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PRESIDENT'S MESSAGE



Dr. Vidya Hari Iyer
President
IDA Madras Branch

It is with immense pleasure I would like to congratulate my team of young dentists of IDA - Madras Branch Editorial board and its Editor-in-chief – Dr. C.K. Dilip Kumar for bringing out this edition of the coveted journal.

I would also like to thank all the authors for sharing and bringing out their scientific research and clinical acumen for the benefit of the general practitioners and budding students. I take this opportunity to also encourage my fellow dentists to share their interesting cases from their clinic in form of case reports.

We at IDA - Madras branch would like to encourage and motivate all the members to actively take part in our e-journal and exhibit their talents and accomplishments. This journal would help each and every one to showcase our talents and grow scientifically which indirectly would increase our productivity.

Last but not the least, I would like to thank all my Executive Committee members who have stood as one at all times and worked as a team throughout 2015. I feel so proud and privileged to be an active member and part of this winning team.

Happy reading.

A handwritten signature in blue ink, appearing to read 'Vidya Hari Iyer'.

Dr. Vidya Hari Iyer

SECRETARY'S MESSAGE



Dr. H. Thamizhchelvan
Hon. Branch Secretary
IDA - Madras Branch

Greetings to all,

Love provided by mother will never change throughout her lifetime, In accordance updating our knowledge should continue throughout our career.

IDA Madras takes pride in saying that we constantly conduct many education programmes and to reach on your desk this e Midas journal fulfils thirst of knowledge.

on behalf of IDA - Madras Branch let me congratulate the enthusiastic editorial team on release of this third issue.

A handwritten signature in blue ink, appearing to read 'H. Thamizhchelvan'.

Dr. H. Thamizhchelvan

LETTER FROM THE EDITOR



Dr. C.K. Dilip Kumar
Editor-in-Chief
IDA - Madras Branch

The progress of a journal from its infancy into maturity depends on various factors. The initial hurdles are launching the journal and finding the right resources to run it. Once the sustenance is established the aim is to make it self-sufficient. The onus shifts to generating its own funds. For this, the primary goal is visibility. The journal needs to be seen, read, downloaded and used for referencing. Once this visibility comes in, the journal is not only respected but also patronized by people far and wide.

The time has come when we need to focus on making our journal more visible through quality work that is submitted, reviewed and published. It's a sincere request from the Editors desk that our contributors submit more original work that would enable us to increase the standards of our indexation.

I believe that this is not an impossible task; it just needs realization by each one of us to take a more serious step in improving the quality of submissions. This combined effort from our part would certainly bear fruit in a period of time in the form of a native journal with not only increased visibility but also popularity. Once again thanks to all our contributors and readers for their patronage.

Dr. C.K. Dilip Kumar

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Cancer Stem Cells - Recalcitrant Cells

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ABSTRACT

The initiation, growth, recurrence and metastasis of cancer have been recently related to the presence of cancer stem cells (CSCs) within the tumor. CSCs are the distinct sub-population of tumor cells that have the ability to undergo self-renewal and differentiation. They also possess the capacity to promote tumorigenesis and recurrence after treatment. Various hypotheses have been proposed regarding their origin. The aim of this review is to discuss the insights of cancer stem cells and to provide a brief review on their features, origin, methods of their identification, association with signaling pathways, properties which rendering them chemo resistance and radio resistance and the development of new therapies that are targeting these cancer stem cells.

Introduction

Cancer is a large group of diseases characterized by uncontrolled growth and spread of abnormal cells¹. The progression of cancer is related to the presence of distinct population of cells known as cancer stem cells. American association of cancer research work shop defined cancer stem cells as a cell within a tumor that possesses the capacity to self renew and to cause the heterogenous linages of cancer cells that comprise a tumor.² Cancer stem cell theory of tumorigenesis was first described in 1994 in acute myeloid leukemia.¹ Accumulated mutations in normal stem cells and their progenitors result in the manifestations of cancer stem cell activity. Several environmental factors and carcinogens cause aberrant mutations which may cause reprogramming of epigenetic mechanism and results in the generation of cancer stem cells.³ Cancer stem cells are thought to be the source for the tumor survival and the regrowth. E.Allegra in 2012 described the characteristics of CSCs as those which possess anchorage independent growth, metastasting capacity, ability to sustain a long life, self renewal and resistant to damaging agents.⁴ Further characterization of cancer stem cells is needed in order to demolish them, which might donate significantly to the therapeutic management of cancers.

Cancer Stem Cell Properties

Stem cells are those cells within organs with the ability to self-renew and give rise to all types of cells within the organ to drive organogenesis. Cancer stem cells are those cells within tumors with the ability to self-renew and give rise to the phenotypically diverse tumor cell population to drive tumorigenesis. Recurrence and metastases of tumor and their cellular heterogeneity might be outcome of cancer stem cell differentiation and asymmetric division of cancer stem cells.

The properties of the cancer stem cell are i) They are small fraction of the cancer cells within a tumor that have tumorigenic potential when transplanted into immunodeficient mice. ii) The CSC sub-population can be separated from the other cancer cells by distinctive surface markers like CD44, ALDH1A1, CD131, CD24 etc.

iii) Tumors arising from the CSCs contain the mixed tumorigenic and non-tumorigenic cells of the original tumor. iv) The cancer stem cell sub-population can be serially transplanted through multiple generations, indicating that it is a self-renewing population.⁵

Cancer Stem Cell Hypothesis

Two separate and mutually exclusive models have been developed to explain the development of tumors. The stochastic model postulates that all cells within a tumor contribute in varying degrees to the maintenance of the tumor. The cancer stem cell model states that, they form a distinct subset of the tumor cells, which are eventually responsible for tumor initiation, progression, and recurrence. Through self-renewal and differentiation, CSCs are responsible for the production of various tumor cells and contribute to tumor heterogeneity.⁶ Cancer stem cells continue to divide asymmetrically creating initially the two different cell populations. One population retains the self-renewing properties of the parental cancer stem cell while the other population is tumor cell with ability to differentiate but they do not have the ability to initiate tumor growth.⁷ Either the stem cell acquires cancer properties or the cancer cell acquires the stemness properties is under debate.

Identification of Cancer Stem Cells

Various methods for identification of CSC sub populations by presence of cell surface and cytosolic proteins by Immunohistochemistry, Isolation and in vitro expansion of cells from tumour specimens, cell sorting by Flow cytometry,⁸ Side population Assay, ALDH Activity, Clonogenic Assay, Mesenchymal differentiating culture conditions for Sarcospheres.⁹

Cancer Stem Cells and Signalling Pathways

CSC self-renewal and differentiation is strongly controlled by multiple regulatory mechanisms, including cytokines from the cancer niche. A number of signaling pathways control cancer, which includes the Hedgehog, Notch, and Wnt/ β -catenin pathway. Wnt signaling plays an essential role in regulating regulating stem cell function. Wnt binding to Frizzled receptors (Fzd) activates disheveled (Dsh), inactivating GSK3 β , thus stabilizing β -catenin, and

thereby inducing target genes. Notch signaling pathway activation contributes to development of a number of stem cells and early progenitor cells. Hedgehog (including Indian hedgehog (Ihh), desert hedgehog (Dhh), and Sonic hedgehog (Shh)) bind the patched receptor (PTCH1), depressing its constitutive repression of smoothed (Smo), leading to activation of the Gli transcription factors.¹⁰

Why Cancer Stem Cells are Recalcitrant?

Various mechanisms such as drug inactivation, changes in cellular targets, inhibition of drug accumulation and activation has been attributed to the recalcitrant property (resistant or unresponsive to treatment) of CSCs. Drug efflux transporter proteins (ABC transporters) are generally found to be overexpressed in drug-resistant cancer cells. Antiapoptotic signaling pathways are concerned in CSC-mediated drug resistance.¹¹ Recently, Wnt and β -catenin signaling was suggested to contribute radioresistance for cancer stem cells.¹²

Strategies to Eradicate CSCs

It is obvious that a cancer treatment that fails to get rid of cancer stem cells may allow regrowth of the tumor. In cases of recurrences, where tumor bulk is removed and chemotherapy is given, a likely explanation is that the cancer stem cells have not been completely destroyed. Therapeutic strategies that specifically target cancer stem cells should eradicate tumors more effectively than current treatments and reduce the risk of relapse and metastasis. Targeting various signaling pathways Notch, Wnt, Hedgehog - Deregulation of signaling pathway networks plays an important role in enabling CSCs to retain stem cell properties. The familiar Notch, Hedgehog and Wnt signaling pathways play fundamental roles in maintaining CSC populations. 2) Targeting Reactive oxygen species (ROS) - alters intracellular environment which facilitates apoptotic death signals. 3) Disruption of supporting niche - The tumor microenvironment can create a niche to harbour and protect CSCs from drug-induced apoptosis. 4) Targeting surface antigens, ligands or antibodies against tumor surface makers have been developed to enhance the specificity of therapeutic strategies. Important interest has been generated in the development of monoclonal antibodies to target CSCs. Monoclonal antibody conjugated to the cytotoxic agent has been developed and widely used to treat AML 5) Blockade of CSC functions, reversal of CSC associated resistance mechanisms.¹³ Lamb. R has proposed that antibiotics that target mitochondria can effectively eradicate cancer stem cells, across multiple tumor types.¹⁴

Conclusion

CSCs are a novel cancer target. Significant links between CSCs, tumor progression, and therapy resistance have necessitates the need for novel therapeutic strategies that target these distinct aggressive cancer subpopulations. Evolution in identifying the CSC-specific surface markers, understanding the mechanism of CSC tumorigenic capacity will be helpful to drive the therapeutic application of targeting these CSCs. Combination therapies targeting CSC and tumor bulk populations are most likely to lead to optimized cancer treatments and to further reduce cancer morbidity and mortality.

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Oral Malodor - A Cause or Disease in Humans

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ABSTRACT

Oral malodor, also called halitosis or bad breath, is the general term used to describe any disagreeable odor in expired air, regardless of whether the odorous substances originate from oral or non-oral sources. Specific groups of bacteria have been identified with the production of oral malodor, in particular, gram- negative anaerobic bacteria. Volatile sulfur compounds (VSCs) resulting from bacterial breakdown of proteins are considered to be the main agents for malodor. This paper reviews the current knowledge, etiology, diagnosis, and possible treatment strategies for oral malodor.

Keywords: Fetor ex ore, Halimeter, stomatodysodia, Tongue coating, Breath tests, diagnosis

Introduction

Malodor is the scientific term for bad breath and has its origin from Latin ("malus," bad, evil + "odorem, odor," smell, scent) and defined as a distinctive smell that is offensively unpleasant. Halitosis is a medical term, first coined by the Listerine Company in 1921, to describe oral malodour or bad breath. Listerine was first formulated by Lister in 1879 as a surgical antiseptic and then by Dr. Joseph Lawrence and Jordan Wheat Lambert in 1879. It was given to dentists for oral care in 1895 and became the first over-the-counter mouthwash sold in the United States in 1914.¹ Halitosis frequently causes embarrassment, and may affect interpersonal social communication.²

Factors Involved in the Etiology of Halitosis

Halitosis is caused due to the presence of odorous gases in the air expelled from the oral cavity. The odorous compounds are mainly divided into;

- 1) Sulphur containing gases (VSCs).
 - a. Hydrogen sulphide
 - b. Methyl mercaptan
 - c. Methyl sulphide
 - d. Dimethyl disulphide
- 2) Non-Sulphur containing gases.
 - a. Volatile aromatic compounds
 - b. Organic acids (acetic and propionic acids)
 - c. Amines (putrescine, cadaverine)³

Microbiota associated with Oral Malodor

Putrefaction is thought to occur under anaerobic conditions, involving a range of gram-negative bacteria such as *Fusobacterium*, *Veillonella*, *T. denticola*, *P. gingivalis*, *Bacteroides* and *Peptostreptococcus*. *Fusobacterium nucleatum* is one of the predominant organisms associated with gingivitis and periodontitis and this organism produces high levels of VSCs. The nutrients for the bacteria are provided by oral fluids, tissue and food debris.⁴

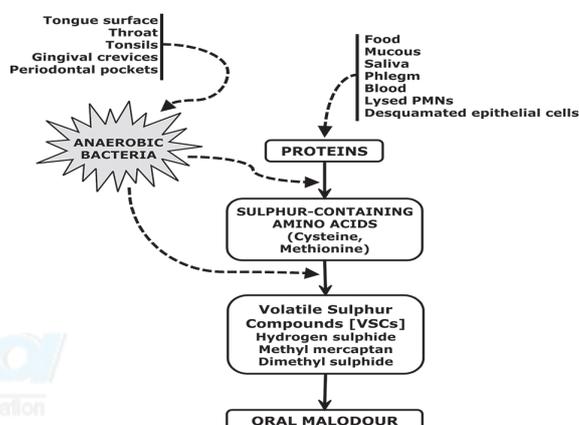


Figure 1: Etiopathogenesis of oral malodour¹

Classification

I. NON-ORAL causes and Oral malodor types:

i) **Physiological halitosis:** is caused by dietary foods and drink, like garlic, onions, curries and spices such as cumin, mace, coriander, cinnamon, or turmeric. It is short lived, temporary and easily reversible.

ii) **Pathological halitosis is caused by systemic disease.**

a) Diabetes mellitus often produces ketones and a sweetish odour. Diabetic control is essential to eliminate this ketosis. Kidney disease may produce an ammoniacal smell, and liver disease has a musty odour.

b) Gastritis with mucosal pathology (like ulcers or neoplasia) may present with OM with a fetid foul odor. Early loss of smell (anosmia) may indicate early onset Alzheimer's, as the CNS connections become less functional.

iii) **Psychological halitosis more accurately called delusional cacosomia.**

a) This OM is subjectively, but falsely, perceived by patients who may have a brain dysfunction or tumor. Taste and smell changes frequently occur with people who are suffering from an intra-cranial neoplasia, either as a primary lesion, a metastasis, or from cancer therapy.

b) In epilepsy, psychological halitosis/delusional cacosomia may be perceived as an aura, which is a

subjective warning sign that a seizure is about to occur. The aura may be any smell but commonly burning rubber, smoke, or a pungent aroma. Once the CNS pathways are dysfunctional subjective reporting of OM remains highly impossible.

Other NON-ORAL causes and Oral Malodor Types

i) Ozostomia: OM caused above the carina. (The carina is the bronchial cartilage that divides the respiratory tree into upper respiratory tract URT and lower respiratory tract LRT) URT infections like pharyngitis, tonsillitis, tonsoliths, rhinitis or sinusitis often cause ozostomia. Blocked and runny noses frequently among children will produce typical ozostomia smells. This is also a pathological halitosis.

ii) Stomatodysodia: OM caused from the lungs below the carina. Tobacco addiction/abuse is the main cause of stomatodysodia, but also infective bronchitis, bronchiectasis, lung abscess, tuberculosis, pleurisy, and/or pneumonia, caused by viruses and/or microbes may also be responsible. This is also a pathological halitosis.

II. ORAL causes and Oral Malodor types:

Oral types are called fetor ex ore (FEO) and fetor oris (FO). FEO and FO are the same. FEO's are caused by odoriferous bacterial biofilms from effects of stagnated microbes with pathology of the mouth affecting teeth, gums and tongue. These conditions and biofilm stagnation areas include Gingivitis, Periodontitis, Pericoronitis / Peri-implantitis, Dead spaces of stagnation on implants, Dorsum of Tongue Pathology, ANUG/NUG (trench mouth), Plaque & Calculus (Biofilm and Calcified Biofilm), Poor Oral Hygiene with stagnation areas, Inadequate brushing & flossing, Reduced salivary flow etc.⁵

Specific Character of Breath Odor

- A "rotten eggs" smell is indicative of VSCs.
- A sweet odor, as that of "dead mice" has been associated with liver insufficiency; besides VSCs, aliphatic acids (butyric, isobutyric, propionic) accumulate.
- The smell of "rotten apples" has been associated with unbalanced insulin-dependant diabetes, which leads to the accumulation of ketones.
- A "fish odor" can suggest kidney insufficiency characterized by uraemia and accumulation of dimethylamine and trimethylamine.⁶

Diagnosis of Oral Malodor

The proper diagnostic approach to a malodor patient starts with a thorough questioning about the medical, dental and halitosis history.⁷ The clinician should ask about the frequency (e.g., every month), time of appearance within the day (e.g., after meals can indicate a stomach hernia), whether others (nonconfidants) have identified the problem (excludes imaginary breath odor), what medications are taken, and whether the patient has dryness of the mouth or other symptoms.⁶

There are a number of methods, from simple to sophisticated, used to detect or diagnose the presence of oral malodor. These are:

Direct Methods

Self-examination

When an intraoral cause has been identified, involve the patient in monitoring the results of therapy by self-examination. The following self-testing can be used:

- Smelling a metallic or nonodorous plastic spoon after scraping the back of the tongue.
- Smelling a toothpick after introducing it in an interdental area.
- Smelling saliva spit in a small cup or spoon.
- Licking the wrist and allowing it to dry.⁶

Organoleptic Method (whole-mouth breath test, spoon test, floss odor test, salivary odor test)

Even though instruments are available, organoleptic assessment by a judge is still the "gold standard" in the examination of breath malodor. In organoleptic evaluation, a trained "judge" sniffs the expired air and assesses whether or not this is unpleasant using an intensity rating, normally from 0 to 5, as proposed by Rosenberg and McCulloch (1992)⁸. It is solely based on the olfactory organs of the clinician: 0 = no odor present, 1 = barely noticeable odor, 2 = slight but clearly noticeable odor, 3 = moderate odor, 4 = strong offensive odor, and 5 = extremely foul odor. The main disadvantage of this method is that it is subjective to the judge's olfaction.

Portable sulfide meter

The portable sulfide meter (Halimeter®) has been widely used over the last few years in oral malodor testing. The portable sulfide meter uses an electrochemical, voltametric sensor which generates a signal when it is exposed to sulfur gases (to be specific, hydrogen sulfide) and measures the concentration of hydrogen sulfide gas in parts per billion. The halimeter is portable and does not require skilled personnel for operation. The main disadvantages of using this instrument are it fails to detect other odorants which contribute to halitosis, such as volatile short-chain fatty acids, polyamines, alcohols, phenyl compounds, alkanes, ketones, and nitrogen-containing compounds.⁹



Figure 2: Halimeter

Gas chromatography

Gas chromatography is the preferable method if quantitative measurements of specific gases are required. This is a highly reproducible, objective, and reliable method in which the concentration of volatile sulphur-containing compounds in samples of saliva, tongue coating or expired breath is measured by producing mass spectra and analyzed by a gas chromatograph.¹⁰

The **Oral Chroma™** portable gas chromatography device analyses individual concentrations of volatile sulphur compounds such as Hydrogen sulfide, Methyl mercaptan and Dimethyl sulfide and displays the concentrations on a display panel.¹¹ The main disadvantages of using this instrument are the equipment is expensive and requires skilled personnel to operate it.¹⁰



Figure 3: Gas chromatography (Oral chroma™)

Electronic nose

Electronic noses are chemical sensors that have been in the recent times for a quantitative assessment of malodor associated with food and beverages. **The FF-1 odour discrimination analyser (Electronic nose, Shimadzu Corporation)** was used by **Tanaka et al (2004)**.¹²

Dark field / phase contrast microscopy

Gingivitis and periodontitis are typically associated with a higher incidence of motile organisms and spirochetes, so shifts in these proportions allow monitoring of therapeutic progress. Another advantage of direct microscopy is that the patient becomes aware of bacteria present in plaque, tongue coating, and saliva. High proportion of spirochetes in plaque has been associated with a specific acidic malodor (**Quirynen & Van Steenberghe, 2006**).⁶

Indirect Method

Bacterial culture, smears and enzyme assays are indirect methods of assessing oral halitosis. These methods will help in the identification of organisms that produce oral malodor. One such technique is (**Benzoyl-DL-arginine naphthylamide**) BANA test.

BANA test

Benzoyl-DL-arginine naphthylamide test is a chair side investigation that assesses the proteolytic activity of anaerobic bacteria. It is a rapid chair side test for evaluation of non-sulfurous malodorous compounds.

To detect malodor, the tongue or inter dental region is wiped with a cotton swab. The sample is placed on the BANA test strip, which is then inserted into a slot on a small toaster-sized incubator. The incubator automatically heats the sample to 55°C for 5 minutes. If *P. gingivalis*, *B. forsythus* or *T. denticola* is present, the test strip turns blue. The bluer it turns, the higher the concentration and the greater the number of organisms. A color guide is printed on the container. It can also be used to evaluate the prognosis of the condition.¹³

Other Methods

Quantifying β -galactosidase activity

Deglycosylation of glycoproteins is considered as an initial step in oral malodour production. β -Galactosidase is one

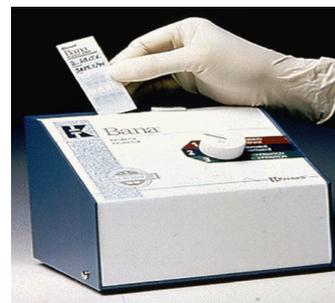


Figure 4: BANA test kit and BANA-Zyme reagent strips

of the important enzymes in deglycosylation. The activity of a galactosidase can be easily quantified with the use of a chromogenic substrate absorbed onto a chromatography paper disc. Saliva applied to the paper disc, may induce a colour change of the paper, which can be recorded by an examiner.¹⁴

Salivary incubation test

The salivary incubation test uses saliva collected in a glass tube. After incubating the tube at 37.8°C in an aerobic chamber under an atmosphere of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen for several hours, the odour can be measured by an examiner.¹⁵

Ninhydrin Method

Amines or polyamines cannot be measured by using sulphide monitoring. The Ninhydrin colorimetric reaction is a simple, rapid, and inexpensive method. A sample of saliva and isopropanol is mixed and centrifuged. The supernatant is diluted with isopropanol, buffer solution (pH 5), and ninhydrin reagent. The mixture is refluxed in a water bath for thirty min, cooled to 21.8 °C, and diluted with isopropanol to a total volume of 10ml. Light absorbance readings are determined using a spectrometer.¹⁵

Polymerase chain reaction

Real-time polymerase chain reaction (PCR) using the TaqMan system can be used for quantitative analysis of volatile sulphur-containing compounds - producing oral bacteria (e.g. *Tannerella forsythensis*).¹⁶

OraTest

This test provides quantitative assessment of the level of microbial activity in the oral cavity. The test involves oral rinsing with a sterile milk sample, followed by expectoration into a test tube containing a oxidation-r. Education indicator (methylene blue). The higher level of micro-organisms, the faster the color changes from blue (aerobic condition) to white (anaerobic condition) at the bottom of the test tube. In addition to co-relation with microbial counts, the OraTest exhibits significant co-relation with plaque and gingival indices.¹⁷

Current Approaches in Diagnosis

- Recent VSC monitors introduced are Tanita breath alert, Osmoscope and diamond probe.
- Another chair side test kit (Halitox reagent kit) measures the halitosis linked toxins. It is quick, simple colorimetric test that detects both volatile sulphur compounds as well as polyamines.

c) The diamond Probe/Perio 2000 system is a dental device designed to detect sulphide concentration of various forms (S, HS, H₂S and CH₃SH) in gingival sulci. The system combines a conventional Michigan "O" Probe style dental probe with a sulphide sensor, which measures probing depth, bleeding on probing and sulphide levels.¹⁸

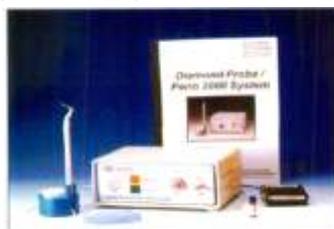


Figure 5: Diamond probe

Halitosis Assessment Protocol

Halitosis associated life-quality test (HALT)

A model used by many for health status is dictated by the Institute for Medical Rehabilitation and Research hierarchy. The Halitosis Associated Life-quality Test (HALT) is a de novo designed tool based on patient interviews and literature review. This new tool is devised to measure oral malodor (halitosis) and associated quality of life (QOL). HALT is a QOL questionnaire with 20 items, each item graded on the commonly accepted Likert scale of 0-5; a higher score indicated a worsening of that single measure. This questionnaire consists of 20 questions covering functional limitation, physical discomfort, psychological discomfort, physical disability and social disability.¹⁹

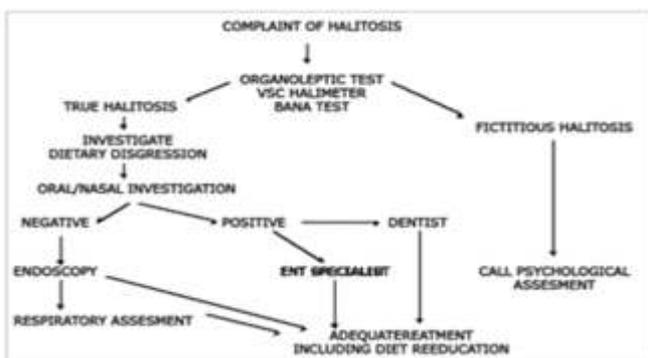


Figure 6: Flowchart suggested for Halitosis Assessment²⁰

Questions
Q1. Mainly mouth breathing
Q2. Frequent tonsillar infections
Q3. Frequent sinus infections
Q4. Worrying about or self conscious about your mouth breath
Q5. Miserable or tense due to halitosis
Q6. Difficulty chewing or limiting certain food due to halitosis
Q7. Change of taste
Q8. Problems speaking (or mouth covering) due to halitosis
Q9. Appearance affected due to halitosis
Q10. Depressed due to mouth breath
Q11. Problems concentrating due to halitosis
Q12. Embarrassed due to halitosis
Q13. Spending time related to halitosis
Q14. Talking from afar due to halitosis
Q15. Avoid going out due to halitosis
Q16. Communication problems due to halitosis
Q17. Mentioned about halitosis
Q18. Suffer financial loss due to halitosis
Q19. Suffer social/personal loss due to halitosis
Q20. Reduced life satisfaction due to halitosis

Figure 7: Halitosis QOL Questionnaire¹⁹

Management of Oral Malodor

General measures

- Identification and treatment of contributing factor
- Avoid foods like onions, garlic and spices
- Avoid habits that may worsen breath odor such as alcohol and tobacco
- Brush your teeth regularly and after meals and keep oral hygiene regular and good
- Rinse at least twice daily with chlorhexidine, triclosan, essential oils or other mouthwashes
- Brush your tongue with tongue scraper.
- Keep your mouth as moist as possible
- Dentures should be kept out at night in hypochlorite or chlorhexidine²¹

The first step towards effectively managing oral .halitosis is to determine the cause for halitosis (oral or systemic) and the nature of halitosis. The therapy consist of: (i) Mechanical reduction of the intra-oral nutrients and micro-organisms; (ii) Chemical reduction of microorganisms; (iii) Inverting volatile fragrant gasses into non-volatile components or (iv) Masking of the malodour.²²

1. Mechanical reduction of the intra-oral nutrients and micro-organisms:

- a) Tongue cleaning, interdental cleaning and toothbrushing are essential mechanical means of dental plaque control.²³
- b) A systemic review by Van der Sleen et al²⁴ demonstrated that tongue brushing or tongue scraping have the potential to successfully reduce breath odour and tongue coating. Due to tongue cleaning, the taste seems to improve again. Interdental cleaning and toothbrushing are also necessary to control plaque and oral microorganisms.

2. Chemical reduction of oral microbial load:

- a) Mouthwashes have been used as chemical approach to combat oral malodor. Antibacterial components in oral rinses such as cetylpyridinium chloride (CPC), chlorhexidine (Halita), triclosan, essential oils, quaternary ammonium compounds, benzalkonium chloride and hydrogen peroxide have been considered along with mechanical approaches to reduce oral malodour.²⁵
- b) In a recent Cochrane review by Fedorowicz (2008)²⁶ only five randomized controlled trials could be found, involving 293 participants. In view of the clinical heterogeneity between the trials, pooling of the results and a meta-analysis of the extracted data was not feasible. Compared to placebo, 0.05% chlorhexidine, 0.05% cetylpyridinium chloride, 0.14% zinc lactate mouthrinse significantly reduced the organoleptic scores, but showed significantly more tongue and tooth staining. It is concluded that this mouthrinse plays an important role in reducing the levels of halitosis producing bacteria on the tongue and can be effective in neutralization of odoriferous sulphur compounds.

3. Rendering malodorous gases non-volatile:

- a) Zinc salt containing mouthwashes, baking soda dentifrices and chewing gum formulated with antibacterial agents and tea extracts like epigallocatechin are used.²¹

b) Supanee et al in 2012²⁷ determined the effect of green tea mouthwash on oral malodor, plaque, and gingival inflammation stating that green tea mouthwash could significantly reduce VSC level in gingivitis subjects after rinsing for 4 weeks.

4. Masking the malodour with mouth sprays and lozenges containing volatiles with a pleasant odor:

a) The use of probiotics to suppress oral malodor is now being recognized. Probiotics, as defined by the Food and Agriculture Organization (FAO), are live microorganisms administered in adequate amounts that confer a beneficial health effect on the host.²⁸

b) Kazor et al (2008)²⁹ compared the bacterial populations on the dorsal surface of the tongue in healthy subjects and people with halitosis. *Streptococcus salivarius* was found to be the predominant species in healthy subjects, but was typically at low levels or absent in those subjects suffering from halitosis. Hence, probiotic bacteria may have potential application as adjuncts for the prevention and treatment of halitosis.

5. Other methods of managing malodor include chewing parsley, mint, cloves, or fennel seeds. Some herbs like alfalfa, cardamom, chamomile, myrrh, rosemary, and sage are also known to reduce halitosis.³⁰

Conclusion

Halitosis or breath malodor may be an indicator for medical problem and in many cases may cause significant social problem. A proper diagnosis and determination of the etiology allows initiation of proper etiologic treatment. Recent developments in the understanding of the etiologies of breath malodor have spawned new techniques for its assessment and management. Hence, dental physicians should take up the responsibility of ruling out the etiology and follow a multiphase approach and redirect the patient accordingly. The role of periodontist/ physician in treating halitosis is ineluctable.

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Barr Bodies and Sex Identification in Intersexed: Current Concepts

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ABSTRACT

The discovery of Barr body offered an important diagnostic technology for medical interpretation of sexual anomalies, such as in intersexuals and transsexuals. Establishing individuality and identification of sex becomes important in several situations like in gender assignment for receiving specific civil rights for transgenders, legitimacy to partake in competitive sports or even in forensic situations. In spite of so much importance being attached to sex identification, very little work has been attached to sex identification, very little work has been done in India about study of sex chromatin in buccal smears in the intersexed. This review emphasizes the need to primarily understand the biology of intersexed individuals, current information of transgender rights in India and the role of cytological evaluation of Barr bodies in preliminary screening of individuals without invasion of privacy.

Introduction

Establishing individuality is imperative in any investigating procedure. Often, determination of the sex of an individual becomes important in following situations: for the purpose of simple identification in the living where the individual of one sex carries the features of the opposite sex; when a person appears to possess the primary sex organs of both the sexes; for the purpose of deciding whether an individual can exercise certain civil rights reserved for one sex only; for deciding questions relating to competing in sex specific athletic and sport events, legitimacy, divorce, paternity disputes, and also to some criminal offences and identification of sex of dead individuals in an advanced state of decay where primary sex organs are lost due to decomposition.[1]

In massive accidents and also in natural disasters, it becomes difficult to identify the bodies. In such instances, buccal smears could help in detecting the sex and thereby establishing the identity.[2]

Demonstration of nuclear sex plays a vital role as far as sexing of the individual is concerned. Nuclear sex can be demonstrated by the study of [1]:

Karyotyping: Direct study of type of sex chromosome in the cell-by-cell culture. This is expensive and is not feasible in all situations.

Fluorescent body (Y chromatin): A demonstration of nuclear fluorescent bodies that is Y chromatin indicates male. This definitely requires special stain and fluorescence microscope.

Polymerase chain reaction: Polymerase chain reaction to amplify DNA sequences of SRY gene, on the sex chromosome. This is similar to karyotyping, as it is not feasible in all situations. It is expensive and inferior to karyotyping but not superior to the chromatin test.

Barr bodies (X-chromatin): In contrast, the study of Barr bodies is advantageous in that it can be studied even under an ordinary compound microscope with simple staining techniques. The easily available material for Barr body

studies is the buccal mucosa, which can be obtained without inflicting trauma on the subject. The buccal smear technique to identify sex was developed by Moore and Barr in 1955 [3].

Role of Cytodiagnosis of Barr Bodies

Barr bodies are known to arise from inactivation of X chromosome in a female cell [4]. Barr bodies are Feulgen positive, heteropyknotic, and basophilic, intranuclear structures, seen in mammalian cells during interphase.

Most often, they are noticed as densely stained condensed chromatin masses adjacent to the nuclear membrane. They can be Plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion. They measure about 0.8 to 1.1 μm in diameter.[1]

The Barr body is not present in the nuclei of males although they also have one X and Y chromosome because X in the males remains uncoiled (extended) in interphase nuclei [5] This discovery of Barr bodies also seemed to offer a new way to identify the true, underlying sex in those whose bodies or lives were sexually anomalous or intersexed individuals (currently classified under a umbrella term as 'transgenders').[6]

Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp.[7]

Sex identification in the medical science using sex chromatin has provided 'good enough' evidence, using simple evaluation of Barr bodies. Patients are encountered occasionally in whom the sex chromatin pattern is unlike that of normal individuals because of a variety of unusual sex chromosome complexes.[6]

The buccal smear test is preferred, because of its simplicity, for routine use as a diagnostic aid or when conducting mass surveys for research purposes.

Defining Some Common Terms

GENDER V. SEX

In everyday language as well as in the law, the terms “gender” and “sex” are used interchangeably. However, it is often important to distinguish the two terms. The term “sex” refers to a person's biological, anatomical, biochemical identity as male or female. The chromosomal sex refers to presence of XX (female) or XY (male) chromosome in the cells. The term “gender” is reserved for the collection of characteristics that are culturally associated with maleness or femaleness [8].

Gender Identity and Gender Expression

“Gender identity” refers to a person's internal, deeply felt sense of being either male or female, or something other or in between and not visible to others. In contrast, a person's “gender expression” is external and socially perceived. Gender expression refers to all of the external characteristics and behaviors that are socially defined as either masculine or feminine, such as dress, mannerisms, speech patterns and social interactions [9].

Intersex

An intersex person is born with sexual anatomy, reproductive organs, and/or chromosome patterns that do not fit the typical definition of male or female. This may be apparent at birth or become so later in life. An intersex person may identify as male or female or as neither. Intersex status is not about sexual orientation or gender identity: intersex people experience the same range of sexual orientations and gender identities as non-intersex people [10].

Biological sex in humans may be determined by five factors present at birth [9]:

- The number and type of sex chromosomes;
- The type of gonads – ovaries or testicles;
- The sex hormones;
- The internal reproductive anatomy (such as the uterus in females), and
- The external genitalia.

People whose five characteristics are not either all typically male or all typically female at birth are Intersex [11].

Transgender

Transgender has become an “umbrella” term that is used to describe a wide range of identities and experiences, including but not limited to: pre-operative, post-operative, and non-operative transsexual people; male and female cross-dressers (sometimes referred to as “transvestites,”); intersexed individuals; and men and women, regardless of sexual orientation, whose appearance or characteristics are perceived to be gender atypical. In its broadest sense, transgender encompasses anyone whose identity or behavior falls outside of stereotypical gender norms. [12]

Epidemiology

At least one in every 2,000 children is born with a sexual anatomy that mixes male and female characteristics in ways that make it difficult, even for an expert, to label them male or female. Although no one is ever born with two

full sets of genitals, male and female, some intersexed infants may have ambiguous genitalia, such as a penis that is judged “too small” or a clitoris that is judged “too large” [10].

Intersex is a group of conditions where there is a discrepancy between the external genitals and the internal genitals (the testes and ovaries). But a lot more people than that are born with subtler forms of sex anatomy variations, some of which won't show up until later in life [11]

Intersex can be divided into four categories [10]:

- 46,XX Intersex
- 46,XY Intersex
- True Gonadal Intersex
- Complex or Undetermined Intersex

46, XX Intersex: These are genetic females (46XX) born with hypertrophied clitoris (to appear like a penis) leading to ambiguity of genitalia. The person has the chromosomes of a woman, the ovaries of a woman, normal uterus and Fallopian tubes, but external (outside) genitalia that appear otherwise. This usually is the result of a female fetus having been exposed to excess male hormones before birth.

46, XY Intersex: The person has the chromosomes of a male and internally, testes may be normal, malformed, or absent. The external genitalia are incompletely formed, ambiguous, or clearly female. This condition is also called 46,XY with under virilization [10]

5-alpha-reductase deficiency: People with 5-alpha-reductase deficiency lack the enzyme needed to convert testosterone to dihydrotestosterone (DHT). Some of the babies have normal male genitalia, some have normal female genitalia, and many have something in between. Most change to external male genitalia around the time of puberty [13]

Androgen insensitivity syndrome (AIS): This is the most common cause of 46, XY intersex (previously called as called testicular feminization). Here the hormones are all normal, but the receptors are insensitive.

Complete androgen insensitivity: As the external genitalia are completely feminine such a baby is not brought to the doctor at birth. Later on, presentation may be for inguinal hernia, or for primary amenorrhea at puberty. These children are best reared as girls as they cannot be distinguished from normal girls at all. The only surgery they require is gonadectomy for removal of testicular tissue and vaginoplasty.

True Gonadal Intersex: These children generally present with ambiguous genitalia and arriving at a diagnosis usually takes time, as gonadal biopsies with or without laparotomy/laparoscopy and karyotyping is required before conclusively proving the diagnosis. The person may have XX chromosomes, XY chromosomes, or both. The external genitalia may be ambiguous or may appear to be female or male [10]

Complex or Undetermined Intersex Disorders of Sexual Development: Many chromosome configurations other than simple 46, XX or 46, XY can result in disorders of sex development. These include 45, XO (only one X chromosome), and 47, XXY, 47, XYY -- both cases have an extra sex chromosome, either an X or a Y. These disorders do not result in a condition where there is discrepancy between internal and external genitalia. However, there may be problems with sex hormone levels, overall sexual development, and altered numbers of sex chromosomes [8].

Children with intersex disorders and/ or with abnormal sex chromosomes are at an increased risk for development of malignancy, particularly in their gonads. It is seen more commonly in: complete AIS, with malignant germ cell tumours, usually in adult life, all gonadal dysgenetic disorders in patients having a Y chromosome, fertility is unlikely in many intersex conditions, with the exception of CAH.

Transgender and their Civil Rights

Transgender people experience a mismatch between their gender identity or gender expression and their assigned sex. The term originally referred to biological men who are satisfied with their male genitalia, but who wish to be seen and to live in the world as women. Such individuals prefer to undergo sex reassignment surgery and hormonal therapy to become a 'transsexual'. There are several other terms in India used to describe transgenders as '**Hijras**', '**Aravanis** and '**Thirunangi**', '**Jogtas/Jogappas**', '**Shiv-Shakthis**' [14]

In India, transgenders face sexual discrimination, sexual harassment, social ostracisation, abuse, and exploitation of various types. In a landmark judgment, the Supreme Court of India created the "third gender" status for transgenders and intersexed individuals with specific civil rights accorded to them in various arenas of social life.

- The SC asked the Centre to treat transgender as socially and economically backward.
- The apex court said that transgenders will be allowed admission in educational institutions and given employment on the basis that they belonged to the third gender category [14]

The analysis of international precedents on transgender rights delineates two prominent models for legal recognition of their gender identity and for obtaining sexual reassignment surgery to those who need it.

Gender Dysphoria/Diagnosis Model (BASED ON WPATH)

In this model patients must receive approval from medical professionals to undergo surgery or have changes to their ID documentation after diagnosis of gender identity disorder (GID) or gender dysphoria.

Self-Identification Model (BASED ON YOGYAKARTA PRINCIPLES)

A self-identification model of gender recognition followed

in India, sees right to self-determination of one's own gender is a fundamental right for all people and are not required to be diagnosed with gender dysphoria or gender identity disorder and have the right to access both hormonal treatment and surgery. Individuals over the age of eighteen years merely have to submit to the concerned government department a request to alter their birth certificate to reflect the self-determined gender [14]

Barr Body and Competitive Sports

Indian sports have been shaken by gender test controversies in recent years. Shanthi Soundarajan, female athlete who won the silver medal in 800 meters- at 2006 Asian games failed the gender verification test and was stripped of her medal. It was reported that she had AIS (androgen insensitivity syndrome, 46 XY with intersex), presenting as a phenotypic female adult owing to failure of androgen receptors [15]

Gender verification test for all female athletes began in response to suspicions that some countries sent men who masqueraded as women to gain competitive advantage in certain Olympics events. There was probably ambiguity of the external genitalia in most of these cases, possibly as a result of male pseudohermaphroditism [16]

The International Association of Athletic Federation (IAAF) made it mandatory for all female athletes to undergo genital examination by gynecologists. This outrageous practice was humiliating for the athletes and the International Olympic committee (IOC) substituted this test with a buccal smear test to test for Barr bodies. But even this test was not of much help in resolving controversial issues, as it was found that some people exhibited some sort of intersex conditions, where there existed phenotypic females with male sex chromatin patterns eg; AIS, XY gonadal dysgenesis, etc [17]

It was argued that such players have no athletic advantage as a result of their congenital abnormality and should not be barred from competitions. Sex chromatin testing does unfairly exclude many genuine athletes. Laboratory tests infact still fail to address and conclusively resolve the issue. Therefore the IAAF did a reappraisal and reached the conclusion that gender verification is not needed at all and as such screening for gender is no longer required at IAAF competitions since 2009 [16]

Nevertheless the IOC has promulgated a new rule according to which women who test in the male range for testosterone and whose bodies respond to this hormone, may not be eligible to compete as females. Such precautions are set to level the playing field preventing people who identify as women but have an unfair male like advantage [15]

More and more experts are now reaching the consensus that sex determination test of players must take in to account all aspects such as chromosome, genitals, gonads and hormones in order to reach unambiguous and undisputed decision.

Under the current policy normal XX females with an naturally high testosterone due to practice could be declared ineligible to compete as woman and the same current criteria would however favour players with XY having AIS would be deemed to be women for the purpose of sports as they exhibit low levels of testosterone [15]

Current Role of Cytogenetics and Karyotyping

The determination of the nuclear sex, a relatively easy procedure, is an important tool in investigating these developmental abnormalities if its usefulness and limitations are clearly recognized. Now that full chromosome analysis is routine, it has become apparent that there exists a possibility of both false-positive and false-negative sex chromatin patterns; therefore the pattern does not always reflect the true cytogenetic state of the individual. It cannot indicate the autosomal constitution of the individual, and it does not reflect the sex chromosome status, for it tells us nothing about the Y chromosome, and structural abnormalities of the X chromosome are not evident with routine methods. The sex chromatin test is an aid to the cytogeneticist in interpreting the chromosome analysis. The presence and frequency of Barr bodies is helpful in deciding whether the possibility of sex chromatin mosaicism is great enough to warrant cytogenetic analysis of more cells or examination of other tissues. Karyotyping is especially essential in certain conditions where a phenotypic female without sex chromosome may have 46 XY complement and the presence of Y chromosome cause a substantial role of gonadal malignancy [18]. Such female should have prophylactic gonadectomy. Again, individual with 47XXY who may appear like normal phenotype males in many situations are known to have criminal tendencies as observed in studies. Such individuals in the transgender population can also be screened for the additional X chromosome by mean of buccal smear test, validating the need for karyotyping.

Conclusion

In the Indian scenario, assigning gender is not easy, because most patients with ambiguous genitalia may end up as females. The problem is further complicated by the timing of presentation as it may be noticed at birth or noticed at puberty. Both need a thorough diagnostic work-up before assigning gender. The latter may have another difficulty in that the child may have been raised as a male, but eventually be assigned female sex. Ideally, a team of health care professionals with expertise in intersex should work together to understand and treat the child with intersex and to understand, counsel, and support the entire family.

Children with intersex disorders and/ or with abnormal sex chromosomes are at an increased risk for development of malignancy, particularly in their gonads. Clearly, intersex is a complex issue. Even after waiting for months to announce the birth of a child to relatives, parents are unclear if it is a girl or a boy. They are disturbed by the fact that if it is a boy his sexuality may be inadequate and if it's a girl she may not menstruate or procreate.

On the principle that a sound basis for good management of a medical problem is provided by knowledge of its biological background, the pathogenesis of anomalous sex has to be studied at its fundamental level, using the nuclear sex.

The determination of nuclear sex is an easy procedure and can be an important tool in investigating these developmental abnormalities. Sex determination using Barr bodies in buccal scrapes is a simple method providing up to 95-98% accuracy; this makes it significant accessory to other methods of sex determination. In borderline cases, it is necessary to utilize other, more elaborate methods. It can be used as a guide to karyotyping. It is essential to have such a test for large screening purposes, as it is simple, easy, cost effective, non-time consuming and non-invasive procedure.

Since the test involved does not invade the privacy, and does not violate the human right of these individuals in any way, it can be advocated to the policy makers in appropriate platform. In a country like India where assigning gender to transgender has been made following self identification model, as opposed to the gender dysphoria/ diagnosis model, it is prudent to use this nuclear sex screening tool for isolating those intersexed individuals who are at additional risk for certain malignancies or those in whom aggressive behavior or altered mental health predilection is evidenced, which would otherwise go unnoticed in the absence of a diagnosis model.

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Non Neoplastic Salivary Gland Disorders

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ABSTRACT

Non-neoplastic salivary gland disorders make up about 6% of diseases of the salivary glands. These disorders are divided into the following groups: developmental, obstructive, infectious / systemic, idiopathic and auto-immune. Clinically these lesions may mimic a neoplastic process involving salivary gland and the treatment process may vary accordingly. Sialadenosis is distinguishable from sialadenitis by its clinical, radiological, and morphological characteristics. Mucoceles represent the majority of these cysts (75%). HIV-associated salivary gland disease includes lymphoepithelial lesions and cysts involving the salivary gland tissue and/or intraglandular lymph nodes, and Sjogren's syndrome-like conditions, diffuse interstitial lymphocytosis syndrome, and other reported lesions of the major salivary glands. This review article discusses the clinical features, histopathological features and treatment of various non-neoplastic salivary gland diseases.

Keywords: Non-neoplastic enlargement, sialadenosis, sjogren's syndrome, labial biopsy.

Introduction

Tumors of salivary glands constitute a heterogeneous group of lesions of great morphologic variations. Tumors of salivary glands have an annual incidence of around 1-6.5 cases per 100,000 individuals. Non neoplastic disorders of the salivary glands are divided into the following groups;^[1]

- a. Salivary duct cysts
- b. Sialadenosis
- c. Sialolithiasis
- d. Sialadenitis
- e. HIV associated salivary gland diseases
- f. Oncocytosis
- g. Necrotizing sialometaplasia

WHO classification of tumor like lesions of the salivary gland;^[2]

1. Sialadenosis
2. Oncocytosis
3. Necrotizing sialometaplasia
4. Benign lymphoepithelial lesions
5. Salivary gland cyst
6. Chronic sclerosing sialadenitis

Table 1: Classification of Salivary gland disorders;^[3,4,5]

1.	DEVELOPMENTAL
	a. Aplasia
	b. Hyperplasia
	c. Developmental lingual mandibular salivary gland depression'
	d. Anterior lingual depression
	e. Heterotopic salivary gland tissue
	f. Accessory salivary gland tissue
	g. Oncocytosis
	h. Adenomatoid glandular hyperplasia
	i. Polycystic disease of the parotid gland
1.	NON NEOPLASTIC
	a. OBSTRUCTIVE
	i. Mucus escape phenomenon
	ii. Mucus retention phenomenon
	iii. Sialolithiasis
	b. INFECTIOUS & SYSTEMIC DISEASES
	i. Tuberculosis
	ii. Cat-scratch disease
	iii. Cytomegalovirus
	iv. Salivary gland cyst as a manifestation of HIV
	v. Mumps
	vi. Sarcoidosis
	vii. Cystic fibrosis
	viii. Sialadenosis
	c. IDIOPATHIC
	i. Necrotizing sialometaplasia
	ii. Benign cyst of salivary gland
	iii. Angiolymphoid hyperplasia and Kimura's disease
	iv. Chelitis glandularis
	d. AUTOIMMUNE
	i. Sjogren syndrome
	ii. Mikulicz disease

Developmental Salivary Gland Disorders

1. APLASIA (Agenesis): Any one or group of salivary glands may be absent, unilaterally or bilaterally. It manifest with the development of xerostomia. A diagnosis of salivary gland aplasia is made after the exclusion of the common causes of xerostomia medications, sjogren syndrome and radiation. CT scan and MRI will indicate the gland's absence. This condition occurs as a unknown cause or may develop along with associated syndromes such as Hemifacial microstomia or LADD syndrome or Treacher Collins syndrome.

2. PALATAL GLAND HYPERPLASIA: An unusual localized hyperplasia or hypertrophy of minor accessory salivary glands in the palate can occur. The causes for this alteration are endocrine disorders, gout, dm, menopause, hepatic diseases, starvation, alcoholism, sjogrens syndrome, adiposity and aging process.

1. HETERTOPIC SALIVARY GLAND: Salivary gland tissue located in sites other than those of normal anatomical locations. "Willis et al" proposed three reasons for heterotopias;

- a. Abnormal persistent & development of vestigial structures
- b. Dislocation of a portion of a definitive organ
- c. Abnormal differentiation of the normal tissues.

Locations: Paraparotid lymphnodes, Middle ear, Intraosseous hetertopic salivary gland tissue, External auditory canal, Mediastinum, Pituitary gland, Prostate, Thyroid and parathyroid gland.

2. ACCESSORY PAROTID GLANDS: Refers to lobules of the parotid salivary gland tissue that are separated from the main body of the gland but drain into stenson's duct. "Frommer et al" pointed out that anterior extensions of the parotid gland tissue along the parotid duct are normal variants and are not considered accessory. It arranges from 0.5 to 3cms and may be superior or ablong. Clinically, lesions of accessory parotid tissue present as masses in the cheek that occur in the central third of a line drawn from the mid tragus to the point midway between the ala of the nose

and vermilion lip border. The histologic features are similar to that of the primary parotid gland tissue.^[6]

3. ONCOCYTOSIS: It is a metaplastic, sometimes hyperplastic, developmental or transformational process that is characterized by focal replacement of normal glandular tissue with enlarged eosinophilic epithelial cells with granular cytoplasm. Oncocytosis is seen with greater frequency in older people and is considered a sequela of aging. The majority of cases occur in parotid but it may be found in any major and minor salivary glands. Histologically, oncocytosis may present as scattered foci of enlarged, eosinophilic epithelial cells or as a solitary foci of metaplastic oncocytes. The cells retain their acinar arrangement. Oncocytosis stain with PTAH and with PAS. Ultrastructural findings are characterized by cytoplasm that is packed with large, pleomorphic mitochondria containing filamentous tubular and vesicular cristae. Oncocytes have been considered as a product of degenerative changes. Cells also contain high levels of mitochondrial enzymes because of increased mitochondria.^[7,8]

4. POLYCYSTIC DISEASE OF THE PAROTID GLAND:

Least common of the benign cystic lesions of the parotid gland and is a developmental malformation of the ductal system. Females are more commonly affected & occur bilaterally. Recurrent painless swelling of the involved gland occurs. Histopathologically, the architecture of the gland is preserved. Lobules are markedly distended. Honey combed or lattice like appearance. Cystic lumina contain flocculent, eosinophilic material and few scattered macrophages. Eosinophilic bodies with concentric and radial patterns similar to spheroliths and microliths.^[9]

OBSTRUCTIVE SALIVARY GLAND DISORDERS

Most common disorders of the major and minor salivary glands.

Occurs as a result of:

- Trauma to the salivary gland ducts
- Stasis of saliva
- Partial or complete obstruction of the ducts.

Three more common primary mechanical obstructive disorders are:

- Mucus escape phenomenon
- Mucus retention cyst
- Sialolithiasis.

1. MUCUS ESCAPE PHENOMENON (MUCOCELE)

It is defined as a pooling of mucus in a cavity within a connective tissue that is not lined by an epithelium.

Traumatic severance of a duct with resultant pooling of mucus in the surrounding tissue is its cause. Clinically, Major and minor (common) salivary glands are affected. 96% affects the minor salivary glands in the lower lip. Superficial lesions present as raised soft tissue swelling with translucent bluish hue. Deeper lesions appear nodular with overlying normal mucosa. Painless mucosal swelling will appear from few days to weeks.

Ranula – Swelling in the floor of the mouth most commonly associated with sublingual glands but submandibular gland can also be affected. (*Plunging or Cervical ranula*). Histologically, Non epithelial lined (Not a true cyst). Circumscribed cavity in soft tissue filled with eosinophilic material staining positive for mucin staining. Presence of acute and chronic inflammatory cells. Consists of compressed fibrovascular connective tissue. As the lesion matures, granulation tissue progressively grows into the cavity and slowly obliterates the defect & is known as “*Organizing mucocele*”.

Treatment: Removal of both the lesion and the adjacent minor salivary glands and its duct.^[10,11]

2. MUCUS RETENTION CYST: It is a true salivary gland cyst lined by an epithelium. “Eversole et al” termed this lesion as an “*Oral Sialocyst*”. This lesion develops as a result of partial obstruction of a duct and buildup of presence may cause its dilatation without the rupture and is responsible for the proliferation of the ductal epithelium. Clinical features: Parotid gland is the commonest site of involvement. Affects individual from first to ninth decades of life. Has slight predilection. Lesion appears as slowly enlarging, painless, circumscribed often fluctuant soft tissue swelling that may persist from months to yrs.

Pathological features: Presence of an overlying epithelial lining. Unilocular / multilocular / multicystic patterns. Lining epithelium consists of cuboidal to low columnar cells of non keratinizing stratified squamous epithelium. Presence of inflammatory infiltrates.

Treatment: Surgical excision of the true cyst.^[2,12]

3. SIALOLITHIASIS: Sialoliths are calcified masses that develop in the ductal system of the salivary glands.

Also known as:

- Salivary gland calculi
- Salivary gland stones

Sialoliths grow by a rhythmic deposition of inorganic and organic components around a mineralized nucleus.

Clinical features: May develop in the ductal system of major and minor salivary gland. Submandibular gland is the most commonly affected gland due to its course of the ductal system. Has slight female predilection. Swelling and pain in the area of the affected gland may be present. Swelling may be located by bimanual palpation in the affected area.

Pathological features: They are round oval or cylindrical calcified masses that may vary in colour from white to yellow brown. The surface texture may be smooth or irregular. Changes in the ductal wall includes squamous, oncocytic or mucous cell metaplasia of the epithelium. Saliva stasis may lead to retrograde infections via its ducts. Longterm obstruction may lead to fibrosis or subsequent loss of secretory functions.

Treatment: Conservative therapy:

- Moist heat therapy
- Increased intake of fluids

c. Sialagogues

d. Use of pilocarpine to increase the salivary flow.[13,14]

Infectious and Systemic Diseases

The salivary glands are involved when the pathogens or other systemic processes affect the gland parenchyma, its stroma or intraglandular lymphnodes. Lymphnodes are the primary factor involved in most of these diseases.

Table 2: Classification of Sialadenitis^[15]

1.	Bacterial sialadenitis
2.	Viral sialadenitis
3.	Radiation induced sialadenitis
4.	Electrolytic sialadenitis
5.	Chronic sclerosing sialadenitis (Kuttner's tumor)
6.	Immune sialadenitis.

Tuberculosis

Tuberculosis lymphadenitis is the most common extrathoracic form of the disease and cervical lymphnodes including lymphnodes in and around the major salivary glands.^[16] Infection of the salivary glands and lymphnodes occurs in one of the two ways: A focus of mycobacterium TB in oral cavity break through the mucous membrane that ascend to the salivary glands. Hematogenous or lymphatic spread.

Clinical features: Parotid gland is the most commonly affected. Females are most commonly affected. Clinically the lesion appears as painless, cystic or solid nodule that is upto 5cms in diameter. Intracutaneous injection of PPD shows positivity with 10mm or more of indurations in 48hrs.

Histopathologic features: Gland & lymphnodes demonstrate multiple granuloma. Central necrosis surrounded by giant cells of both langhan's and foreign body types, epitheloid cells and lymphocytes.

Treatment: Multi drug anti-TB therapy.

CYTOMEGALOVIRUS: CMV is DNA virus of Herpes viridae family. It is the most common viral disorder of the salivary glands & affects the newborns.

Clinical features: CMV infection is seen in one of the four clinical settings;

1. Congenital infections
2. Perinatal infections
3. CMV mononucleosis
4. In immune compromised patients

Histologic features: Consists of large cells around 25 – 40 micrometers that exhibits central basophilic nuclear inclusions and cytoplasmic inclusions.

Treatment: Antiviral chemotherapy.

Salivary Gland Cysts as Manifestations of HIV^[17]

Persistent generalized lymphadenopathy (PGL) is defined as palpable lymphadenopathy at 2 or more extrainguinal sites that persists for more than 3 months in absence of any infections. In some cases, the initial manifestations may be

localized rather than generalized lymphadenopathy that may precede to months or even years. Parotid swelling can be unilateral or bilateral for a period of 1 to 4 yrs. Presence of multiple parotid swelling or cysts which are frequently bilateral.

Histologic features: Monomorphic round (clear cells) focally pack medullary sinuses or form aggregates along the blood vessels, fibrous septa and peripheral lymphnodes. Mitoses are absent. Lymphnodes in and around the salivary glands contain squamous lined cysts and epimyoeplithelial islands.

MUMPS: Mumps virus is an RNA virus of paramyxoviridae family and is the most common viral infective agent of the salivary glands. Mumps is an acute, contagious disease that is endemic in all areas of the world.

Clinical features: Usually spreads from human reservoirs by airborne droplets of infected saliva. After incubation period, the condition manifests as pain and rapid swelling of one or moth parotid glands during a period of 1 to 3 days.[18] Tasting citrus fruits or other sour liquids that stimulates salivation intensifies the pain. The swelling & systemic symptoms gradually subside in 3 – 7 days.

Histopathologic features: Three sets of changes:

- a. Interstitial changes
- b. Acinar cell changes
- c. Ductal epithelial changes

Other features are dilated ducts with lumina filled with clumps of secretion & desquamated epithelium.

Treatment: Self-limiting condition.

Cystic Fibrosis: It is the most common fatal inherited, autosomal condition. Past few years, new concepts concerning the cause of cystic fibrosis has emerged. Previously it was thought to be a disease of abnormal mucus products and now considered to be involving abnormal fluid and electrolyte transports across the exocrine gland epithelium. Chronic obstructive pulmonary disease, impaired digestive & absorptive & elevated concentrations of salt in sweats. Salivary gland acini are mainly mucous in types, are affected. Sublingual gland shows more pathologic alterations. Consists of ductal ectasia, microliths abd interstitial fibrosis.

Treatment: Aggressive respiratory & physical therapy as well as administration of appropriate antibiotics.

SIALADENOSIS: Non neoplastic and non inflammatory enlargement of the salivary glands. Usually bilateral and may manifest reoccurrence or pain or both.

Table 3: Classification of sialadenosis^[19]

a.	Hormonal sialadenosis
b.	Neurohumoral sialadenosis
c.	Dysenzymtic sialadenosis
d.	Malnutritional sialadenosis
e.	Mucoviscidosis
f.	Drug induced sialadenosis

Slowly evolving, undulating & recurrent swelling affecting fourth decades of life. Hypertrophy of acinar cells and terminal ducts branching yields sialographic "leafless tree" patterns.

Histopathologic features: "Donath and Seifert et al" described as swelling is due to acinar enlargements. Diameter of the acinar cells increase 2-3 times than that of normal. The nuclei tend to be basally situated. Inflammatory cells are absent.

Treatment: Dependent on controlling the underlying cause.

IDIOPATHIC SALIVARY GLAND DISORDERS: These are unrelated group of lesions for which the causes are generally remains unknown.

NECROTIZING SIALOMETAPLASIA: Necrotizing sialometaplasia is non neoplastic, inflammatory, self limiting condition of the salivary glands. Most commonly misdiagnosed as carcinomas, mainly squamous cell carcinoma or mucoepidermoid carcinoma. This pitfall in diagnosis can be avoided if one is familiar with the clinical and histopathological findings.

Clinical features: Mostly involves the minor salivary glands of the hard palate. Etiology: Trauma, radiation therapy or ill fitting dentures. Presents as deep, crater like ulcers of 1-3cms. These ulcers are usually unilateral but may occur bilateral also. Asymptomatic but numbness, pain & burning sensation can be present. Cause is usually unknown, but most of them favors on ischemic pathogenesis.

Histologic features described by "Abrams et al" includes:

1. Coagulative necrosis of glandular acini
2. Squamous metaplasia of the ductal epithelium
3. Pseudoepitheliomatous hyperplasia
4. Mucus pooling with an associated granulomatous inflammatory responses.

"Anneroth & Hansen" proposed five Histologic stages:[20]

1. Infarction (Necrotic) stage
2. Sequestration stage
3. Ulceration stage
4. Reparative stage
5. Healed stage

KIMURA'S DISEASE: It is a chronic inflammatory condition of unknown cause that is endemic in origin. The disease occurs predominately in young & middle aged adults. Typically the lesions are firm, rubbery, subcutaneous, tumor like nodules or masses over a period of one to two yrs. Most common in the peri-auricular regions, but misdiagnosed as salivary gland tumors. Microscopically, the lesions are unencapsulated & ill defined & are characterized by fibrocollagenous tissue, lymphoid tissue & mixed inflammatory infiltrates with numerous eosinophils. The enlarged regional lymphnodes in kimura's disease typically reveal florid follicular hyperplasia & eosinophilic infiltration & sclerosis.

Treatment: Surgical excision, radiation therapy and drug therapy.

CHELITIS GLANDULARIS: Chelitis glandularis was first described by "Volkman", to describe a condition characterized by a suppurative, inflammatory swelling of the lower lip. In 1914, "Suttan et al", postulated that the swelling was due to enlargement of the labial salivary gland. Hypertrophy of the salivary gland is noted. Most commonly affects middle aged and older man.

Chelitis glandularis has been classified into three types:

1. Simple type of chelitis glandularis
2. Superficial chelitis glandularis (Bacalz's disease)
3. Deep suppurative (Chelitis glandularis apostematosa)

Pathophysiology: Although some have speculated that cheilitis glandularis represents a hereditary autosomal dominant condition, composite findings in most cases appear to indicate cheilitis glandularis represents a clinical reaction pattern to chronic irritation of the lip from a spectrum of highly diverse external causes. These include actinic damage, factitial injury, atopy, infection, and tobacco irritation. "Carrington and Horn" reported a case in which an elderly man developed cheilitis glandularis related to actinic damage following vermilionectomy for squamous cell carcinoma of the lower lip. These authors advocate clinical investigation in cases of cheilitis glandularis to rule out neoplastic, immune suppressive, or inflammatory changes due to local factors. This illustrates the concept that cheilitis glandularis is not a separate and distinct disease. Instead, it appears to be a descriptive phenomenon that could represent any one of a host of diverse clinicopathological entities. The possibility of a genetic predisposition for cheilitis glandularis has been raised by some authors. "Parmar and Muranjan", among others, described a genetic syndrome involving "double lip" of both lips in conjunction with ptosis and other physical abnormalities.[21]

Autoimmune Salivary Gland Disorders

BENIGN LYMPHOEPITHELIAL LESIONS / MIKULICZ DISEASE / MIKULICZ SYNDROME AND SJOGREN SYNDROME

The classification now is as follows:

(1) *Primary Sjögren's syndrome (sicca complex)* - this consists only of xerostomia and xerophthalmia with no connective tissue component.

(2) *Secondary Sjögren's syndrome* - this consists of xerostomia, xerophthalmia and a connective tissue disease which in nearly 50% of cases is rheumatoid arthritis but may also be systemic lupus erythematosus, scleroderma and polymyositis

(3) *Benign lymphoepithelial lesion*, otherwise known as myoepithelial sialoadenitis, which is localized to the parotid glands and some regard as a prelymphomatous condition

(4) *Aggressive type*, lymphocytic behavior which again is confined to the parotid glands and is almost a pseudolymphoma.

Clinical features: Sjögren's syndrome is a multisystem disease affecting every system in the body but particularly the oral cavity, the eyes and the salivary apparatus.

The oral symptoms are those of dry mouth with secondary candidiasis, stomatitis, glossitis and subsequent dental caries. The eye symptoms are keratoconjunctivitis sicca; the patient has a foreign body sensation in the eye, burning, redness, itching, photosensitivity and an inability to tolerate contact lenses. Only 40% feel salivary gland enlargement and only 20% show it clinically. It is nearly always in the parotid and those patients with parotomegaly from Sjögren's disease have a much higher chance of developing lymphoma. Two-thirds of the patients never have salivary gland enlargement. Other associated systemic problems are primary biliary cirrhosis, chronic hepatitis, vasculitis, chronic graft versus host disease, cryoglobulinaemia, hypergammaglobulinaemic purpura and polyarteritis. Fifteen per cent will have thyroiditis and many will develop pancreatitis.

Investigations:

San Diego Diagnostic criteria for Sjögren syndrome:^[22]

I. Primary Sjögren syndrome

A. Symptoms and objective signs of ocular dryness

1. Schirmer's test less than 8mm wetting per 5 min
2. Positive rose Bengal staining of cornea or conjunctiva to demonstrate keratoconjunctivitis sicca

B. Symptoms and objective signs of dry mouth

1. Decreased parotid flow rate using Lashley's cups
2. Abnormal findings from biopsy of minor salivary glands

C. Serological evidence of systemic autoimmunity

1. Elevated Rh factor
2. Elevated ANA (AntiNuclearAntibody)
3. Presence of anti-SS-A or anti-SS-B

II. Secondary Sjögren syndrome

Characteristic signs and symptoms of primary SS along with clinical features sufficient enough to allow a diagnosis of rheumatoid arthritis, SLE, scleroderma or biliary cirrhosis.

1. Blood examination: The erythrocyte sedimentation rate is usually raised. A protein profile will show elevation of all the immunoglobulins especially IgG. Rheumatoid factor and antinuclear factor will probably be positive and there may well be a wide range of autoantibodies.

2. Specific immunological tests: When class 2 antigens such as HLA A1 and B8 and DR3 are examined, then almost three times as many patients with sicca syndrome have these antigens when compared with patients with the secondary syndrome. Specific antigens for Sjögren's syndrome are called SSA and SSB. Again these are more common in patients with the sicca syndrome than in those with secondary Sjögren's disease with rheumatoid arthritis.

3. Schirmer's test: This is carried out by putting special strips into the lower fornix. Wetting of less than 5 mm in 5 minutes represent a diagnosis of xerophthalmia. A diagnosis of keratoconjunctivitis sicca, however, cannot be made until the ophthalmologist examines the eye with Rose Bengal dye to see the filamentary keratitis.

4. Salivary flow rate: This is measured using Carlsson-Crittenden cups; these are suction cups placed over the parotid duct. Maximum stimulation is created by getting the patient to suck a lemon. A flow of less than 0.5 mL in a minute represents xerostomia.

5. Labial biopsy: This is performed by obtaining four globules of fat from the back of the lower lip. It can be performed under local anaesthetic and is the diagnostic test for Sjögren's disease. The pathologist must grade it according to the rules laid down.

Grade 1: slight lymphocytic infiltration

Grade 2: less than 50 lymphocytes per 4 mm²

Grade 3: 50 lymphocytes per 4 mm²

Grade 4: more than 50 lymphocytes per 4 mm².

The distribution of lymphocytes is important also because they cannot be diffuse, but must be periductal. In this test, false positives can be obtained in rheumatoid arthritis, scleroderma, subacute lupus erythematosus, sarcoid, amyloid and graft versus host disease.^[23]

6. Radiology: Sialography either shows a normal sialographic pattern or that of 'globular sialectasis'. This does not imply that the patients with Sjögren's disease have sialectasis. What it does imply is that there is an abnormality in the duct allowing leakage of lipiodol into the stroma of the gland.

Treatment: There is little of a specific nature that can be done to help these patients. Bouts of parotid swelling may be treated with steroids but the bouts are seldom so severe that they require other immunosuppressive drugs. Artificial tears and synthetic saliva provide limited comfort and bromhexine 40 mg/day sometimes helps a tenacious cough. The most important feature of treatment, however, is to put these patients on a lymphoma follow-up. Those who have parotid enlargement are at a higher risk of developing lymphoma and diagnostic parotidectomy should be considered.

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Displacement of Root Tip into the Bucal Mucosa – Complication during Therapeutic Extraction – A Case Report

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ABSTRACT

Exodontia is one of the most common minor surgical procedures carried out by the general dental practitioners in their daily clinical practice due to various etiologies. Encountering an unforeseen complication during any routine dental office procedure cannot be underestimated. Amongst various common and uncommon complications discussed in the literature, accidental displacement of teeth or roots into the anatomical spaces during extraction is one amidst them. In this article we report a case of an accidental displacement of a root tip into the adjacent buccal mucosa during a therapeutic orthodontic extraction of the right maxillary first premolar.

Key words: Exodontia, Complications, Displacement, Orthodontic extraction.

Introduction

In the era of modern dentistry, teeth can be maintained as long as possible for functional and aesthetic motives. However it becomes certain at times to extract teeth owing to a variety of reasons like dental caries, pulpo-periodontal lesions, and pathologic lesions around the tooth, before radiation therapy, fractures of the crown and root, teeth in line of fracture, malposed, impacted and supernumerary tooth, and therapeutic extractions for orthodontic, prosthodontic and other reasons.¹

An ideal tooth extraction is the painless removal of the whole tooth or tooth-root, with minimal trauma to the investing tissues so that the wound heals uneventfully with minimal postoperative complications. Extraction of tooth basically involves three main principles.²

1) Expansion of bony socket, 2) Lever of first order and 3) Wedging principle.

Delivering a tooth out of the socket involves a thorough understanding of the tooth anatomy, root morphology, the investing soft tissues and proper knowledge about extraction techniques prior to extraction. Extraction of tooth principally falls under two categories,

1) Closed or Forceps or Intra Alveolar Extraction.
2) Open or Surgical or Trans Alveolar Extraction.

A careful preoperative assessment is mandatory to assess difficulties during extraction and minimize complications, which includes a detailed clinical history, procuring and interpreting a proper preoperative radiograph, a definitive treatment plan, and the choice of anaesthesia. Despite these necessary precautions encountering complications becomes inevitable.

Complications though unexpected, increases the morbidity of the procedure, increases the procedure time making it burdensome for the patient and the dental practitioner. Though it is absolutely challenging to cease its occurrence it is imperious for the clinician to be aware of the complications and should be readily available to manage, if any or refer them to counterparts who are

competent enough to manage them.

Complications accompanying dental extraction can range as listed below:²

- Inadequate anaesthesia,
- Postoperative pain and swelling
- Trismus
- Trauma to the adjacent hard and soft tissues
- Hematoma, and haemorrhage
- Infection
- Wound dehiscence
- Failure to remove the tooth or root
- Fractures of the crown, root, alveolar bone, tuberosity, and mandible.
- Displacement of the tooth or root into adjacent soft tissues, anatomic structures, (maxillary antrum, pterygopalatine fossa),
- Oro-antral communication
- Systemic complications

Case Report

A 20 year old female patient reported to the Department of Oral and Maxillofacial Surgery for therapeutic extraction of the maxillary right first premolar. After proper preoperative assessment, anaesthesia was achieved buccally by field block and palatally by infiltration using 2% lignocaine with 1:80,000 adrenaline. After proper evaluation of adequate anaesthesia, mucoperiosteal flap was raised and tooth was luxated using a dental forceps. Tooth extracted was examined and the apical tip of the buccal root was found to be fractured and was observed for the same in the extraction socket. The fractured tooth is shown in picture (fig.1).

Retrieving the fractured root tip was attempted using periosteal elevator, reamers, and apexo elevators which was not successful. After few attempts, upon palpation the buccal socket was found to be empty. With proper clinical evaluation and assessment the root tip was finally palpated at the mucogingival junction in relation to the apex of the tooth extracted.

Patient was informed about the root displacement and was reassured for recovery of the root tip.

The root was palpated and a stab incision was given 5mm above the muogingival junction and the root tip was delivered through the soft tissue with the haemostat (fig.2, 3). The retrieved part was checked and was confirmed for total extraction of the tooth and root completely out of socket (fig.4, 5). Suture was placed in the incision region with 3-0 vicryl. Post-operative instructions given and medications were prescribed. Patient was called for follow up with uneventful recovery and satisfactory healing.

Discussion

Though proper controlled force is applied, fracture of the tooth or root while performing dental extractions can be attributed to the variations in tooth and root morphology, bony anatomy, operator's experience, and patient's age, and gender associated factors.⁵ Retained root fragments in clinical practice necessitates key decision in attempting the removal of these fractured roots which is dependent on the risk benefit ratio for an individual patient.⁴

Maxillary molars and premolars are most commonly fractured tooth during extractions and are most likely to be found during routine radiographic examination. Intra-operative fracture of these tooth are likely due to its curved root morphology, and thin and multiple roots. Excessive force or improper technique or faulty usage of instruments is the most common cause for iatrogenic displacements of root or tooth into anatomical spaces.³

The architecture of the maxilla features a trabecular pattern that is more vulnerable to fractures and depicts non-pathological perforations, which are not rare anatomical variations, so there is a risk of displacement into adjacent anatomical sites.³ The buccinator muscle originates from the alveolar process of the maxilla and inserts inferiorly on the alveolar process of the mandible and posteriorly onto the anterior border of the pterygo-mandibular ligament. The attachment of muscles determines the movement of the displaced tooth into anatomical spaces. The displacement of the root tip can be attributed to the existing fenestration in the maxillary alveolar wall and below the attachment of buccinator muscle exiting through the vestibular space.



Fig.1



Fig.2



Fig.3



Fig.4



Fig.5

Studies have concluded that roots with vital pulp tissue healed effectively with cementum formation over the fractured dentinal surface when excluded from oral fluids.⁴ In consideration to orthodontic extractions retained root tips might deter orthodontic tooth movement, which dictates tooth removal with minimal damage to the buccal and palatal cortical plates. There is no evidence in literature⁴ as to quantify the size of the root that can be left behind.

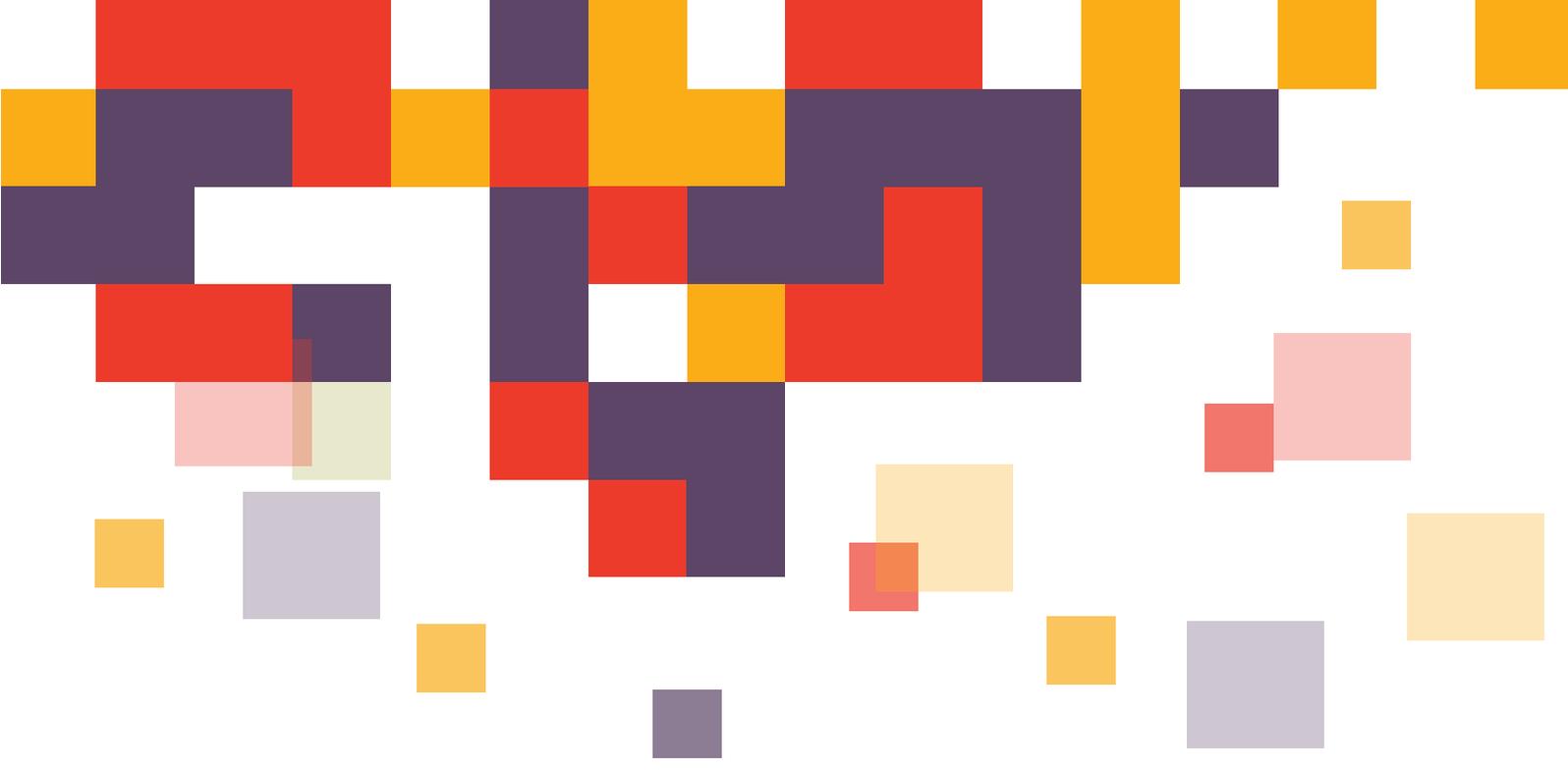
It mandates necessarily to precisely localize the fragment with the aid of radiographs (panoramic or occlusal or CT). Nerve injury or displacement to deeper tissues or damage to the buccal artery or minor salivary glands must be taken into consideration during retrieval procedures. In our case immediate retrieval of root tip was done and a thorough clinical examination and palpation aided to localize the displaced fragment under the mucosa. A stab incision was placed and the root tip was retrieved with a haemostat.

Conclusion

To conclude, exodontia practise may have complications varying from simple to severe ones. During extraction, it is imperative to pay attention to details, protect the soft and hard tissues, and appropriate force to be delivered with caution while using dental forceps and elevators. The clinician must possess the clinical insight to recognize imminent complications and should keep in mind the dictum *"to do no harm"* to serve effectively to the patients and render healthcare at its best as professionals.

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