a chronic disease of multifactorial etiology that directly and indirectly debilitates the human quality of life. Newer treatment modalities are needed to overcome the permanent damage caused to the periodontal tissues and also regeneration of tissues. Hence, in this study the quantity of β -catenin is compared in severe periodontitis and normal gingiva that maybe a potential target in regeneration.

Objectives

To compare the mRNA levels of β -catenin in Stage IV Generalized Periodontitis patients and in clinically healthy patients using their gingival tissue samples

Methods

Samples were collected from the patients after taking their consent when they were undergoing minor surgical procedures. The tissue bits were processed to obtain mRNA and were quantified and later converted to cDNA. RT-qPCR has been utilized in our study to compare the quantity of β -catenin in the study groups.

Results

The mRNA levels of β -catenin are significantly higher in stage IV Periodontitis compared to clinically normal healthy patients' gingiva.

Conclusion

β -catenin can be a potential target in treatment of Periodontitis to regenerate the lost tissues and/or to reduce the destruction of tissues. Keywords: Beta-catenin, Bone loss, periodontitis, Gingiva, RT-PCR, Wnt pathway

Introduction

Periodontitis is characterized by increased expression of inflammatory mediators leading to destruction of tooth supporting tissues namely the gingiva, cementum, periodontal ligament and alveolar bone.¹ Affecting 743 million individuals worldwide, it represents the sixth most prevalent disease.² Polymicrobial infection preventable factors such as tobacco use, diabetes, obesity, unhealthy diet is the prime etiology of Periodontitis and many genetic and life style.³ Currently, the treatment of periodontitis includes scaling, root planning and concurrent use of antibiotics to prevent its progression, however the recurrence rate is too high even after surgical treatment of few cases.⁴

Also this destructive process cannot be reversed. Persistent inflammation and immune mediators may lead to malignant transformation of the periodontal cells and give rise to oral or breast cancer.⁵ Hence, understanding and studying the underlying molecular mechanisms of the disease is crucial to identify newer potential targets to prevent or reverse the loss of tissues.⁴

One such target could be β -catenin, as higher levels of β -catenin is thought to be related to increased levels of Matrix Metalloproteinase which is one of the primary causes of extracellular matrix degradation.¹ The Wnt/ β -catenin signaling pathway regulates growth,

development and homeostasis in humans and hence, controls cell fate. Currently, Wnt signaling and its regulators are known to play important role in tissue repair and regeneration, due to its direct effect on osteoblasts and osteoclasts through the RANK/RANKL/OPG system.⁶ These cells produce Secreted related protein (sFRP), Dickkopf 1 (DKK1), and sclerostin that inhibit Wnt

signaling thereby increasing the breakdown of β -catenin in the cytoplasm and finally inhibiting the pathway.⁷ Negative feedback occurs to compensate for the increased breakdown of β -catenin. These processes should increase the β -catenin expression during the disease process and forms the basis of this study. This study quantitatively delineates the role of β -catenin signaling in the form of mRNA expression in periodontal soft tissue of healthy and stage IV Generalized periodontitis affected patients using real time-quantitative Polymerase Chain Reaction (RT-qPCR).

It is a highly specific, sensitive, rapid tool that can detect even minor quantity of increase in β -catenin. Disturbance in the Wnt signaling has been proven as an etiology in many bone and metabolic disorders.⁴ But its role in Periodontitis has not been successfully elaborated.

Objectives

1. To determine the mRNA expression of β -catenin in gingival tissue of stage IV Generalized Periodontitis using RT-qPCR.
2. To determine the mRNA expression of β -catenin in healthy gingival tissue using RT-qPCR.
3. compare the level of mRNA expression of β -catenin in the above groups using RT-qPCR

Materials and Methodology

Source of data

Samples were collected from the Department of Oral & Maxillofacial Surgery and Department of Periodontology of our college when the patients underwent minor surgical procedures under Local Anesthesia

Study design

This is an ex vivo case control comparative study with 34 samples in total. The samples were selected by purposive sampling technique and were categorized into 2 groups as follows,

Group I (Case): 17 subjects with stage IV Generalized Periodontitis

Group II (Control): 17 Subjects with clinically healthy gingival tissue

Ethical clearance was obtained from the Institutional Ethical Committee (No.GDCRI/ IEC-ACM (2)/10/2020-21) according to Helsinki Declaration (Brazil, 2013).

Inclusion criteria

- Gender: Both male and female subjects
- Age group: 30-65 years
- Subjects: Clinically and Radiographically diagnosed, untreated cases of stage IV Generalized Periodontitis (WHO 2017 criteria)
- Subjects willing to give informed consent spontaneously were included

Exclusion criteria

- Patients who have undergone long term treatment for Periodontitis
- Patients with co-morbid conditions like uncontrolled Diabetes, Hypertension
- Patients undergoing treatment for any cancer or taking anticoagulants or immunosuppressants
- Patients with a habit of smoking
- Lactating and pregnant women

Methodology

Sample collection and storage

A written consent was taken from the subjects after explaining the procedure in their language before initiating the procedure. The samples were collected during extraction of teeth (periodontal tissue that remains attached to the extracted tooth surface or the unsupported tissue present after completion of extraction) or during a Periodontal procedure (small bits of tissue that were discarded following flap surgery). Approximately 5mm of tissue were collected in 1.5ml eppendorf tubes containing RNAlater and kept in -80°C until it was processed.

RNA extraction

Samples were taken out from RNAlater and immediately transferred to 1.5ml eppendorf tubes containing TRIzol and stored in -80°C overnight. Next day the tubes were taken out from -80°C and kept over ice tray for thawing (Figure 1).

After the samples thawed completely, tissue grinder was used at 4-8 RPM at 4°C to homogenize the tissue bits into fine paste (Figure 2).

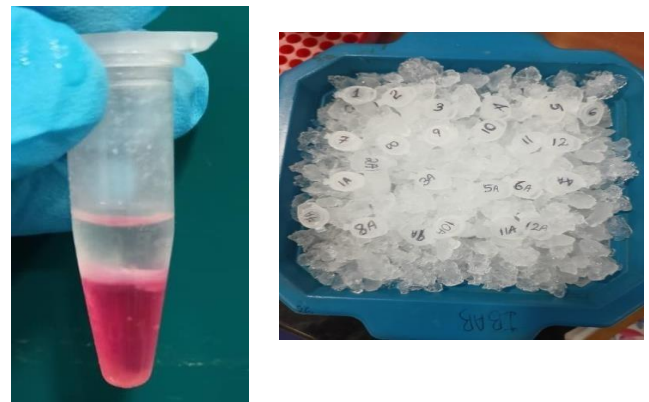


Figure 1: Samples kept for thawing



Figure 2: Homogenization of tissues in TRIzol

200µl of chloroform was added to the tubes and vortexed vigorously. The tubes were then incubated at room temperature for 2 minutes and then were centrifuged at 15,000rpm for 15 minutes at 4°C. Three different layers were formed in the tube after centrifugation (Figure 3).



Figure 3: Arrow- Superior most layer formed by RNA

The superior most aqueous layer that contained only RNA was transferred to a new centrifuge tube carefully. To the aqueous layer equal amount of isopropanol was added and mixed by gentle inversion and then 1µl glycogen was added for pellet visibility. Tubes were placed in -80°C for 1 hour and then centrifuged at 15,000rpm for 15 mins at 4°C. Pellet was formed in the bottom and excess liquid was removed from the tube. 500µl 80% ethanol was added to the pellet and centrifuged at 5000 rpm for 5 minutes at 4°C. Ethanol was removed carefully without disturbing the pellet. The pellet was air dried for 10 min and 20µl DEPC water was added. The tubes were placed on heat block at 65 degrees for 3 minutes. RNA was obtained (Figure 4).



Figure 4: Obtained RNA

RNA quantification

RNA quantification was done using Nanodrop spectrophotometer by absorbance at 260nm and the purity was checked by the 260/280 absorbance ratio. 1 microliter of DEPC water was first put on the well of nanodrop and a 0 reading was obtained for the purpose of blanking. The well was wiped with tissue paper to avoid contamination. 1 microliter from each sample was put on the well one after the other carefully and by avoiding contamination. The readings for each sample were noted (Table 1).

Table 1: A260/280 ratio to check the purity of samples

Group	No. of samples	Mean of A260/280 ratio	Standard Deviation	Ttest (p value)
Case	17	1.89	0.219	0.13
Control	17	1.93	0.241	9

cDNA synthesis from RNA

After quantifying RNA, the volume of RNA from each sample to be taken for cDNA synthesis was calculated by standardizing it against 1000ng. cDNA synthesis was done following the kit protocol (TaKaRa PrimeScript™ RT Reagent Kit # RR037A)

Standardization of the primers

Standardization of the primers was done in NCBI prime blast and sequences were obtained according to RT-PCR requirements and commercially obtained from JUNIPER Life Sciences in a lyophilized form. Glyceraldehyde- 3-Phosphate Dehydrogenase (GAPDH) was used as a house keeping gene for normalization.

CTNNB1 (β -CATENIN)

Forward TCTGAGGACAAGCCACAAGATTACA

Reverse TGGGCACCAATATCAAGTCCAA

GAPDH

Forward

GTCTCCTCTGACTTCAACAGCG

Reverse

ACCACCCTGTTGCTGTAGCCAA

Preparation of RT-qPCR master (10 μ l) mix	
2x Sybr Green (EnTurbo™)	5 μ l
Primer μ l	0.8
cDNA	1 μ l
DEPC water	3 μ l
50x Rox	0.2 μ l

Setting up RT- qPCR (Applied Biosystems StepOnePlus Real Time PCR system)

10 μ l of master mix was added to each well from each sample in triplicates separately for GAPDH and β -catenin in 96 well plates (Figure 5). A standard run was setup and ct values were obtained

Figure 5: Loading 96 well plate In RTqPCR machine



Results

The Ct values of GAPDH and β -catenin were used to calculate the fold increase of β -catenin in chronic periodontitis tissue in relation to clinically healthy gingiva. Mean fold increase of β -catenin was 10.350.

Subsequently student's t test was used to calculate the statistical significance. p value

< 0.05 was considered statistically significant. The obtained p value was 0.002, which shows that the mRNA levels of β -catenin are increased in tissues of chronic periodontitis patients compared to normal individuals (Table 2).

Table 2: Statistical Correlation of mean fold increase of the two groups

	<i>FI Normal</i>	<i>FI CP</i>
Mean	1	10.35068088
Variance	0	116.9937579
Observations	17	17
Pearson Correlation	0	
Hypothesized Mean Difference	0	
df	16	
t Stat	-3.56439931	
P(T<=t) one-tail	0.001293309	
t Critical one-tail	1.745883676	
P(T<=t) two-tail	0.002586619	
t Critical two-tail	2.119905299	

Table 3: Age correlation of samples in cases and controls

Group	N	Min age	Max Age	Mean Age	Std Deviation	T test (p value)
Control	17	32	62	50.18	8.72	0.369
Case	17	39	65	51.24	7.30	

DISCUSSION

Periodontitis is an infectious disease resulting in inflammation in the supporting tissues of the teeth, progressive attachment and bone loss followed by loss of teeth. The 2017 World Workshop formed by joint association American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) on the Classification of Periodontal and Peri-Implant Diseases and Conditions developed a new classification of periodontitis designated by a multifaceted staging and grading system.⁸

According to this criteria, our cases were patients diagnosed with Stage IV Generalized Periodontitis, with features like Interdental Clinical attachment loss of >5mm, radiographic bone loss extending to middle third of root and beyond, probing depths >6mm and >30% of teeth involved. Patients with a history of smoking and co-morbid conditions were excluded to limit the associated risk factors and maintain homogeneity in the study samples. Periodontitis is a progressive disease that not only results in exfoliation of teeth and loss of facial height it also increases the risk of malnutrition, leads to poor quality of life, lowers self-confidence and adversely affects social and general health.⁹

Though the existing treatment modalities like scaling, root planning, guided tissue regeneration and periodontal bone grafting in few cases and adjuvant use of antibiotics slows down and prevents further loss of periodontal tissues they do not reverse the disease process.⁴ A better treatment option would be to find newer treatment modalities that will replace the lost bone and periodontal tissues and reduce the disease burden, improving social and economic well-being of the patient. Wnt signaling pathway is central and crucial to all cells of the human body and maintain homeostasis, normal growth and development of the organs and normal embryonic development. Any disturbance in this pathway will give rise to an ever increasing list of pathologies ranging from periodontitis, odontogenic cysts and tumors to a variety of deadly cancers.² Two types of Wnt pathways have been identified in humans; the canonical (β -catenin dependent) and non-canonical (β -catenin independent) pathways.¹⁰ Here we talk about the canonical wnt pathway which is activated when extracellular Wnt proteins interact with the Frizzled (Fz) and lipoprotein receptor-related proteins (LRP) 5/6 (transmembrane co-receptor complex). This

interaction leads to activation of the destruction complex, and thereby release of β -catenin which enters the nucleus and transcribes Wnt- target genes. In the absence of the interaction with Wnt, the destruction complex phosphorylates β - catenin leading to its degradation in the cytoplasm.^{4,10}

β -catenin a component of the Wnt signaling pathway is thought to be directly and indirectly involved in maintenance of the periodontal health by acting in the following ways⁴:

1. Human genetic studies show that a mutation in LRP5, a trans membrane protein of the Wnt signaling pathway, leads to osteoporosis and that may affect the alveolar process as well thereby increasing the breakdown of β -catenin.¹¹

2. Sclerostin and DKK1 are Wnt signaling inhibitors, whose expressions were increased in rats with experimental periodontitis.^{12.}

3. Receptor Activator of Nuclear Factor- κ B Ligand (RANKL)/ Receptor Activator of Nuclear Factor- κ B (RANK)/osteoprotegerin(OPG) system regulates osteoblasts and osteoclast function thereby regulating bone remodeling. Intriguingly, this mechanism is controlled by the Wnt signaling.^{13.}

4. Gene levels of cementum protein1 (CEMP1) and cementum attached protein (CAP), involved in cementogenic differentiation are controlled by the activation of the Wnt signaling.¹⁴

5. Proteins involved in the Wnt signaling are potent factors regulating self-renewal and homeostasis of periodontal stem cells.¹⁵

Hence, normal activation of Wnt signaling is required to maintain homeostasis of the periodontal tissues. Inflammation caused by microbes and associated factors involved in periodontitis deregulate the pathway and⁽ⁱ⁾

increase the breakdown of β - catenin in the cytoplasm through several mechanisms.⁴ Studies have shown that sclerostin, Secreted related protein (sFRP) and Dickkopf 1 (DKK1) are Wnt signaling inhibitors which associate with bone degradation and are increased in Periodontitis. They are considered as ideal targets for treatment of bone diseases and regenerative treatment.¹⁶ IHC studies on gingiva of Periodontitis patients have concluded that Wnt/ β -catenin signaling is essential and should be active in diseases like chronic and aggressive periodontitis, because it helps in bone makeover.² Studies on liposomal Wnt3A, increased the rate of healing in post extraction socket and osteotomy in ovariectomized mice.¹⁷ The standardization of our study was maintained by checking the purity of the obtained RNA using Nanodrop spectrophotometer by checking absorbance ratio (A260/280) and the values obtained were well within the optimal range of 1.7 to 2.18 Obtained p value between the group was > 0.05 which showed all the samples were uniformly pure. Age correlation was done to check if there's any statistical difference within the groups and the p value obtained was greater than 0.05 which shows no difference between exists in the study subjects. In our study we have for the first time used RT-qPCR to quantitatively analyze the difference between mRNA levels of β - catenin in clinically healthy gingiva and gingiva of severe periodontitis patients. Our study showed significantly higher mRNA levels of β -catenin in stage IV generalized periodontitis compared to the control group ($p < 0.001$). This significant fold increase of mRNA levels of β -catenin in the case group could be due to one or both of the following reasons:

Increased cytoplasmic breakdown of β -catenin due to loss of wnt signals in the extracellular space when the wnt inhibitors are activated.

There might be a negative feedback

mechanism that will increase production of β -catenin in the cells. In future human studies can be conducted with confidence by activating the Wnt pathway or by blocking the inhibitors of the pathway in treatment of periodontitis. Drugs targeting the same can be developed to reverse or prevent bone loss and supporting tissues.¹⁹

Conclusion

This study implies that reducing the breakdown of β -catenin and thereby also reducing negative feedback increase of β -catenin can be key factors in reducing tissue destruction in periodontitis. In other words, stimulating Wnt pathway may help in regeneration of periodontal tissues and reverse the disease.

Acknowledgment: None to be made

Conflict of interests: No
Conflict of interests

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Delayed replantation of avulsed permanent tooth with 3 days extraoral dry time and 18 months follow up – A case report.

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Introduction:

With the carefree, adventurous zeal of childhood, the lack of judgement and the absence of overthinking of each move, there arises a downside which includes an increased incidence of accidents. Sudden impact involving the face may result in the exarticulation/avulsion of the tooth and may force the tooth completely out of the socket. Avulsion is defined as the total displacement of the tooth out of the socket.¹ Avulsion injury occurs most commonly in the permanent dentition of 7-14 year-old children,² when there is loosely structured periodontal ligament surrounding erupting teeth with a resilient alveolar bone that provides only minimum resistance to extrusive forces.³ It has a reported incidence of 0.5-16% of all dental injuries and the maxillary central incisor being the most commonly avulsed tooth.⁴ Avulsion, most frequently involves a single tooth but multiple avulsions are occasionally encountered and it is more common in children with an increased overjet

and proclined anteriors.² Injury to lip and fracture of alveolar socket are also frequently associated findings.

Treatment of avulsion injury is directed towards minimizing the effects of periodontal ligament attachment damage and pulpal infection.³

The present case highlights an 18-month follow-up of delayed replantation (after 72 h) in an avulsed maxillary right central incisor.

Case Report:

A 12-year-old girl reported to our department for treatment of traumatically avulsed upper front tooth after she slipped and fell at home before 72 hrs. The patient's medical history was non-contributory. She was not under any medication and had no drug allergies. The extra oral examination conducted showed abrasions on the lower lip. Intraoral examination revealed avulsed right

permanent maxillary central incisor, Ellis class II fracture in left permanent maxillary central incisor and Ellis class I fracture in left and right permanent maxillary lateral incisor (Fig 1a). The avulsed tooth was stored dry, wrapped in a piece of paper. Upon inspection, it was noted that the tooth had closed apex and there was a crown fracture involving enamel and dentin (Fig 1b). A well-preserved socket wall, with no particle remnants of the avulsed tooth in the socket and no associated fracture of the alveolar bone was confirmed radiographically (Fig 1c).

Since the patient was a young girl, replantation of the avulsed tooth was planned to relieve her from psychological trauma. The treatment plan formulated was explained to the patient's mother with thorough emphasis on possible effects of delayed replantation. A special informed consent was signed. Local anesthesia (2% lignocaine with 1 : 1,00,000 adrenaline) (Indoco Remedies Ltd., Mumbai, India) was administered. The contaminated coagulum was gently curetted (Fig 2a) and rinsed from the socket with a sterile isotonic saline solution (Aculife Healthcare Pvt Ltd, Gujarat, India). Since, this was a case of delayed replantation, extra oral endodontic treatment of the tooth was performed. The necrotic PDL was delicately removed with a gauze sponge dampened with sterile saline solution. The complete extirpation of necrotic pulp was performed. The tooth was cleaned and shaped, followed by root canal filling with gutta-percha (Dentsply India Pvt Ltd., Haryana) by lateral condensation technique. The access cavity and apex of the tooth was sealed with glass ionomer cement (GC Corporation, Tokyo, Japan). The tooth was then placed in doxycycline solution for 20 minutes (Fig 2b) followed by placement in APF gel.

(Pascal international, Inc., USA) for 5 minutes (Fig 2c). The tooth was replanted slowly u n

d e r digital pressure. The alignment and occlusion of the tooth was checked and then splinted to the adjacent teeth with orthodontic wire and composite (3M ESPE Filtek Z250 XT) extending from right permanent canine to left permanent canine (Fig 2d). Intra oral periapical radiograph was made at this point to confirm the position of the replanted tooth (Fig 3a). The patient was prescribed systemic antibiotics for 5 days with analgesics on demand basis. The patient was kept on soft diet for 2 weeks; proper oral hygiene instructions (prescribed chlorhexidine 0.12% mouth rinse twice a day for 1 week and a soft toothbrush to brush her teeth after each meal) were given and a follow up visit was scheduled after 2 weeks.

At 2 weeks recall the splint was found to be intact. Splint was removed and radiograph was taken. Although no radiographic evidence of resorption was apparent (Fig 3b), there was grade I mobility of the replanted tooth clinically. At the third appointment (after another 2

weeks) the tooth was asymptomatic and showed normal mobility. Radiographic examination revealed normal root and intact periodontal ligament of the replanted tooth (Fig 3c). However, the adjacent left permanent maxillary central incisor showed periapical radiolucency, hence root canal treatment was initiated for that tooth. Calcium hydroxide powder (Merck, Darmstadt, Germany) was mixed with sterile saline in a 3:1 ratio to produce a thick homogenous paste. The mixture was placed into pulp chamber using plastic carrier and packed into root canal with file and paper points (Mani Inc., Japan). Finally, the access cavity was sealed with Cavit (3M ESPE, Seefeld, Germany). The patient was recalled after 2 weeks and obturation was carried out (Fig 3d).

During the next visit a direct composite restorative technique was planned to restore the fractured anterior teeth. The unsupported enamel was removed with the aid of a 45-degree bevel (Fig 4a).

Preliminary impressions of both the arches were made using alginate (Algitex; DPI Mumbai, India), study models were made in dental stone and mock preparation of the fractured maxillary anterior teeth were done with modeling wax (Fig 4b) and checked for occlusal interference. Further, the cast was duplicated by using putty impression material (Aquasil soft putty/regular set; Dentsply, India). The labial surface of the putty template was removed up to middle third of the crown, to assist in the restoration of the lost tooth structure. A clinical try-in of the template was done to ensure adequate fit (Fig 4c). After appropriate shade selection of the composite material, this putty template was used to reconstruct the fractured teeth (Fig 4d).

The patient was recalled after 3 months. However, the patient was unable to keep appointments due to change of location and presented herself after 18 months. During this period, the patient remained asymptomatic. The replanted tooth showed no mobility or signs of resorption (Fig 5a, 5b).

She was then recalled after 6 months, yet again the patient did not keep their appointment, as she was transferred to boarding school for her high school education. The patient and parent were explained fully of the prognosis.

Discussion:

Avulsion is not a very common, but a very serious form of dental injury. It is preferable to immediately and atraumatically replant the avulsed tooth into the socket at the accident site.³ But most of the cases that we come across; we see that this is not the scenario. In fact, sometimes parents come 3-4 days after

the injury may be with/ without tooth. There is definitely a lacking knowledge regarding this. The prognosis of an avulsed tooth is governed by the extraoral time, which in turn is related to the status of the PDL cells.⁵

From a clinical perspective, since avulsions occur infrequently, the average dental practitioner will not instinctively know how best to treat each (rare) case that he/she encounters and differentiate the protocols to be followed under special situations.

Maintaining the tooth and surrounding bone for a few years can be considered a successful treatment in the growing patient till final consolidative treatment in form of implants or fixed partial denture can be initiated. Therefore, an avulsed tooth that is maintained until growth is completed should be considered a successful outcome because tooth loss before this time often includes loss of the alveolar bone as well as further resorption of the bone in the site.

Keeping the above said in mind, that the decision to replant avulsed tooth is always the right decision even if extraoral dry time is more than 60mins; in the present case replantation was planned. The patient's mother had brought the tooth wrapped in a paper to the dental hospital. after 3 days. The tooth was not stored in any physiologic media. Hence, the condition of PDL cells was considered nonviable. The treatment regimen adopted here was the same, using the decision tree as published by Lee et al,¹⁷ in 2001, which was followed by an updated decision tree by McIntyre et al,³ in 2007, to provide clinicians with user-friendly flow-charts that simplify the best possible outcomes for managing these teeth in children and adolescents as reference base, with few modifications.

Before replantation of the avulsed 11, the root surface was cleared off all debris and extraoral

root canal treatment were carried out. Placement of the tooth in topical doxycycline has shown to reduce the frequencies of both ankylosis/osseous replacement and Inflammatory root resorption. Soaking of the avulsed tooth in fluoride for 5-20 minutes is known to delay the process of ankylosis.³ The treatment was completed after following proper protocols, anterior esthetic composite build up was done and the patient was instructed for regular follow ups. The subsequent follow-up visits confirmed the asymptomatic and stabilized nature of the tooth. There was no sign of mobility or pain.

Delayed replacement of a tooth that has been avulsed may determine rapid root resorption or, more frequently, dental ankylosis with subsequent bone substitution.⁶ The mechanism can be explained as, slower moving cementoblasts cannot cover the entire root surface in time, hence in certain areas, bone will attach directly onto the root surface. In time, the entire root will be replaced by bone; a process which has been termed replacement resorption.

If replantation and ankylosis become evident before the end of jaw growth, the replaced tooth will appear infraoccluded.¹ The clinical protocol dictates that replanted teeth be monitored clinically and radiographically at 2 weeks (when the splint is removed), 4 weeks, 3 months, 6 months, one year, and yearly thereafter for at least five years. But in this case the patient failed to keep up for follow up appointments, hence long term follow up could not be monitored.

Conclusion:

Since the key to success lies in the status of periodontal ligament, utmost care was taken to preserve the integrity of the periodontal ligament. In the case presented, radiographic and clinical findings after 18 months revealed absence of root resorption, ankylosis or

mobility of the replanted central incisor. Although root resorption and ankylosis were not observed after 18 months, these events might occur in the long term. Therefore, longer periods of clinical and radiographic follow-up are required. These factors are highly dependent on the compliance of the patients in question which point to the possible reason for dearth in literature.

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Figures :

Figure 1a: Preoperative intraoral photograph of the patient showing missing 11 and fractured 21, 22, 12.

Figure 1b: Avulsed maxillary right central incisor associated with fracture of enamel and dentin.

Figure 1c: IOPA with respect to the exarticulated ¹¹



Figure 2a: Socket preparation

Figure 2b: Placement in doxycycline solution

Figure 2c: Placing the tooth in APF gel.

Figure 2d: Tooth replanted and splinting done.

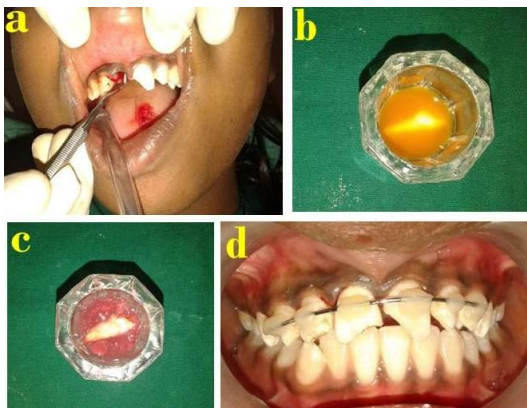


Figure 3a: IOPA to confirm the position of replanted tooth after splinting.

Figure 3b: 2 weeks follow up radiograph.

Figure 3c: Splint removal and 4 weeks follow up radiograph.

Figure 3d: IOPA showing obturation of 21.



Figure 4: Composite build up by template technique.

Figure 4a: Preoperative photograph

Figure 4b: Mock preparation of the teeth on study model

Figure 4c: Trial fit of putty template Figure 4d: Postoperative photograph

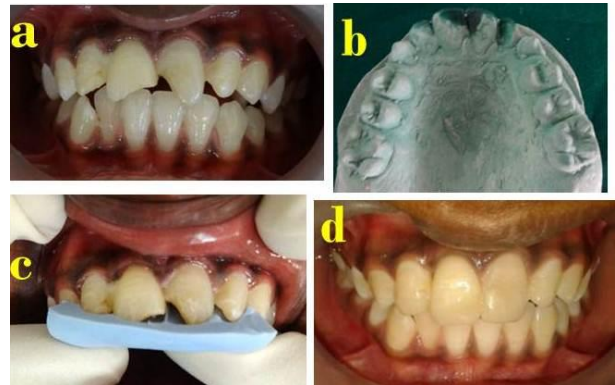


Figure 5a, 5b: 18 month follow up photograph and radiograph.



Association of upper airway dimension and sleep disordered breathing in subjects with TMD - a pilot study

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ABSTRACT

Background -Sleep-disordered breathing (SDB) is a condition that refers to partial or complete cessation of breathing that occurs during sleep. Among the various contributing factors the anatomic constraints have a major contribution. Also there has been increasing evidence of association of this and temporomandibular disorders. The present study is a prospective observational study to correlate the above said disorders.

Methods- Subjects reporting with symptoms of TMD were examined and diagnosed according to DC TMD criteria. Ten patients diagnosed with TMD were subjected to radiographic evaluation of TMJ with the use of cone beam computed tomography of TMJ of both sides. Following which subjects were subjected to Cephalometric radiography for airway analysis. In addition all subjects were assessed for sleep disordered breathing with the use of Epworth sleepiness scale.

Results- there was negative correlation observed with pharyngeal space when compared with sleepiness scores amongst the various parameters assessed.

Conclusions- there is a definite correlation of reduced airway space and occurrence of TMD which needs to be quantified in a larger sample of subjects, which will help to comprehensively manage the subjects with TMD.

Introduction

Temporomandibular disorders comprise a group of disorders that affect the TMJ and cause musculoskeletal pain.¹This painful condition influences the quality of life as well. Experimental studies have established that sleep disruption and pain occur in a bidirectional relation.²It is advised that other than polysomnography studies, best way to screen obstructive sleep apnea or sleep

disordered breathing is questionnaires. The various factors causative of TMD include age, genetic factors, gender predisposition, stress, anxiety, occlusion, poor posture, rheumatoid arthritis, and dysfunctional breathing.³ Cephalometric films can provide details on Structural narrowing depicting the anterior- posterior dimension of the airway, this will aid in the early recognition of obstructions leading to sleep apnea.

Sleep apnea (SA) happens to be the most common type of sleep-disordered breathing, which presents with repetitive shallow breathing and cessation of breaths during sleep.^{4,5} Polysomnography (PSG) stands out as the confirmatory investigation for establishing the diagnosis of SA .⁶ SA is known to be related to a plethora of diseases including diabetes mellitus, hypertension, arrhythmia, ischemic heart disease, stroke, metabolic syndrome, chronic kidney disease, erectile dysfunction, hormonal dysfunction, and dementia.³ Various studies in the past have shown the association between sleep disorders and TMD while only few of them discussed the association between the two.^{3,5} Sleep-disordered breathing (SDB) has been rising among various populations and is a matter of concern. It can be associated with Comorbidities like hypertension, myocardial infarction, stroke, memory loss, diabetes, depression, insomnia, and daytime drowsiness.⁷ The present study aims to correlate the degree of radiographic changes of temporomandibular disorders with sleep disordered breathing assessed by Epworth's Sleepiness scale⁸ and lateral cephalometric analysis of pharyngeal space.

The objectives of this, study is to explore and associate the pharyngeal airway dimensions and the position of the TMJ in subjects with

sleep disordered breathing (SDB- assessed by Epworth's Sleepiness scale).

The specific objectives include (1) assessment of upper airway dimensions in SDB on a cephalogram, (2) determine the TMJ condylar positions in SDB (3) investigate the association between upper airway dimensions and TMJ condyle position.

Materials and Methods

Study Design and participants - The design of the present study is a cross- sectional prospective one where in patients were recruited from outpatient section of department of oral diagnosis in a dental school in a span of 10 months.

The study was initiated following the ethical clearance from Institutional review board of XXX Institute with reference number IRB No. 2020/UG/OM/62 issued on 03.02.2020. Informed consent was sought from all participants which was in accordance with human ethics approval from Institutional review board. 10 patients presenting with symptoms of TMD were selected for the study. **Study setting-** The study was conducted over a span of 10 months from 1-01-2020 to 31-10-2020. The subjects reporting to the department of Oral medicine & Radiology, fulfilling the inclusion criteria were further evaluated for sleep disordered breathing with Epworth sleepiness scale questionnaire, cephalometric assessment of airway, and CBCT assessment of bilateral Temporomandibular joints.

Data collection

The following data were collected for all subjects: demographic data (age, gender, ethnicity), status of any deleterious habits, physical factors, and features relating to sleep

disturbances and temporomandibular joint disorders.

All patients were assessed with Sleep Disordered breathing questionnaire (SDB).⁸ All the subjects will be assessed for sleep disordered breathing with the use of Epworth's Sleepiness scale.

Participants-

INCLUSION/Eligibility CRITERIA-Patients presenting with signs and symptoms of TMD diagnosed with DC TMD Criteria⁴ and willing to participate in the study.

Exclusion Criteria -

1. Patients unwilling for investigations.
2. Pregnant women

Sample selection - Sample of ten participants was selected based on the feasibility of the time duration of the study

Data sources/ measurement- Radiographic analysis with CBCT - CBCT images of both the TMJs were procured for analysis. Images were obtained from Kodak 9000C. The technical parameters used were: 90 kV, 8 mA, 10.8 seconds, scan time < 12 s, and field of view (FOV) of 4 × 6 cm. The maximum dose of exposure was 231 mg/cm². The imaging was performed in an upright position to facilitate, a natural head position, and ensuring teeth were in light contact in a closed mouth position. The obtained images were assessed for linear measurements and osseous changes using built in software within the machine: CS Imaging software (CS imaging patient browser 7.0.18.5.d11, CS data manager 4.2.9.0).

The linear measurements included:

1. condylar height
2. condylar length
3. condylar width.

Osseous changes included: Flattening, erosion and osteophytic changes. Medial axial and lateral axial space measurements were also included. (Fig 1)

In addition all subjects were subjected to Cephalometric radiograph for airway analysis. Lateral cephalometric radiographs were taken using a standardized technique (jaws in centric relation, teeth occluding, lips relaxed and the head in natural head position).

1. The following variables were measured which included 12 linear measurements: (Fig 2) Tongue in sagittal dimension length Height of the tongue
2. The soft palate length
3. Thickness of the soft palate
4. Position of the vallecula in horizontal plane
5. Position of the vallecula in vertical plane
6. Depth of the hypopharyngeal airway space
7. Position of hyoid bone in horizontal plane
8. Position of the hyoid bone in vertical plane

The four area measurements included

1. tongue area
2. Soft palate area
3. Oral area
4. Nasopharyngeal area

area measurements were calculated on jpeg images of Cephalograms using GIMP software.

Study size - Sample size was not estimated at the beginning of the study. A sample of 10 was taken based on feasibility.

Statistical Analysis: All the cephalometric linear measurements were tabulated and descriptive statistics like mean and standard deviation were calculated. Correlation

between pharyngeal space and sleepiness score was analyzed by

Pearson's correlation coefficient. Probability value of less than 0.05 was considered as statistically significant. Software used SPSS (Statistical Package for Social sciences version 20.0, IBM Corp., Armonk, NY:IBM Corp)

Results

The values of cephalometric measurement data is depicted in Table 1. The various parameters regarding TMJ is depicted in Fig-3. Amongst the various areas assessed there

Table 1

Linear measurements	Mean	Standard Deviation
Tongue in sagittal dimension vt	75.3	10.68
Height of the tongue	36.61	2.11
Soft palate length	35.82	3.59
Thickness of the soft palate	10.86	0.55
Position of the vallecula in horizontal Plane	17.02	4.75
Position of the vallecula in vertical plane	78.82	8.55
Depth of the hypopharyngeal airway space	16.59	5.35
Position of the hyoid bone in horizontal Plane	32.22	5.61
Position of the hyoid bone in vertical plane	83.17	8.42
Area measurements		
Oral area	24.61	8.99
Soft palate area	2.26	0.62
Tongue space	6.64	2.38
Pharyngeal space	3.50	2.23

was negative correlation observed with pharyngeal space when compared with sleepiness scores. The correlation coefficient was -0.1905 (p-value 0.59) which shows a non significant negative correlation indicating that pharyngeal space may be inversely correlated to sleepiness (Fig-4). The comparison of various areas assessed and the sleepiness score has been depicted in Fig-5.

Discussion

There has been increased evidence based correlation of inter relation and co-existence of TMD & sleep disordered breathing.²

Dysfunctional TMJ may comprise of many problems, comprising teeth grinding, headaches, ear pain and sleep disturbances. A disordered temporomandibular joint may be attributed for sleep apnea owing to position of tongue influenced by the alignment of the upper and lower teeth; this malpositioned can block the airway leading to disturbed sleep cycle.³ Yeon-Hee Lee¹⁰ et al in a detailed review suggest that OSA can have typical presentations in orofacial region and that OSA in patients with Sleep Related Breathing Disorders may co-occur with TMD through several mechanisms. Radiographic screening of the upper airway will substantiate data delineating the anatomy and degree of constrictions at different levels along the airway track.^{5,9} To the Authors' knowledge this is a first of its kind study attempting to evaluate the relationship of TMD and sleep disordered breathing in local population. Truong L et al⁵ in their study emphasized that there was an association between airway volume and minimum crosssectional dimensions (area) implying that the constricted airway's cross-section

significantly determining upper airway dimensions and also with regards to the TMJ, condyles were more posteriorly seated condyle positioned, in SDB patients.

Although the study had a minimal sample size, there was a negative correlation observed with pharyngeal space when compared with sleepiness scores which implies the coexistence of these diseases. The significance of the present study lies in the fact that sleep disturbance may enhance sensitivity to pain in subjects with chronic pain conditions. This in turn triggers a self-perpetuating cycle of disturbed sleep, heightened pain, and depression.

Amongst the various mechanisms linking these two disorders, inadequate and/or altered sleep might heighten pain sensitivity, leading to hyperalgesia, a key feature in majority TMD patients.¹¹

The shortcomings of the present study were non inclusion of BMI and the position of condyle needs to be established on a larger sample size.

It's also important to educate the patients regarding the early recognition of symptoms of SDB to prevent complications. The study supports the fact that simple radiographs such as cephalograms can support the identification of SDB with certain assessments. This will not only aid in quantifying and correlating the severity of the disease, but also aids in educating the patient and making them aware of the existing problems. The authors would suggest long-term assessments in larger set of populations, with correlation clinical/ orofacial features along with relevant radiographic manifestations, the data of which will add clarity to the coexistence of these two disorders.

In view of the results from the study the authors would suggest that – It is imperative to reassess the airway parameters in patients reporting with TMD symptoms, so oral physicians or dentists should investigate for the comprehensive picture in patients presenting with TMDs.

- TMDs can crop up or get initiated during certain treatments such as orthodontic treatments or in situations when occlusion is not restored appropriately, hence airway assessments should be a part of structured assessment requisites in addressing TMDs.
- When complete assessment of sleep disordered breathing or sleep apnea is not feasible, use of questionnaire to evaluate the patient's status initiates the process for further investigations.

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Figures Legends

Fig1- Linear measurements recorded on lateral cephalogram



Fig 2- Dimensions of the TMJ as per DVT measurements



Fig 3 -Comparison of area measurements of various spaces with sleepiness score.

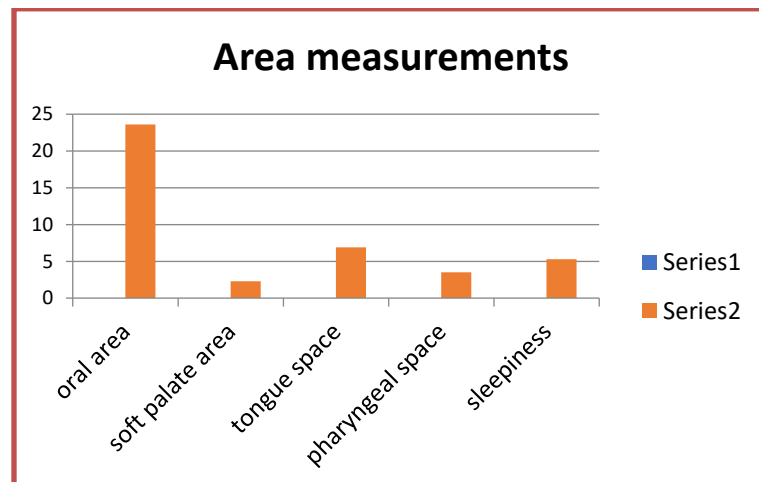


Fig 4- The dimensions of TMJ on CBCT

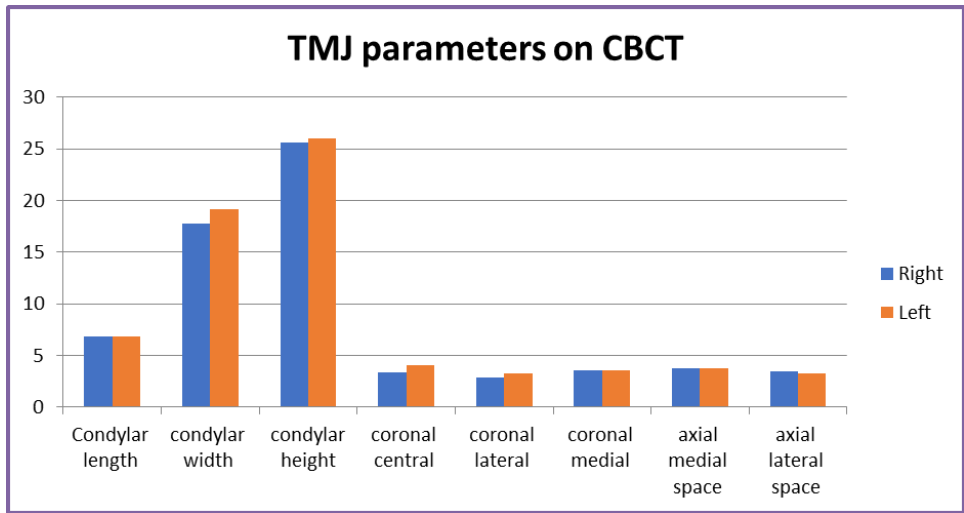


Fig 5 Correlation of pharyngeal space with sleepiness score.

