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THE JOURNAL:

This is an official publication of the KMCT Dental College, Manassery P.O., Mukkom, Kozhikode 673 602, Kerala, India. The journal caters to scientific research and its application to practice for clinicians. This is a quarterly publication, with 4 issues per year.

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SUBSCRIPTION:

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KMCT Dental College
Manassery P.O., Mukkom
Kerala, India

DENTAL BITES

VOLUME 5, ISSUE 3

July-September, 2018

A publication of

Manassery P.O., Mukkom, Calicut-673602, Kerala

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EDITORIAL

This edition of journal comes to you when our state has witnessed one of the worst natural disasters of the century. The recent rains, landslides and floods have played havoc, that left thousands of people homeless. The government machinery, the NGOs and each and every association across the state are trying their level best to tide over this crisis. We the faculty and students as professionals, have social responsibilities to our fellow citizens. It is our duty to reach out to them in times of distress. There's a saying which goes like this-"When the going gets tough, its the tough that gets going".

In these hard times, what spills out of us?- gratitude, humility, empathy, peace? Or does bitterness, anger hatred or reactions? It is up to you to choose. Folks, let's work towards filling our cups with kindness, words of affirmation, gentleness and concern for others. All the obstacles we face today stand definitely nothing to us if we join our hands together. Never expect life to be fair to everyone always. So let's work together to rebuild our state using whatever possible ways and means provided to us.

Dr. Manoj Kumar K P
Chief Editor

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GLIDE PATH – THE TRUMP CARD TO ENDODONTIC SUCCESS!!!

*Dr. Amitha Krishna, **Dr. Ramesh Kumar M, ***Dr. Elsy P Simon, ****Dr. Ravi S V

Abstract

What is a glide path? Why is it important? Glide-path is a smooth radicular tunnel from canal orifice to physiologic terminus (foraminal constriction). Its minimal size should be a "super loose No. 10" endodontic file. The Glide path must be discovered if already present in the endodontic anatomy or prepared if it is not present. Glide path creation is essential for prevention of rotary file separation and most effective rotary use. Creating a .02 tapered glide path is critical for the safe and effective use of nickel-titanium rotary shaping instruments. Routine glide path establishment and enlargement with glide path files can increase the lifespan of rotary instruments with a reduced risk of instrument fracture. Aim is to provide information about glide path with which we can maintain the original canal anatomy with less modification of canal curvature and fewer canal aberrations.

Key Words: glide path, instrument fracture, rotary file separation

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Introduction

In aeronautics, Glide Path is the approach path of an aircraft when landing, usually defined by a radar beam. It's purpose is to establish a tightly controlled horizontal and vertical "corridor" that will take the incoming aircraft to the proper location at the end of the runway. And just as turbulence can cause an aircraft to veer off its Glide Path, in Endodontics the lack of a properly established glide path will often lead to ledge formation, transportation of the natural canal anatomy, and blockage of the canal with dentin debris, or a combination of all three negative consequences. Just as the preparation of the Glide Path lays the groundwork for the proper shaping of the canal, proper access preparation is an essential precursor for identifying the canal orifice (s) and the subsequent preparation of the Glide Path.[1]

The surprising fact is that the subject of Glide path has no formal training in the Endodontic curricula of most Dental schools. The purpose of Endodontics is to prevent or heal lesions of endodontic origin.[2] Cleaning and shaping of the root canal is the single most important phase of endodontic therapy. The goal of root canal instrumentation is to obtain a continuous tapering funnel flowing with the shape of the original canal from the coronal access to the apex. It is the starting point of radicular preparations. Without it, cleaning and shaping becomes unpredictable or impossible because there is no guide for endodontic mechanics.[3]

What is endodontic glide path?

The endodontic Glide path is a smooth radicular tunnel from canal orifice to physic-logic terminus (foraminal constriction). Its minimal size should be a "super loose No. 10" endondontic file. The Glide path can be short or long, narrow or wide, essentially straight or curved.[2] Glide path creation is essential for prevention of rotary file separation and most effective rotary use.[3]

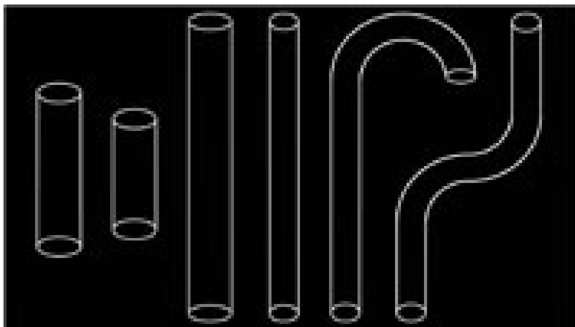
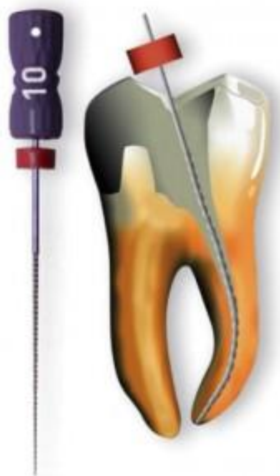


Fig 1: Various glide paths

Why is the endodontic glide path important?

First, without the endodontic Glide path, the rationale of Endodontics cannot be achieved.



The rationale states that "any endodontically diseased tooth can be predictably saved if the root canal system can be non surgically or surgically sealed, the tooth is perio-dontally sound or can be made so, and the tooth is restorable".[3]

Nickel-titanium (NiTi) rotary instruments were introduced to improve root canal preparation.[4] In clinical practice these instruments are associated with an increased risk of fracture, mainly because of bending normal stresses (failure by fatigue) and torsional shear stresses (failure by torque).[5]

Various aspects might contribute in increasing these stresses, such as excessive pressure on the handpiece,[6] a wide area of contact between the canal walls and the cutting edge of the instrument, or if the canal section is smaller than the dimension of the non-active or non-cutting tip of the instrument,[7] the latter case might lead to a taper lock, especially with regularly tapered instruments.[8] The risk of taper lock might be reduced by performing coronal enlargement[9] and creating a glidepath before using NiTi rotary instrumentation,[4] both manual and mechanical.

Once the canal orifices have been identified, and before placing a single instrument into the canal, study the pre-treatment radiograph carefully. Even though the radiographic and physiologic termini of the canals likely do not correspond exactly, understanding what problems you might encounter when negotiating the canals will help you plan the most efficient technique to overcome these potential impediments to successfully creating an effective Glide Path.[1]

What the rationale of Endodontics requires is the entire length of the root canal system be cleaned and shaped. Glide path is pre requisite to this mechanical objective.[2] A glide path is achieved when the file forming it can enter from the orifice and follow the smooth canal walls uninterrupted to the terminus.[10] The glide path assures the operator that the tip of the file will not become locked as it moves apically and that the canal is free and clear of significant debris and blockage, could lead to iatrogenic events.[11]

The lack of glide path establishment may result in:

- Ledge Formation.
- Blockage of root canals.
- Transportation.
- Zip Formation.
- Perforation.

Instruments used

Table 1: Instruments used for glide path

Manual	Mechanical
K- Files	Path Files
C + files	G –files
C- Pilot files	Safe siders
C files	V-files
Pathfinders TM	Hi-5 Files
Senseus Profinders	Pre Shaper
K-Finders	Endo Wave
S-Finders	
D-Finders	
Pathfinders TM CS	

Throughout Glide Path preparation and subsequent shaping of the canal, copious irrigation should be maintained. Sodium hypochlorite is the irrigant of choice and is best combined with a carbamide-peroxide and EDTA-containing gel.[2] The EDTA chelates calcium salts from calcified areas within the root canal, and due to its effervescent properties, it allows for pulp tissue, dentinal shavings, and debris to be easily removed, facilitating the movement of the instruments down the canal.[1]

Methods of preparation of glide path

The skill for consistent Glidepath preparation is to understand and master the 4 manual motions :

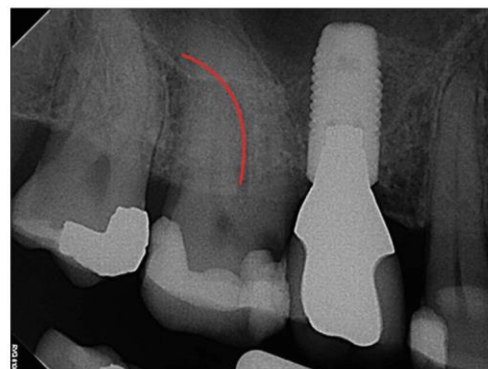


Fig 3: Preparation of glide path

1. **"Follow"** Identify the entrance to the canal and remove any dentin or enamel triangles that are preventing straight-line access. Irrigate thoroughly with sodium hypochlorite before *gently* "slipping and sliding" down the canal. Take the smallest file that fits the canal easily, and slightly pre curve the apical a few millimetres using metal cotton pliers. Allow it to go whatever direction it wants.

2. **"Smooth"** Once RT file position has been validated, make short amplitude vertical stokes until the file is *loose*. If the file is at first too tight to easily make short strokes, ie, the file is apparently binding against 2 or more walls, then wiggle the handle left and

right without any up or down motion. The minimal Glide path file size for safe rotary shaping is a loose No. 10 file. Rotary files rarely glance over shelves or ledges and must be meticulously removed before proceeding. An excellent series of manual files for smooth and progressive Glide path enlargement are the ProFile Series 29 invented by Schilder.

3. **"Envelope**" If the file does not easily "follow" to the RT, stop short of maximum resistance. If you force, you may block or ledge. So, *do not force or push*. The proper next step is to remove the file using the "envelope of motion." The envelope will wear away restrictive dentin by withdrawing and carving to the right, or clockwise, direction. Envelope is the only motion of the 4 manual motions that removes dentin on the outstroke. Your unimpeded files are your eyes in Endodontics. Now "follow" to the RT with your smallest file, smooth, and finish Glide path. If you cannot "follow" to RT, you will almost always at least "follow" closer toward the RT. Envelope again and repeat until you reach RT, smooth, and finish the Glidepath.

4. **"Balance"** Sometimes a file size larger than a super loose No. 10 is desired. The dentist may feel safer with a larger size or the walls may not feel as smooth as possible. If you want to have a smooth No. 15 as your Glide path size, for example, then use balance motion. It is safe and predictable. Originally this motion was referred to as Balanced Force or the Roane Technique, named after Dr. James Roane, the first person to describe this manual motion. Simply put, turn the handle of the file clockwise, and then turn it counter clockwise using slight apical pressure so that the file will not "unscrew" its way out of the canal. During the clockwise motion, the file blades cut into the dentin; during the apical counter clockwise motion, the dentin is collected into the file's flutes. This can be repeated several times as the file is "balanced" apically. The file is then turned clockwise and removed having carved a wider Glidepath. That same file is then used in a "smoothing" motion and the Glide path is once again finished and ready for rotary shaping.

A new approach to increasing Glide path size is mechanically vs. manually. One recent and successful method is the introduction of 3 Path Files .When properly used, these robust and efficient rotary Glide path files can take even further risk out of rotary shaping. As with every dental instrument, the dentist must precisely follow the manufacturer's directions for use.[2]

According to Mounce, there are several advantages for using stainless steel K-files to prepare a glide path:

- K-files have excellent tactile sensation
- Low potential for file separation
- When a small size K-file is removed from the canal, the file often has an impression of the canal, there by guiding the operator to the curvatures present in the canal.
- The stiffness of hand steel files aids in negotiating blockages and calcifi-cations.[12]

In 2006 West recommended using a K-file with an initial watch winding motion to

remove restricted dentin in very narrow canals, followed by a vertical in and out motion with a 1mm amplitude and gradually increasing the amplitude as the dentin wall wears away and the file advances apically. [11] Kinsey and Mounce described a technique using a reciprocating handpiece attached to small size K-file for glide path preparation. The main advantage of using the reciprocating handpiece is to reduce glide path preparation time and hand fatigue with narrow, multiplanar root canals compared to the conventional manual technique.[13]

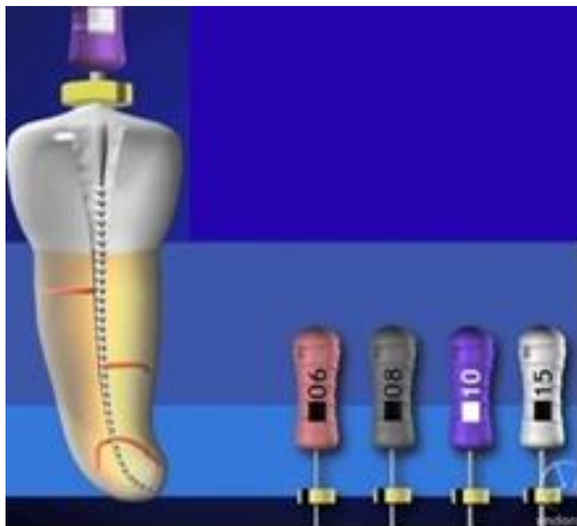


Fig 4: Working with progressively decreasing file sizes to obtain glide path

Rotary

Initiate the preparation of the glide path using a pre-curved No. 10 stainless steel K-type hand file that matches the canal curvature obtained from the preoperative. If canal constrictions prevent the No. 10 K-file from easily reaching the estimated working length (EWL), use a No. 08 K-file along with copious irrigation until it easily reaches the EWL. Because the No. 08 is not as stiff as the No. 10, whatever prevented the No. 10 from reaching the estimated working length initially may distort the No. 08, and you may require a number of instruments to achieve your goal. You may even have to resort to using No. 06 instruments to ensure that you remain within the natural canal and are not creating your own pathway. Try the No. 10 K-file again, and if it reaches the EWL, continue using the No. 10 until it easily reaches the EWL. To facilitate the removal of debris, especially in long and narrow canals, it may be necessary to alternate between the No. 08 and No. 10 files.

In narrow canals, meticulously creating the Glide Path has been a slow and tedious procedure. Once a No. 10 K-file is worked to the EWL, Z-Pathfinder3 nickel-titanium engine-driven files, which have an innovative design comprised of cutting edges on three different radii that leave a large and efficient area for debris removal, and increased flexibility due to their small diameters (No. 12 and No. 17) and their slight 0.03 taper, quickly and efficiently complete the preparation of the Glide Path. The Pathfinders are used by slowly progressing apically without pressure. When resistance is encountered, remove the file from the canal, place a small amount of RC Cleaner in the canal, irrigate with sodium hypochlorite, and again progress slowly toward the apex using the Pathfinder file. In most cases the use of the P1 file with its 0.12 apical tip will be sufficient to allow the placement of a No. 15 K-type file to the EWL. If this is not the case, complete the Glide Path preparation with the P2 Pathfinder. Its 0.03 taper and No. 17 apical

tip size will create a canal shape that will permit the effortless placement of the No. 15 K-type file (0.02 taper and No. 15 apical tip size) to the EWL, and this is important as the No. 15 is the minimum size apical tip that can be properly verified on a radiograph.

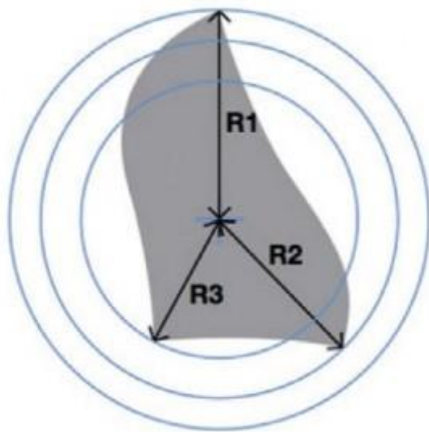


Fig 5: Cross section of the Z-Pathfinder showing the three different radii

Recently introduced G-files (Micro Mega), G1 and G2 are the rotary Ni-Ti files used to create the glide path. The system consists of two rotary instruments that can be used for glide path enlargement after and establishment of a glidepath with a number 10 K-file.[3]

Glide path preparation using a reciprocating hand piece

Establish working length and patency of root canals with 08 or 10 K-file. Select the smallest size K-file that fits tightly into the root canal. Pre-curve the tip of the file and work the file down the canal using a 'watch-wind' motion until the file has reached working length. Attach the M4 reciprocating hand piece to the handle of the file. While keeping the file at working length activate the hand piece. Let the hand piece 'watch-wind' the K-file for 5-10 seconds until you feel that the file becomes loose in the root canal. Keeping the hand piece activated, withdraw the file approximately 0.5mm from canal and move it back to length. Withdraw 1mm and move it passively back to length. This process can be repeated until the file can be withdrawn and moved back to length over a distance of 3-3.5mm. This will confirm glide path preparation with a number 08 K-file. Negotiate (by hand) the next ISO sized precurved K-file in the canal – up to working length. Repeat procedure. Follow the same procedure with size 15 K-file. To check if a glide path was established the clinician must be able to place size 15 or 20 K-file in root canal up to working length, withdraw the file 1.5mm from canal and push it back to working length without any difficulty (by hand). Repeat the above but withdraw the file 3mm and then 5mm from working length. When the file can travel 5mm in the root canal without any obstruction, a successful glidepath preparation is achieved.[14]

Conclusion

Every step in endodontic instrumentation is important in order to achieve the desired end result. However, creating the correct glide path for the canal you are treating will make each of the remaining steps in your treatment protocol much easier to perform, resulting in a cleaner and well-shaped canal that can be ideally obturated.The endodontic glide path is the secret to radicular rotary safety. This article has offered a definition of glide path, explained why it is important in producing optimum endodontic

mechanics, and described how to prepare a glide path for radicular shaping.

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HALITOSIS - A REVIEW

*Dr. Theerdha V Thulasi

Abstract
Halitosis is a term defining disagreeable or offensive odour spread from the breath. Halitosis may occur due to physiological reasons as well as pathological and psychological factor, thus the underlying factors of halitosis should correctly be diagnosed and the patient should be directed to the related physician. Although halitosis has multifactorial origins, the source of 90% cases is oral cavity such as poor oral hygiene, periodontal disease, tongue coat, food impaction, unclean dentures, faulty restorations, oral carcinomas, and throat infections. The most important issue for treatment of halitosis is detection etiology or determination its source by detailed clinical examination. The aim of this review is to describe the etiological factors, prevalence data, diagnosis, and the therapeutic approaches related to halitosis.

Key Words: halitosis, treatment, literature review

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Introduction

Halitosis is a general term used to define an unpleasant or offensive odour emanating from the breath regardless of whether the odour originates from oral or non-oral sources. Other terms used are bad or foul breath, breath malodour, oral malodour, foetor ex-ore, and foetor oris. Halitosis should not be confused with the generally temporary oral odour caused by intake of certain foods, tobacco, or medications. Medications known or suspected of causing halitosis are suplatast tosilate, cyclosporine, and a fish oil derivative in the treatment of Crohn's disease.[1]

Halitosis can be classified into categories of genuine halitosis, pseudo-halitosis, and halitophobia. Genuine halitosis is diagnosed if obvious malodour with intensity beyond socially acceptable level is perceived. If obvious malodour is not perceived by others, although the patient stubbornly complains of its existence, it is diagnosed as pseudo-halitosis. Should a patient, after treating either genuine or pseudohalitosis resulting in no objectively noticeable foul odour, still believe that he or she has halitosis, very likely the diagnosis is halitophobia.[1]

Prevalence

Most adults suffer from genuine halitosis occasionally, while an estimated 10–30% of the population suffers from this problem regularly.[2]In the general population, halitosis has a prevalence ranging from 50% in the USA to between 6% and 23% in china, and a recent study had revealed a prevalence of self-reported halitosis among

Indian dental students ranging from 21.7% in males to 35.3% in females.[3-6] Miyazaki concluded that there was increased correlation between older age and malodor with aging resulting in greater intensity the of odor.[7] In above 60 years age group of the Turkish individuals, the incidence was around 28% .[8]

Classification of halitosis

The International Society for Breath Odor Research established a method of classifying halitosis through scientific analyses.[8,9] The classification system allows the dental team to identify causative factors and establish potential treatment protocols (Tables I and II).

Etiology of halitosis

1. Intra-oral causes

Intraoral conditions are the cause of 80–85% of halitosis cases.[10] Halitosis is formed by volatile molecules which are caused because of pathological or non-pathological reasons, and it originates from an oral or a non-oral source. These volatile compounds are sulfur compounds, aromatic compounds, nitrogen-containing compounds, amines, short-chain fatty acids, alcohols or phenyl compounds, aliphatic compounds, and ketones.

Periodontal infections are characterized by a tremendous increase in Gram-negative bacteria that produce volatile sulfur compounds (VSCs). The association between anaerobic bacteria that produces VSCs and halitosis has been well-documented.[11] Most important VSCs are hydrogen sulfide (H2S), methyl mercaptan and dimethyl sulfide.[12] They produce bacteria by enzymatic reactions of sulfur-containing amino acids which are L-cysteine and L-methionine. In addition, some of the bacteria produce hydrogen sulfide and methyl mercaptan from serum. The dorsum of the tongue is the biggest reservoir of bacteria as a source of malodorous gases.[10] Pericoronitis, oral ulcers, periodontal abscess, and herpetic gingivitis are some of the pathologies that result in increased VSCs. Diamines such as putrescine and cadaverine are also responsible for oral malodor as with the increase in periodontal pocket depth; oxygen tension decreases which results in low pH necessary for the activation of the decarboxylation of amino acids to malodorous diamines.[12]

Odontogenic infections include retention of food debris in deep carious lesions and large interdental areas, malaligned teeth, faulty restorations, exposed necrotic pulp, over wearing of acrylic dentures at night, wound infection at the extraction site and ill-fitting prosthesis. The absence of saliva or hypofunction results in an increased Gram-negative microbial load, which increases VSCs, a known cause of malodor. Several mucosal lesions such as syphilis, tuberculosis, stomatitis, intraoral neoplasia and peri-implantitis allow colonization of microorganisms that releases a large amount of malodors compounds.[12,13] Other contributing factors of halitosis are exposed tooth pulps and non-vital tooth with fistula draining into the mouth, oral cavity pathologies, extractions/healing wounds or prosthetics or dentition factors such as orthodontic fixed appliances, keeping at

night or not regularly cleaning dentures, restorative crowns which are not well adapted, non-cleaning the bridge body, and interdental food impaction.

2. Halitosis of non oral orgin

Nearly 8% of the halitosis cases caused from an extra-oral source. Respiratory system problems, gastrointestinal disease, hepatic disease, hematological or endocrine system disorders and metabolic conditions can all be the causes of halitosis.

Respiratory system problems can be divided into upper and lower respiratory tract problems. They are sinusitis, antral malignancy, cleft palate, foreign bodies in the nose or lung, nasal malignancy, subphrenic abscess, nasal sepsis, tonsilloliths, tonsillitis, pharyngeal malignancy, lung infections, bronchitis, and bronchiectasis lung malignancy.[19-21] Bacterial activity in this pathology causes halitosis which leads to putrefaction of the tissues or causes tissue necrosis and ulcerations and production of malodorous gases, which are expired causing halitosis.[22,23]

Gastrointestinal diseases cause halitosis. Pyloric stenosis, duodenal obstruction, aorto-enteric anastomosis, pharyngeal pouches, zenker's diverticulum, hiatal hernia cause food retention. Reflux esophagitis, achalasia, steatorrhea, or other malabsorption syndromes may cause excessive flatulence or *Helicobacter pylori* infection causes gastric ulcers and VSC levels increase in oral breath. Levels of VCS's in oral breath may be higher in patients with erosive than nonerosive oesophagogastro-duodenal mucosal disease although VSC levels are not influenced by the degree of mucosal damage.[24,25]

Diagnosis of halitosis

Diagnostic methods of halitosis enable differentiation of genuine halitosis, pseudohalitosis and halitophobia. Therefore, assessment of diagnosis and severity of halitosis is important to prevent incorrect or unnecessary.[26]

Organoleptic Measurement

Organoleptic method is accepted as the gold standard for halitosis measurement.[27] The results of organoleptic measurements showed a strong correlation with the breath VSCs levels. The advantages of this method are that it is cheap, that no equipment is needed and that there is a wide range of odour.[28] On the other hand, the test has disadvantages such as subjectivity, nasal saturation, the lack of quantification and the repeatability of the test. In addition, the measurement method is unpleasant for practitioner and patient.[29]

Gas Chromatography

Gas chromatograph (GC), which is an objective, reproducible and reliable method, analyses air, incubated saliva, tongue debris or crevicular fluid for VSCs.[30] In this method the measurements are carried out with instruments equipped with a flame photometric detector or mass spectrum. The concentration of VSC (10 ng/mL) is assessed on the basis of hydrogen sulfide and methyl mercaptan preparations prepared as standard.[31] In practice, the patient is told

to close his mouth and hold his breath for 30 seconds. After the aspiration with the help of a gas-tight syringe of breath is injected into the GC column at 70 °C.

Sulfide Monitoring

In this method, the patient's mouth is first closed for 5 minutes. Then the disposable tubes of the device are placed in the patient's mouth and nose. At the same time when the air in the mouth is collected, the patient is asked to breathe through the nose. Sulfur components in the breath are detected by electrochemical reaction.[32-34] This method is reproducible and easy to use. However, the ability to detect only sulfur-containing compounds can lead to an incorrect assessment of the source and intensity of oral malodor. As oral malodor may also contain substances other than VSCs.[35]

Indirect method to detect halitosis

a) Chemical Sensors

Chemical sensors and sulfur monitors have a similar working principle. The probes of chemical sensors are sulphur sensitive. Sulphur components detected by the probe produce electro-chemical voltage. The voltage measured by the electronic unit is shown as digital scores on the device's screen.[36-38] Chemical sensors, also known as electronic nose, could measure ammonia, methyl mercaptan compounds and each volatile sulphur-containing compound from breath air.

b) Benzoyl-DL-Arginine-A-Naphthylamide Test

Benzoyl-DL-arginine-naphthylamide-BANA test is very useful for clinical practice. Samples taken with a cotton swab from the tongue surface to detect halitosis and subgingival plaque samples taken with curette for periodontal risk assessment are placed on the BANA test strip. The samples placed in the incubator are heated at 55 °C for 5 minutes. The presence of *Treponema denticola*, *Porphyromonas gingivalis* or *Bacteroides forsythus* is proved when the test strip turns blue. There is a positive correlation between the darkness of the blue and microorganism concentration.[39]

c) Quantifying β-galactosidase Activity

Several studies have shown that β-galactosidases are effective in the production of VSCs.[40-42] Halitosis formation begins with deglycosylation of glycoproteins.[41] Glycoproteins are proteolyzed by the removal of Oand N-linked carbohydrates from side chains. One of the main enzymes responsible for this separation is β-Galactosidase.[42,43]

d) Ammonia Monitoring

In this method halitosis measurements can be made with a portable monitor that detects the amount of ammonia produced by the oral bacteria. Physicians ask patients to stop eating and drinking at least 2 hours before measurements. Then patients use the mouth rinse containing urea solution for 30 seconds and close the mouth for 5 minutes. A disposable mouth piece attached to an ammonia gas detector containing a pump that draws 50 mL of air through a tube is placed in the mouth of the patient. Ammonia measurement results are taken from the scale in the detector tube.[45] Ammonia monitoring measurements show similar results with gas

chromatographic method and different results with organoleptic method.

e) Ninhydrin Method

The ninhydrin method is used for the measurement of amino acids and lamines. Isopropanol is added to the saliva collected from the patient and the mixture is centrifuged. Isopropanol, buffer solution (pH 5) and ninhydrin reagent are used to dilute the supernatant. The mixture is refluxed in a water bath for 30 minutes then cooled to 21.8 °C and isopropanol is added to dilute. Light absorption readings are measured with a spectrometer.[39] This method is similar to organoleptic scores and sulphide monitor measurements.[46]

Halitosis Associated Life-quality Test Questionnaire

Halitosis associated life-quality test (HALT) questionnaire was developed to understand the effect of halitosis on quality of life.[47] In addition, this method provides a comprehensive evaluation of the physical, social and psychological negative effects of halitosis.[48] HALT was created with a Likert scale of 0-5; a higher score showed a worsening and a greater impact on an individual's quality of life.[47] This questionnaire consists of 20 specific items and has a maximum score of 100. In addition, the follow up of halitosis treatment is based on the difference in scores between sessions.

Treatment

Proper diagnosis is essential for effective treatment of halitosis. If there is significant periodontal diseases and dental caries, which contribute to halitosis it should be treated. Ensuring adequate oral hygiene is the most important element for oral malodor treatment.[49] Among methods of treatment are chemically and mechanically reducing the amount of microorganisms, products that mask the odor, and chemical neutralization of VSCs.[50]

The primary objective of antimicrobial therapy is to reduce proteolytic, anaerobic flora on the tongue surface. Treatment management should include components such as a tongue scraper that reduce the mechanical load and antimicrobial mouth rinse that reduce the chemical load.[51] Frequently used antimicrobial mouth rinse are chlorhexidine (CHX), essential oils, triclosan and cetylpyridinium chloride (CPC), metal ions and oxidizing agents .The gold standard CHX is also accepted for mouth rinse used for halitosis treatment.[52] By the combined use of CHX and CPC a decrease in the VSCs level was found, due to the reduction of both the aerobic and anaerobic bacterial load.

In a study using a combination of zinc at 0.3% concentration and CHX at 0.025% concentration shows a drop of H2S levels of 0.16%, 0.4%, 0.75% at 1 hour, 2 hour,3 hour respectively. It has also been shown that consumption of daily tablets containing probiotic *Lactobacillus salivarius* WB 21 may help control factors associated with oral malodor.[53]

Discussion

The ultimate goal of halitosis research should be to control this condition in the worldwide population by effective

preventive measurements and treatment. 80–90% of all halitosis cases have an oral aetiology, and in the management of halitosis attention should primarily be paid to all forms of microbial degradation of organic substrates in the oral cavity. The importance of non-oral halitosis should not be underexposed, as the non-oral halitosis might be a manifestation of a serious disease or problem.

Organoleptic measurement, gas chromatograpy and sulphide monitoring are the three primary measurement methods of genuine halitosis. And there difference in their correlation coefficients are explained by the following factors: variability in patients groups (age, race, intensity o halitosis), variability in equipment used (caliberation), differences between judges(calibration, sniffing capacity), and variability in breath gases exhaled by patient.

Variety of parameters are used for the measurement of halitosis and each methods has its specific advantages and shortcomings. Organoleptic measurement is considered as the gold standard among the parameters used for halitosis measurement. Gas chromatograpy are reliable method but expensive and not very practical method. Sulphide monitoring is a relatively inexpensive and easily used method, but has the demerit that important odours are not detected.

Conclusion

Breath malodor has important socio economic consequences and can reveal important diseases. It is estimated 10–30% of the population suffers from genuine halitosis problem regularly and in that approximately 80–90% of all cases halitosis is caused by oral conditions, defined as oral malodour. A proper diagnosis of the etiologic agent will allow proper treatment. It need a multidiciplinary approaches as the cause may not be single or localized.

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METHODS OF ESTIMATION OF ADVANCED GLYCATION END PRODUCTS

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Abstract
Diabetes mellitus leads to the development of complications. One of the major mechanisms of development of these complications is the formation of advanced glycation end products (AGEs). AGEs accumulate in long lived proteins of tissues and cause cross linking and development of inflammation and thickening of basement membranes. This leads to the development of complications like atherosclerosis, retinopathy, neuropathy and nephropathy. Clinical studies have demonstrated that the level of circulating AGEs may be linked to various diabetes complications. Despite intensive investigations, the elucidation of the structure of specific AGE remains a problem. Till now, there is no ideal way to measure various AGE moieties. This review article explains various methods for estimation of advanced glycation end products.

Key Words: diabetes, advanced glycation end products, spectrofluorimetry

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Introduction

WHO reports Diabetes mellitus as one of the most common public health problems which will affect a total population of 220 million worldwide by the year 2020.[1,2] One of the major mechanisms of development of complications in diabetic subjects is the formation of advanced glycation end products (AGEs). AGEs accumulate in long lived proteins of tissues and cause cross linking and development of inflammation and thickening of basement membranes. This leads to the development of complications like atherosclerosis, retinopathy, neuropathy and nephropathy. Diabetic patients have higher levels of AGEs than nondiabetic subjects because hyperglycemia and oxidative stress both contribute to their accumulation. N-carboxymethyl lysine (CML), pentosidine and methylglyoxal (MG) are compounds that commonly are used as AGE markers.

Glycation refers to the nonenzymatic reaction between a protein and a reducing sugar such as glucose or fructose that leads to the formation of advanced glycation end products (AGEs) (Fig. 1).[3] Glycation reaction is also known as the Maillard reaction, after the name of French chemist Louis– Camille Maillard, who described it first in 1912. It is a multi- step process that involves a series of rearrangements and cyclization reactions.[4] In the first step, the free amino groups of amino acids like lysine or arginine present in a protein react with the carbonyl group of reducing

sugars to form the Schiff base. The Schiff base being a highly unstable product undergoes rearrangements to form Amadori product. The Amadori product may subsequently undergo oxidative and nonoxidative degradation or rearrangement, polymerization and other spontaneous reactions, giving rise to a heterogeneous group of substances loosely described as AGEs.[5]

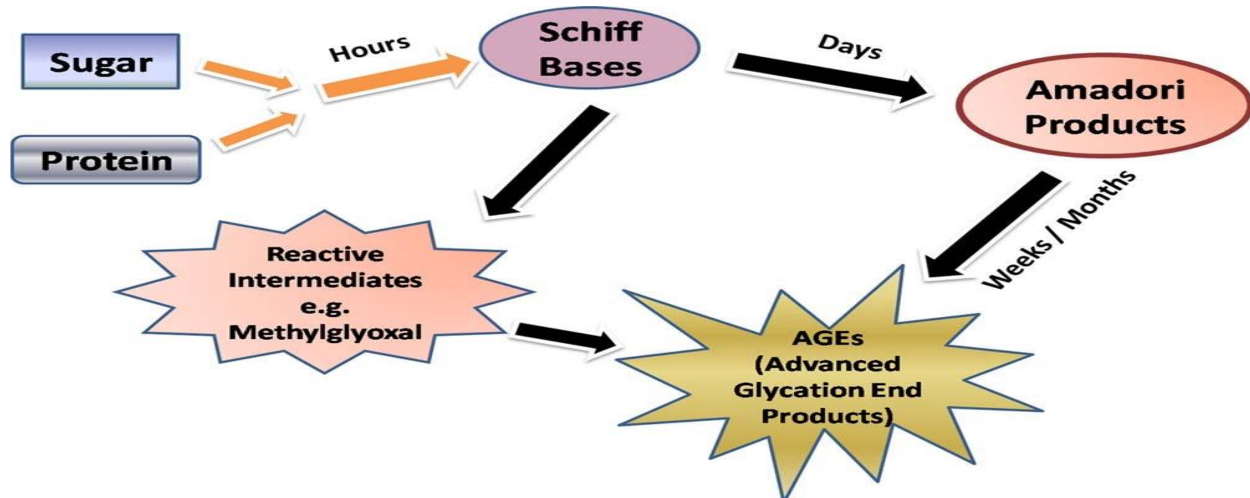

Fig 1: Formation of AGEs

AGEs are divided into the following three main groups:

(i) cross-linking AGEs which fluoresce such as pentosidine and crossline;

(ii) non-cross-linking AGEs like Ne-carboxymethyl- lysine (CML) and

(iii) nonfluorescent cross-linking AGEs like imidazolium dilysine cross-links[7-9]

In addition to the formation of AGEs, glycation also leads to the synthesis of reactive dicarbonyl metabolites such as glyoxal, methylglyoxal and 3-deoxyglucosone (3-DG). Some of the most abundant AGEs are CML, Ne-carboxyethyl-lysine (CEL), fructosyl-lysine (FL), hydroimidazolones derived from glyoxal, methylglyoxal such as G-H1, MG-H1 and 3DG-H, and the imidazolium cross-links that are derived from glyoxal, methylglyoxal and 3-deoxyglucosone (MOLD, GOLD and DOLD).[10]

General mechanisms through which the AGEs exert their action include the following:

(i) by altering the structural features of a protein via forming cross-links, interacting with their receptors RAGEs (receptors for AGEs) which belong to the family of immunoglobin and are found on the cell surfaces of macrophages, monocytes, vascular smooth muscle cells and endothelial cells and

(ii) by intracellular accumulation as illustrated in Fig. 2.[11]

Measurement of the products of non-enzymatic glycation has a twofold meaning: on one hand, measurement of early glycation products can estimate the extent of exposure to glucose and the subject's previous metabolic control; and on the other hand, measurement of intermediate and late products of the glycation reaction is a precious instrument in verifying the relationship between glycation products and tissue modifications.[12] AGEs can be measured by a variety of techniques including Spectrofluorimetry, ELISA, HPLC or mass spectrometry.[13] The purpose of this review is to evaluate the different methods available to detect the advanced glycation end products.

Methods

A. Spectrofluorimetric Methods

Determination of total advanced glycation end products (AGEs):

To 100 µl samples add 10 µl of 100 % (w/v) TCA in each tube. The supernatant containing sugar, test sample and the interfering substances was removed after agitation and centrifugation (15000 rpm, 4 °C), then the precipitate of AGEs-BSA was dissolved with 400 µl buffer (PBS) to serve for screening. The fluorescence intensity of glycated materials was measured at 370 nm excitation and 440 nm emission using Varian spectrofluorometer, Cary Eclipse model. The IC50 (the concentration that resulted in 50% inhibition of the activity) was estimated for each test sample from the least-squares regression line of the logarithmic concentration plotted against the remaining activity.[14]

% inhibition = 1 _ (fluorescence of the solution with inhibitors/fluorescence of the solution without inhibitors) X 100%.

In vitro glycation of bovine serum albumin

The bovine serum albumin (BSA, 10 mg/ml) was incubated in fructose (100 mM) and sodium azide (0.02 %) with or without CuSO4 (100 µM) in 0.2 M phosphate buffer (pH 7.4).The samples in capped vials were protected from light and incubated at 37°C for 21 days. Samples were then dialyzed against sodium phosphate buffer at 4°C for 48 h. This removed reversibly bound and unbound sugars from the BSA solutions. Following dialysis, the samples were stored at -20 °C in small aliquots prior to analysis. The dialysis experiments were repeated three times and the results were similar. After dialysis, the protein concentration of samples was determined in triplicate by BSA assay.

BSA-glucose assay

To 5 g BSA and 14.4 g D-glucose were dissolved in phosphate buffer (1.5 M, pH 7.4) to obtain the control solution with 50 mg/mL BSA and 0.8 M D-glucose. 2 mL of the control solution was incubated at 370C for 7 days in the presence or absence of 1 mL of test sample in phosphate buffer (1.5 M, pH 7.4). The test solution also contained 0.2 g/L NaN3 to assure an aseptic condition.[15,16] AG (1 mm) and rutin (100 lM) were used as positive controls. After 7 days of incubation, fluorescent intensity (excitation, 330 nm; emission, 410 nm) was measured for the test solutions. Percent inhibition of AGE formation by test compound was calculated using the

following equation, % inhibition = 1 _ (fluorescence of the solution with inhibitors/ fluorescence of the solution without inhibitors) X 100%.

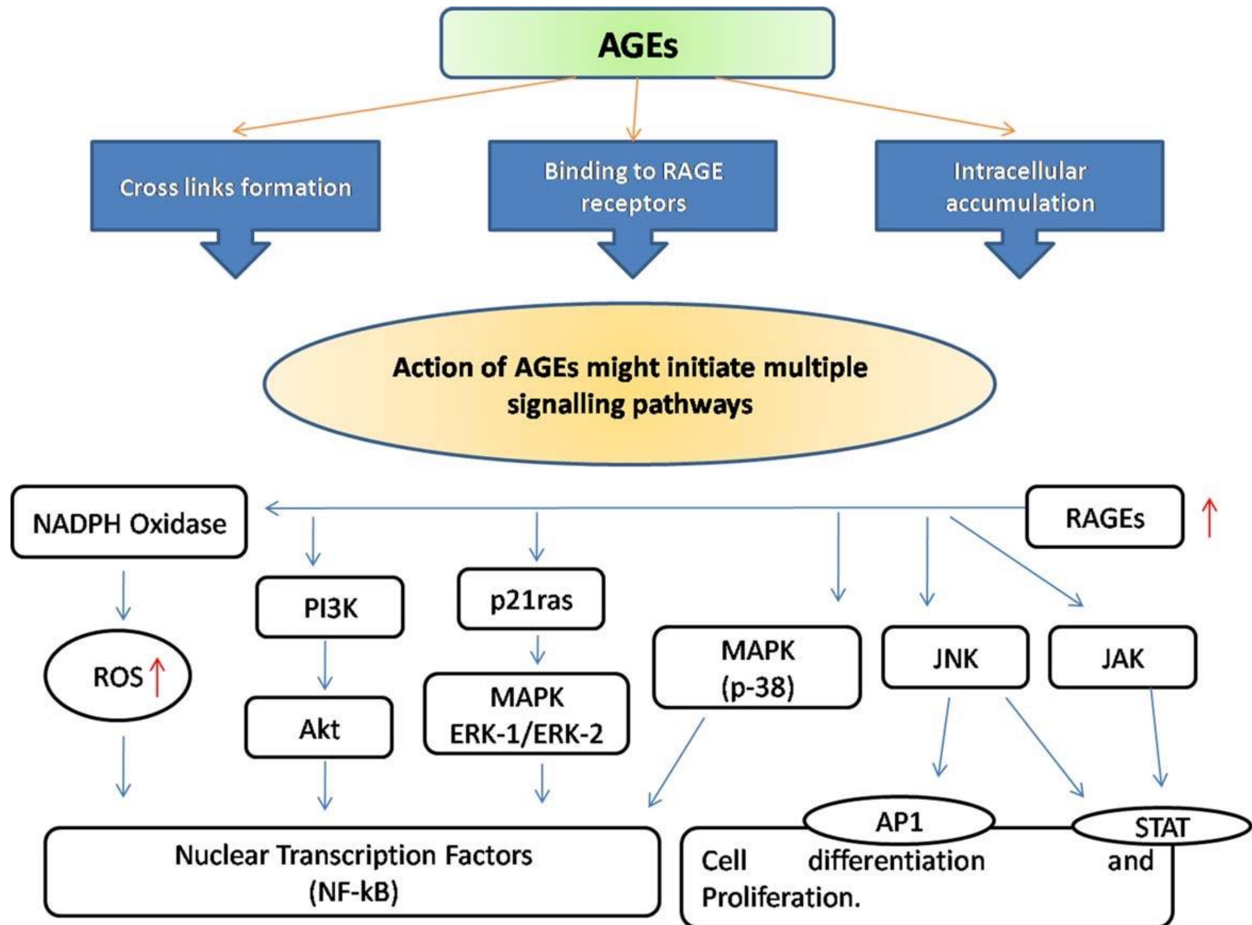

Fig 2: Downstream signal cascade activation of multiple signaling pathways through AGE–RAGE interaction

BSA-MGO assay

To 40 mg BSA mixed with 31 µL MGO in phosphate buffer (0.1 M, pH 7.4) to obtain the control solution with 1 mg/mL BSA and 5 mM MGO. 2 mL of the control solution was incubated at 370C for 6 days with/without test sample in phosphate buffer.[17] The test solution also contained 0.2 g/L NaN3 to assure an aseptic condition. AG (1 mM) and rutin (100 lM) were used as positive controls.

% inhibition = 1 _ (fluorescence of the solution with inhibitors/ Fluorescence of the solution without inhibitors) ×100%.

In vitro glycation of proteins

A 10% homogenate of goat lenses was prepared in phosphate buffer saline, pH 7.4 and centrifuged at 10,000 g for 30 min at 4oC. The lens total soluble protein (TSP) was used for in vitro glycation. Each 1ml reaction mixture contained 10 mg of TSP, 0.2M phosphate buffer, pH 7.4, 0.1M-

fructose, 50 mg of penicillin and streptomycin and 0•01% sodium azide. Reaction tubes were incubated in the dark at 37oC for 3 weeks. At the end of the incubation, unbound sugars were removed by dialysis against the same buffer. Protein concentration was determined by the Lowry method using BSA as standard.[18] Stock solutions of all the reaction contents were filtered through 0.20 mm syringe filters.[19-21]

Estimation of Serum AGEs

The blood was centrifuged and the serum was diluted with 1:50 ratio with phosphate buffer saline at ph 7.4. The amount of AGE was measured in a spectroflurometer then fluorescence intensity was measured at the exciation wavelength 350nm and emission 440nm against PBS.[22]

Urinary AGE estimation

A fasting urine samples were centrifuged and stored at -800C until assayed. To measure the urinary fluorescent AGEs, the urine samples were diluted to 1:10-1:200 by phosphate buffer saline, and the fluorescence intensity was measured at 440 nm after excitation at 370 nm with a 96-plate spectrophotofluorimeter (Spectra Max Gemini EM; Molecular Devices, Sunnyvale, CA) at room temperature. The fluorescence was expressed as the relative fluorescence intensity in arbitrary units (AU).[23,24]

Estimation of retinal AGEs

The retinal tissue was homogenized in Tween buffer pH-8, followed by centrifugation at 8000g for 15 min at 40C. Estimate the protein levels in soluble protein in homogenate. Dilute the protein up to 1mg/ml and the amount of AGE was measured in a spectroflurometer at an excitation/ emission wavelength of 370/440 nm against buffer blank. BSA preparation (1 mg/ml in distilled water) was used as a reference, and its fluorescent intensity was defined as 1 arbitrary unit (AU).[25] The fluorescence intensities of the samples were measured and expressed as arbitrary units (AU)/mg protein.

Estimation of renal AGEs

Kidney was minced and delipidated by shaking gently with mixture of chloroform and methanol (2:1 v/v) overnight. The delipidated tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000g for 15 min at 40C. Estimate soluble protein in homogenate and dilute the protein up to 1mg/ml and the amount of AGE was measured in a spectroflurometer at an excitation/ emission wavelength of 370/440 nm against 0.1 N NaOH blank, BSA preparation (1 mg/ml in distilled water) was used as a reference, and its fluorescent intensity was defined as 1 arbitrary unit (AU). The fluorescence intensities of the samples were measured and expressed as arbitrary units (AU)/mg protein.[26]

B. HPLC methods

Estimation of AGEs in serum and urine

The serum samples were diluted 1:25 in phosphate-buffered saline (PBS). Size selective permeation chromatography was performed in a Merck-Hitachi HPLC system, using a Superdex 75 10/300 column (Amersham Bioscience, Uppsala, Sweden), eluted at 0.5 mL/min with PBS. Fluorescence signals in mV were recorded

in a Merck-Hitachi detector (F-1080), at 350 nm excitation and 430 emission wavelengths. A molecular weight calibrator was employed (Molecular Weight Marker kit MW-GF-70, Sigma-Aldrich, St Louis, USA), containing the following proteins: bovine serum albumin (BSA) (66 kDa), carbonic anhydrase (29 kDa), cytochrome c (12.4 kDa) and aprotinin (6.5 kDa). In the tested samples, the molecular weights of the chromatographic peaks obtained were assigned, according to their retention times, using a semi-logarithmic regression curve based on the molecular weight standard proteins. The reproducibility of the assay was ensured by injecting an AGE- Bovine Serum Albumin standard (catalogue No) 121800, EMD Biosciences, La Jolla, CA) together with the subjects samples.[27]

For urine samples a similar procedure was employed, except for dilution (1:5) and elution at 0.7 mL/min. As all urinary signals were beyond the separation range of the column, their molecular weight was not identified by the molecular weight standards employed. Data were expressed as area under the curve (AUC)/ total serum protein or urinary creatinine respectively.

For detection of serum and urine small sized AGE-peptides, a flow injection assay

Flow injection assay in the HPLC system was employed. The samples were treated with trichloroacetic acid, and then centrifuged, and the aqueous layer was injected at a flow rate of 0.5 mL/min into the flow system, driven by a Merck-Hitachi L-6200 pump to the fluorescence detector. The 50mg/L of AGE-BSA after hydrolysis with proteinase K as a standard (Mp De LM, 2007). Results were expressed as fluorescence intensity/g creatininuria x 1010.

Estimation of pentosidine

Plasma or dialysate protein was subjected to acid hydrolysis. Protein was precipitated on ice with 10% TCA (Tri Chloro Acetic Acid). The pellets were washed twice with 5% cold TCA and acid hydrolyzed in 1 ml 6 N HCl for 16 h at 1100 C. Acid was removed by vacuum centrifugation, The hydrolyzed pellet was dissolved in 250 ml of water/0.01 M heptafluorobutyric acid. The hydrolysate was filtered with 0.45- mm nylon microfilterfuge tube. The equivalent of 0.8 mg of plasma protein was injected onto an HPLC system, Waters Division of Millipore.[28] The column used was a 25×0.46 cm C-18 Vydac type 218 TP (10 μm). The HPLC was programmed with a linear gradient from 0 to 35 min of 10–17% acetonitrile in HPLC water and 0.1% heptafluorobutyric acid as a counter ion. Pentosidine eluted at ~30 min as monitored by fluorescence excitation at 335 nm and emission at 385 nm.[29] Results were calculated per milligram added protein or milligram collagen protein.

C. ELISA techniques

The measurement of CML

The antigen was diluted to 10 μg/mL in 50 mM sodium carbonate buffer, pH 9.5–9.7 and loaded in a 96-well polystyrene plate (0.2 mL per well). The plate was coated overnight at 40C. After coating, the wells were washed three times with PBS (phosphate buffer saline) and then blocked

with gelatin for 2 h at 370C. Rabbit anti-CML antibody was diluted at a titer of 1:500 in antibody diluent and loaded in each well.[30] After the overnight incubation at 40C, wells were washed three times with NP-40. An alkaline phosphatase conjugated antibody to rabbit IgG was then added as the secondary antibody at a titer of 1:1000 in antibody diluent, incubated for 2 h at 370C and washed with NP-40 again. The wells were developed with p- nitrophenyl phosphate substrate solution (pH 10.4). The reaction was terminated by adding 2 M sodium hydroxide and the absorbance at 405 nm was determined by a micro-plate reader.[31,32]

Determination of CML

BSA (2mg/mL) and glyoxal (5 mM) were incubated with test sample in PBS at 370C for 2 days, followed by the determination of CML by ELISA.[33] The 96-well microtiter plate was coated with 100 with PBS containing 0.05% Tween 20 (washing buffer). The wells were incubated with 0.1 mL of anti-CML antibody, 6D12 (1 μg/mL), or anti-pentosidine antibody (1 μg/mL) dissolved in washing buffer for 1 h. The wells were then washed with washing buffer three times and reacted with HRP-conjugated anti-mouse IgG antibody, followed by reaction with 1, 2-phenylenediamine dihydrochloride. The reaction was terminated by the addition of 0.1 mL of 1M sulfuric acid, and the absorbance at 492 nm was read by a micro-ELISA plate reader. The CML content of the samples were quantified by acid hydrolysis with 6N HCl for 24 h at 1100C, followed by amino acid analysis on a Hitachi L-8500A instrument equipped with an ion-exchange HPLC column (2622 SC, 4.6×80 mm; Hitachi) and a ninhydrin postcolumn detection system, as described previously.[34]

Plasma AGE evaluation

The Hanson Advanced Glycosylation End Products immunoassay kit (Hanson Hong Biomedical Co, Ltd, Taipei, Taiwan) was used and the results are expressed as unit/mL. Anti- AGE reagent (0.25 mL) was added to each labeled colorimetric tube and the OD value (H1) at 340 nm was recorded for each tube. Each serum was diluted 1:4 after standing for 30 min. Diluted serum (15 mL) was added to the differently labeled colorimetric tubes that contained 0.25 mL of the anti -AGE reagent from which the OD value had been recorded as H1. The OD value was obtained at 340 nm immediately after 360 sec incubation; this value was recorded as the H2 value. To obtain the true OD value of the diluted serum in accordance with the standard curve, the following equation was used: H2 - H1 = H0. This value for the diluted serum was multiplied by 4 to obtain the final result.[35]

Estimation of renal CML

The kidney was homogenized in ice-cold buffer (0.1 mmol L−1 KH2PO4/K2HPO4, pH 7.0, plus 29.2 mg ethylenediamine tetraacetic acid in 100mL of distilled water and 10mg digitonin in 100mL of distilled water, final volume, and 2,000 mL) to produce a homogenate. The kidney homogenates were then centrifuged at 10,000 g for 10 min at 4ºC. Then, the supernatant was tested for CML using the anti-CML rat autoantibody ELISA kit which

employs the semi quantitative enzyme immunoassay technique. The absorbance of the resulting yellow product is measured at 450 nm.

D. LC-MS technique

AGE analysis

Pentosidine, GOLD, MOLD, CML were evaluated on total tissue extracts after hydrolysis with 0.6M trichloroacetic acid and 50 µl of hydrochloric acid 6M for 12h at 60°C. The chromatographic separations were run on an Ultimate 3000 HPLC (Dionex, Milan, Italy) coupled to a high-resolving-power mass spectrometer LTQ Orbitrap (Thermo Scientific, Rodano, Italy), equipped with an atmospheric pressure interface and an ESI ion source. The samples were analyzed by using a Reverse Phase C18 column (Phenomenex Synergi 150×2.1 mm, 3 µm particle size) at a flow rate of 200µl/min. A gradient mobile phase composition was adopted: 95/5 to 40/60 in 25 min, 5 mm heptafluorobutanoic acid/ acetonitrile.[36]

E. Western Blotting

AGE modified proteins in 1D-SDS gels 3 µl test samples (20–30 µg total protein) were mixed with 1 µl of 4× sample buffer (0.125 M Tris-HCl, 2% SDS, 40% v/v glycerol, 0.8% bromophenol blue, pH 6.8). Following incubation at AT for 10 min, samples were loaded onto a pre-cast Bio-Rad 4%–12% Bis-Tris 1.0 mm mini gel. Electrophoresis was performed at 100 V in running buffer (25 mM Tris base, 0.1% SDS, 192 mm glycine, pH 8.3) until the dye front reached the end of the gel. After soaking the gel in equilibrating buffer (25 mm Tris base, 192 mM glycine, 20% methanol, pH 8.3) for 30 min, the proteins were electrotransferred to NC membrane using a Bio-Rad mini-gel transfer apparatus in transfer buffer (250 mm Tris base, 1.92 M glycine, 20% methanol, pH 8.3) at 100 V, 4°C for 1 h. The membrane was washed twice in MilliQ water, then blocked for 2 h with protein-free blocking buffer at AT. Following three rinses with TBST, the membrane was incubated with rabbit anti-human AGE polyclonal antibody, goat anti-rabbit IgG (diluted 1: 40,000 in protein-free blocking buffer) and substrate as in dot-immunobinding assay. Bands were visualized and analyzed as described above.[37] The membranes were stained with Ponceau S, a general protein staining dye, after western blotting to visualize the major proteins in test samples.

AGE modified proteins in Dot-immunobinding assay

The test samples were diluted 1:20 in PBS (pH 7.4). The 4 µl of the diluted test samples and various concentrations of glycated BSA were dotted on nitrocellulose (NC) membrane at 1 cm intervals and allowed to dry for 1 h at ambient temperature (AT). Unreacted protein binding sites on the membrane were blocked by immersing the membranes in 3% BSA in Tris-buffer saline (TBS, 10 mM Tris base and 150 mM NaCl, pH 7.5) and incubated for 2 h at AT, followed by washing three times with 0.05% Tween-20 in TBS (TBST). NC membranes were incubated with rabbit anti-human AGE polyclonal antibody diluted 1:1,000 in blocking solution containing 0.5% BSA (BMBA) for 2 h at AT. After washing three

times with TBST, the membrane was incubated with secondary antibody (goat anti-rabbit IgG peroxidase-labeled; Bio-Rad, Hercules, CA) diluted 1:10,000 in BMBA for 1 h at AT. Luminal/enhance and peroxidase buffer solutions in a 1:1 ratio were added to the membrane after another three washes and incubated for 3–5 min. The chemiluminescent spots were detected using a Versa Doc Imaging System (Bio-Rad). Quantity-one software (Bio-Rad) was used to analyze the image. Standard curves were generated using the AGE-BSA standards (1,000, 500, 250, 125, 62.5, 31.2 and 15.6 ng/ml) and were used to calculate the concentrations of AGE modified proteins in test samples. The results were also divided by total protein concentration to convert to amount (μg) of AGE modified proteins per mg of total proteins.[38]

Conclusion

Measurement of early glycation products has shown helps in the metabolic monitoring of diabetic patients. This will certainly provide important information on the pathogenesis and progression of chronic complications in diabetic patients and guide physicians and dentists in providing treatment for them.

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GUIDED TISSUE REGENERATION IN PERIAPICAL SURGERY

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Abstract
Most periapical radiolucent lesion associated with infection of the root canal system will heal uneventfully after endodontic treatment. However, some case may require peri radicular surgery in order to remove pathological tissue from the periapical region and simultaneously eliminate any source of infection that could not be removed by orthograde root canal treatment. The major objective of this surgery is to obtain peri radicular tissue regeneration, including the formation of a new attachment apparatus, by exclusion of any potentially noxious agent within the physical confines of the affected root. The use of guided tissue regeneration (GTR) techniques has been proposed as an adjunct to endodontic surgery in order to promote bone healing. Studies assessing the added benefits of GTR for the outcome of endodontic surgery are significantly variable in their treatment protocols, follow-up periods, and inclusion criteria, thus generating inconsistent and confusing results. GTR techniques favourably affected the outcome of surgical endodontic treatments in cases of large periapical lesions and through-and through lesions. Additional large-scale prospective clinical studies are needed to further evaluate possible benefits of GTR techniques in endodontic surgery.

Key Words: guided tissue regeneration, periapical surgery, endodontic surgery

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Introduction

The American Academy of Periodontology has defined guided tissue regeneration (GTR) procedures as those used to regenerate lost periodontal structures through differential tissue response.[1] It is a technique for enhancing and directing cell growth to repopulate specific parts of the periodontium that have been damaged by periodontal diseases, tooth diseases, or trauma.[2] In periodontology, guided tissue regeneration (GTR) principle using a barrier membrane has been extensively studied and successfully used. Application of guided tissue regeneration concepts to periapical surgery is primarily based on extensive studies of periodontal regenerative therapy.[3] The ultimate goal of GTR in periapical surgery is, regeneration of periradicular tissues including cementum, periodontal ligament, and alveolar bone.

GTR achieved better results in through and-through lesions compared with lesions breaking through the buccal or lingual wall only.[4] Numerous studies on the clinical effectiveness of GTR techniques to promote healing and improve the outcome of surgical endodontic treatments have been published.

The objectives of membrane application in endodontic surgery resemble those in periodontology and implantology:

(1) facilitate tissue regeneration by creating an optimum environment (stable and protected wound); and

(2) exclude non desired fast-proliferating cells that interfere with desired tissue regeneration.[5]

Biology of Periapical Wound Healing After Periapical Surgery

The principle of periapical wound healing after periapical surgery is similar to that of connective tissue wound healing elsewhere in the body. It is a host's ''programmed event,'' which begins with (1) hemostasis or coagulation phase, (2) inflammation phase, (3) proliferative phase, (4) regeneration and/or repair phase, and last (5) remodeling or maturation phase. Wound healing usually involves recruitment and differentiation of progenitor/stem cells into tissue committed cells. Wound healing can result in either regeneration or repair. Regeneration represents the replacement of damaged tissue by the cells of the same tissue. Repair represents the restoration of the destroyed tissue by new tissue different from the original tissue. It does not reconstitute the architecture and functions of the original tissue.[2]

Regardless of the size of periapical lesions, persistence of root canal infection is the primary cause of inflamed periapical tissues not to heal after endodontic therapy.[7] Complete periapical wound healing after periapical surgery should include regeneration of alveolar bone, PDL, and cementum.

For regeneration of the periapical tissues after periapical surgery, one of the important requirements is recruitment and differentiation of progenitor/ stem cells into committed pre-osteoblasts, pre-PDL cells, and pre-cementoblasts. Homing of progenitor/stem cells into the wounded periapical tissues is regulated by factors such as stromal cell–derived factor 1, growth factors/cytokines, and by microenvironmental cues such as adhesion molecules and extracellular matrix and associated noncollagenous molecules.[2]

Factors that may affect healing of periapical lesion

1. Lesion Size

In small periapical lesions, resident osteoblasts, periodontal ligament cells, and cementoblasts might be capable of restoring damaged periapical tissues. However, in large periapical lesions, periapical wound healing requires recruitment and differentiation of progenitor cells/stem cells into osteoblasts, cementoblasts, and periodontal ligament cells. Andreason and Rud proposed that if the size of the osseous defect is too large, osseous regeneration of the wound will not occur and the defect will heal by fibrous connective tissue repair.[6]

2. Lesion Type

The results of the current studies suggest that GTR may be beneficial for through-and-through lesions, whereas there was no significant advantage for the use of GTR for four-wall defects. A possible explanation may be related to the colonization of the healing wound by periodontal progenitor cells, a prerequisite for the formation of new

cementum, a new periodontal ligament apparatus, and new alveolar bone. A key factor for this process is the periosteum because it may act both as a source of osteocompetent cells and as a barrier against the infiltration of epithelial cells into the healing site. The use of a barrier for the treatment of large defects or ''through-and-through'' lesions may be advised because it is aimed to improve the self-regenerative process by blocking undesired proliferation of gingival connective tissue or migration of oral epithelium into the defect.[6]

Membrane barriers in periapical surgery

The application of a membrane barrier in periodontal regenerative therapy is to prevent apical migration of gingival epithelial and connective tissue cells onto the denuded root surface and to facilitate the repopulation of the damaged root surface with PDL progenitor/ stem cells to differentiate into PDL cells and cementoblasts.

The epithelial cells from gingivomucosal epithelium are not able to proliferate into the apical bony defect after periapical surgery if there is no periodontal involvement. A membrane barrier will not selectively allow specific cell types, such as PDL progenitor/ stem cells, bone marrow mesenchymal stem cells, or endosteal osteoprogenitor cells, to repopulate a root surface damaged by apical periodontitis or by surgical root resections during periapical surgery.

There are three types of cases encountered when performing endodontic microsurgery where consideration should be made as to whether utilizing guided tissue regenerative materials will make an impact.

Case Type 1: Uncomplicated Osteotomy

The uncomplicated osteotomy represents surgical cases that have only an isolated endodontic lesion; there is no periodontal defect. The majority of outcome studies in endodontic surgery are cases that are selected to have these isolated endodontic lesions, and in these studies there is no utilization of GTR materials. Studies on the outcome of endodontic microsurgery find success rates in the 93% range when no GTR techniques are utilized, indicating that in cases where an isolated endodontic lesion is present, there is no increase in outcome and no change in the favour of regeneration versus repair whether a graft or membrane or bioactive agent is utilized in conjunction with contemporary microsurgical techniques.

Case Type 2: Complicated Osteotomy

Complicated osteotomy cases represent those where an isolated endodontic lesion is present with no periodontal component, but the lesion size is considered large and/or the buccal and palatal/ lingual plates of bone are resorbed (through and through) lesions. Animal studies as well as histologic evidence strongly support the use of a graft and membrane in complicated osteotomy cases.

Case Type 3: Periodontal Involvement

The final type involves cases where a defect of the supporting alveolar bone is present, such as a denuded root where no buccal plate is present initially or following degranulation or resection. The evidence indicates that a periodontally involved case benefits from the application of GTR in three ways: Facilitated healing, increase in success rate, and an improvement in the

periodontal status of the tooth. Clot stabilization has been attributed to the benefit of GTR with regards to a facilitated healing process.

Scar tissue formation is a pathologic process occurring during tissue repair and is sometimes observed in through-and-through human inflammatory periapical lesions after endodontic surgery .In animal studies at 3- to 5-month observation periods, if membrane barriers were not used to cover the bony defects both buccally and palatally/lingually, when through-and-through osseous defects were created in jawbones, the defects were filled with fibrous connective tissue. This is most likely due to the lack of available osteogenic progenitor/stem cells rather than to fast movement of fibroblasts. It has been suggested that immune regulation, up-regulation of collagen production by myofibroblasts, down-regulation of matrix metalloproteinases, and up-regulation of transforming growth factor (TGF)–b, as well as dysregulation of apoptosis of myofibroblasts might play an important role in fibrosis or scar tissue formation. Studies have shown no difference in bone tissue healing if only the buccal cortical plate was destroyed, whether or not a membrane barrier was used in periapical surgery.

Clinically, the best application of membrane barriers in periapical surgery appears to be in combined endodontic-periodontal or periodontal-endodontic lesions[8] or large periapical lesions communicating with the alveolar crest. In this kind of apicomarginal bony defect, the PDL and cementum are destroyed. Accordingly, application of a membrane barrier is indicated during periapical surgery to prevent apical migration of junctional epithelium along the denuded root surface into the periapical wound and to induce selective repopulation of cells of the connective tissue attachment. In combined endodontic-periodontal or periodontal-endodontic lesions, the use of a membrane to manage the lesions is directed at the periodontal tissue rather than periapical tissue regeneration. Oh et al have presented an excellent review of the application of guided tissue regeneration in combined endodonticperiodontal lesions with apicomarginal bony defects. It should be noted that a true new connective tissue attachment can only be demonstrated by means of histologic rather than clinical examination.[9]

In case dehiscence, the nature of a dehiscence (naturally occurring or pathologic) is an important factor in determining whether application of a membrane barrier is necessary. If dehiscence is naturally occurring, a fibrous connective attachment is present between the root surfaces and the mucosa. A membrane barrier is not required during periapical surgery because fibrous connective tissue reattachment will occur onto the root surface after reposition and suturing of a surgical flap. However, if the cause of dehiscence is pathologic as a result of marginal periodontitis, a membrane barrier is suggested to prevent apical migration of junctional epithelium along the root surfaces during periapical surgery.[10]

Types of barrier membranes

The barrier membranes used for GTR can be broadly divided into three generations of membranes.

1. First Generation Membranes

The first generation of barrier membranes developed in the 60s and 70s aimed to achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host. In the first GTR attempts, a bacterial filter produced from cellulose acetate (Millipore) was used as an occlusive membrane by Nyman et al., in 1982. Later studies have utilized membranes of expanded polytetrafluoroethylene (e-PTFE) specially designed for periodontal regeneration (Gore Tex Periodontal Material). Other non-resorbable membranes are titanium reinforced ePTFE, high-density-PTFE, or titanium mesh. The major drawback is the need for second surgery for the removal of the membrane.[11]

2. Second Generation Membranes

The second generation of barrier membranes was designed to be resorbable to avoid the need for surgical removal. There are two broad categories of bioresorbable membranes: the natural and the synthetic membranes. Natural membranes are made of collagen or chitosan. Several complications, such as early degradation, epithelial downgrowth along the material, premature loss of material, were reported following the use of collagen membranes.[11]

Synthetic barrier materials made of polyesters [e.g., poly (glycolic acid) (PGA), poly (lactic acid) (PLA), poly(-caprolactone) (PCL), and their copolymers] were evaluated in animal and human studies and are commonly used.

3. Third Generation Membranes

As the concept of tissue engineering has developed, third-generation membranes have evolved, which not only act as barriers but also as delivery devices to release specific agents such as antibiotics, growth factors, adhesion factors, etc., at the wound site on a time or need basis in order to orchestrate and direct natural wound healing in a better way.

Barrier membranes with Antimicrobial activity

Incorporation of 25% doxycycline into a GTR membrane, which was composed of polyglycolic acid and polylactic acid, would seem to have a beneficial effect on periodontal bone regeneration in dogs.

When applied clinically, tetracycline-loaded expanded polytetrafluoroethylene (ePTFE) membranes reduced bacterial contamination and increased clinical attachment gain.[11]

Barrier membranes with Bioactive Calcium Phosphate incorporation-

Studies on the membrane prepared by Liao et al., demonstrated that the addition of nano-carbonated hydroxyapatite (nCHAC) improved both the biocompatibility and the osteoconductivity of the membrane. This three-layered membrane had a porous side (to allow cell in growth) which contained nano-carbonated hydroxyapatite/ collagen/ PLGA, a pure PLGA non-porous side (to

discourage cell adhesion), and a transitional layer consisting of nCHAC/PLGA.[11]

Barrier membranes with Growth Factor release

Growth factors or morphogens modulate the cellular activity and provide stimuli to cells to differentiate and produce matrix toward the developing tissue. Growth factors have an essential role in the healing process and tissue formation. They influence tissue repair and disease, including angiogenesis, chemotaxis and cell proliferation; and control the synthesis and degradation of extracellular matrix proteins. Several bioactive molecules have demonstrated strong effects in promoting periodontal wound repair in preclinical and clinical studies. These bioactive molecules include PDGF, IGFI, basic fibroblast growth factor (FGF-2) , TGF-1 , BMP-2, -4, -7 and -12, and enamel matrix derivative (EMD) that have shown positive results in stimulating periodontal regeneration.[12]

Chi et al conducted a study on Guided Tissue Regeneration in Endodontic Surgery by Using a Bioactive Resorbable Membrane and showed that bone regeneration was rapid and extensive. This can be attributed at least in part to the use of the bioactive membrane that contains an array of growth factors that enhance cell proliferation, inflammation, recruitment of progenitor cells, and metalloproteinase activity.[13]

Conclusion

When a periodontal defect is encountered while performing endodontic microsurgery, based on clinical studies in endodontics and many in the field of periodontics, and guided tissue regenerative materials are implemented; a histologic, radiographic, and clinical benefit is realized. To fully understand the rational basis of regenerative procedures, we need to have more information concerning the variety of molecular and cellular biologic processes associated with the formation of each component of the periapical tissues. More conclusive animal studies are needed to determine which types of periapical lesions could benefit from using membrane barriers and/or bone grafts to regenerate new tissues, including PDL and cementum in periapical surgery. It appears that we still lack controlled clinical trials with a high level of evidence concerning membrane barriers in periapical surgery.

An ideal barrier material has to meet the following essential design criteria:

- It should be biocompatible.
- Act as a barrier to exclude undesirable cell types from entering the secluded space adjacent to the root surface.
- Allow the passage of nutrients and gases.
- Allow tissue integration into the material without penetrating all the way through in order to prevent rapid epithelial down-growth on the outer surface of the material, provide stability to the overlying flap.
- Capable of creating and maintaining a space adjacent to the root surface, comes in configurations that are easy to trim and to place.

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CONCEPT OF ORAL FIELD CANCERIZATION- A PERPLEXING DILEMMA

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Abstract

The development of multiple neoplastic lesions in the oral cavity, following surgery or adjuvant chemo radiotherapy still represents a critical clinical challenge in the management of patients with oral squamous cell carcinoma (OSCC). The treatment has a high propensity for local failure, which is attributed to recurrence at the primary site or the development of second primary tumor (SPT). Oral field cancerization implies that oral cancer does not arise as an isolated cellular phenomenon but rather as an anaplastic tendency involving many cells at once and results in the multifocal development of cancer at various rates within the entire field in response to a carcinogen especially tobacco. There always exists a 'field 'with genetically altered cells with a high risk of developing premalignant and malignant lesions. It may often happen that an individual stem cell is genetically altered and can cause the formation of a clone or a patch which is likely to turn into a tumor. This explains the higher recurrence rates following tumor resections. It is essential to identify and to treat this field in order to have greater chances to prevent cancer and achieve a better outcome. This review briefly discusses the origin, principle, and various theories used to explain this effect along with the clinical implications of field cancerization.

Key Words: oral cancer, field cancerization, review

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Introduction

One of the most common malignancies in humans is head and neck squamous cell carcinoma (HNSCC). The average 5-year survival rate of HNSCC is one of the lowest among aggressive cancers and has not improved during the last two decades. HNSCC develops in the mucosal lining of the oral cavity, larynx and pharynx. HNSCC comprises about 5% of diagnosed cancer cases in developed countries. Worldwide, there is a prevalence of approximately 20 HNSCC cases per 100,000 individuals per year.

HNSCC is ranked at number five on the list of the most prevalent cancer types. The prognosis of squamous cell carcinoma patients is adversely influenced by development of a new tumor.

Squamous cell carcinoma may arise as a recurrence of an incompletely resected index tumor or second primary tumor (SPT) or

second field tumor (SFT). Depending on both the location of the first primary tumor and the age of the patient the incidence rate of SPT is 10-35%. These findings led to the field of cancerization theory.[1]

The concept and term "field cancerization" was first put forwarded by Slaughter and his coworkers in 1953. It is also known as field effect, field defect, field carcinogenesis, or condemned-mucosa.[2]

The oral cavity is one of the predominant and prevalent sites of development of potential malignancies, since it comes into direct contact with many carcinogens. The squamous cell carcinoma is one of the most common malignancies developed in the oral cavity with an average survival rate of about 5 years. Despite monitoring the original tumor site following an advanced surgical and non-surgical therapy, the overall mortality rate remains unchanged probably due to the recurrence of the tumor either locally or at a remote site. The development of recurrences and second primary tumors, even when surgical margins are histopathologically tumor-free corroborates the concept oral of field cancerization.[3]

Slaughter et al proposed that the normal tissue adjacent to the tumor harbors certain pre-neoplastic genetic finger prints which can eventually lead to development of local recurrence or second primary tumors. Slaughter and his group formulated the concept of field cancerization based on the following observations. [4]

1. Tumor adjacent mucosa is molecularly 'abnormal'.
2. Multifocal areas of precancerous changes develop due to a prolonged and widespread exposure to carcinogens.
3. Oral cancer often consists of multiple independent lesions that sometimes coalesce.
4. Formation of second primary tumors and recurrences can be explained by the presence of residual abnormal tissue after surgery.[3]

Summary of slaughter's findings are as follows:[4]

1. Oral squamous-cell carcinomas in 783 patients have been reviewed from the gross and microscopic standpoint.
2. Eighty-eight instances of independent multiple tumors were found, an incidence of 11.2 per cent, which is far beyond the statistical possibility of chance occurrence.
3. Microscopic evidence of multicentric origin was demonstrated by serial section in all excised tumors less than 1 cm. in diameter.
4. Abnormal and hyperplastic, often atypical, epithelium was found to surround all oral cancers for varying distances.
5. Multicentric origin through a process of field cancerization would seem to be an important factor in the persistence or recurrence of epidermoid carcinoma following therapy.

Field cancerization is defined as "increased risk of cancer development in the entire upper aero digestive tract due to multiple genetic abnormalities in the whole region after prolonged exposure to carcinogen" .[5]

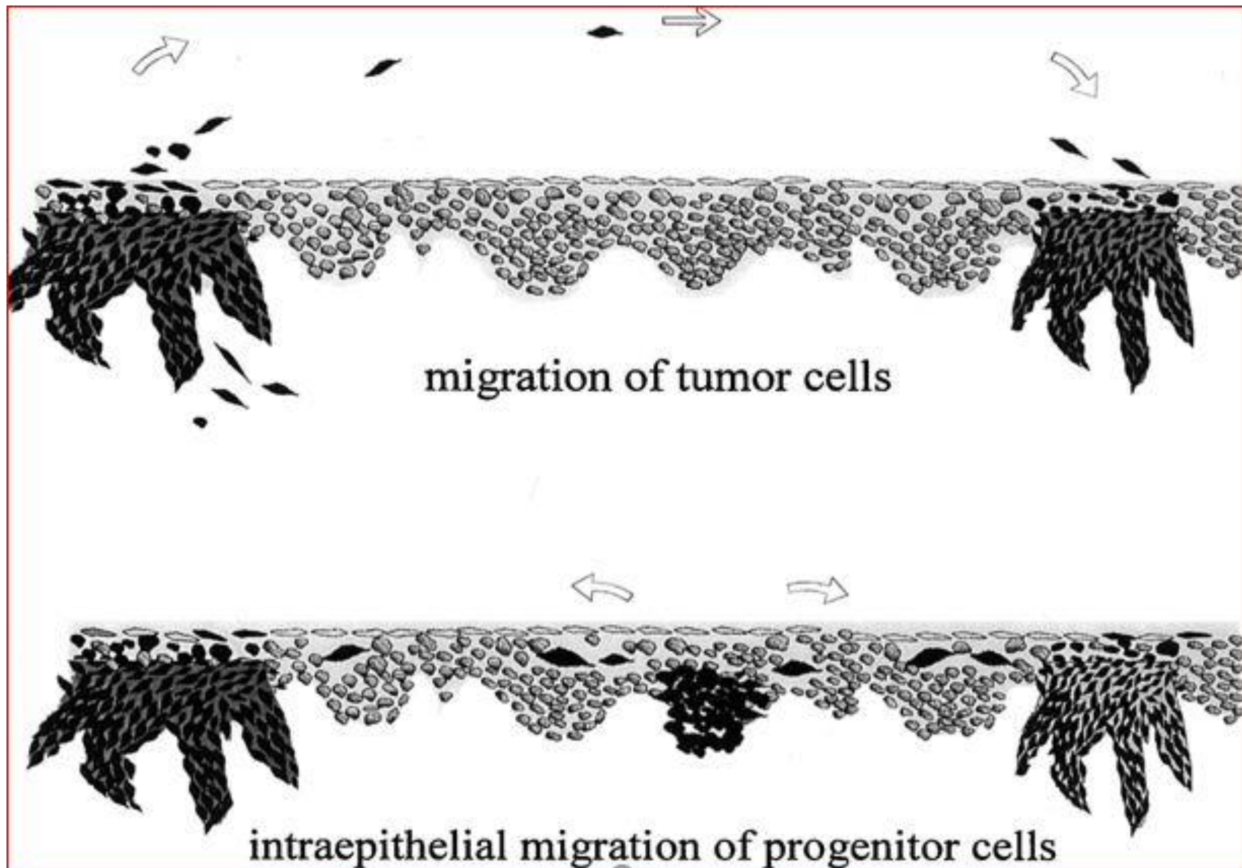

Fig 1: Migration of tumour cells

Principle of Oral Field Cancerization

Exposure to carcinogens results in an altered field in which the epithelium has multiple independent foci of abnormal tissue that can subsequently give rise to premalignant and Malignant lesions. It has been frequently used to explain the occurrence of multiple primary cancers and recurrences following complete excision of oral cancer. This concept of field cancerization has been applied for lung, esophagus, vulva, cervix, anus, colon, breast, bladder, skin, pharynx, larynx, and oral cavity. The most common carcinogen implied in oral cancer has been tobacco followed by alcohol.[2]

The mucosal changes in the entire upper aero digestive tract (UADT) were generally considered to be the result of exposure to carcinogens that caused multiple genetic abnormalities in the whole tissue region. The occurrence of multiple tumors can be explained by two competing hypotheses.[5]

- Monoclonal theory in which a single cell is transformed, and through the mucosal

spread, gives rise to multiple genetically related tumors.

- Polyclonal theory in which multiple transforming events give rise to genetically unrelated multiple tumors.
- An alternative theory for the occurrence of multiple malignant lesions has been proposed and is based on the premise that any transforming events is rare and that multiple lesions arise due to widespread migration of transformed cells through the whole aero digestive tract.[1]

Monique et al hypothesized two types of migration might be involved in already genetically transformed cells[6] (Fig 1):

(a) Migration of tumor cells by, for eg. Saliva.

(b) Intraepithelial migration of the progeny of the initially transformed cells.

Pathogenesis, diagnosis, and clinical implications

Normal tissue adjacent to the primary tumor harbors pre-neoplastic alterations that can lead to the development of local recurrence and SPTs. Studies have suggested that in the initial phase, a cell acquires genetic alterations, divides and forms a patch, a clonal unit of daughter cells. Additional acquired alterations transform the patch into a proliferating field that gradually displaces the normal mucosa, and from this field a tumor develops.[6]

There is occurrence of multiple primary tumors, second primary tumors, and second field tumors due to field effect. Second primary tumors (SPT) are cancers geographically distinct and separate, not connected by neoplastic epithelial changes from original primary cancer. The term SPT suggests that these tumors and the index tumors have developed independently.[7]

Warren and gates criteria (1932) for defining SPT

- Each of the tumors must present a definite picture of malignancy.
- Each of the tumors must be distinct.
- The probability of one being a metastasis of the other must be excluded.

Additional criteria

- Distance between each should be 1.5-2 cm or more
- If a tumor recurred at the same anatomic site, then for it to be considered a second primary tumor, at least three years had to have elapsed between detection of the tumors.

They can be synchronous, i.e., neoplasms that are diagnosed at the same time or within 6 months of primary lesions, or can be metachronous, i.e., lesion develops after 6 months of the index tumor.[7] These tumors increase the morbidity of the already treated patient, may alter the therapeutic approach to the index tumors, and adversely affect the prognosis by negating the benefit of successful control of the primary cancer[2] (Fig 2A, Fig 2B).

Tumor margins and multiple recurrences

In the field change concept, the significance of negative surgical margins becomes less important, as the resected tumor that appears

adequately excised may leave microscopic islands of cancer distant from surgical site, and even though the entire tumor has been removed at the primary operation, a recurrence may develop from the field of preconditioned epithelium.[9]

Use of intermediate end- point markers

Risk assessment and early detection of subtle cellular and molecular changes before clinical cancer develops using intermediate end point markers has been reported to combat field change effect (Table 1).[10]

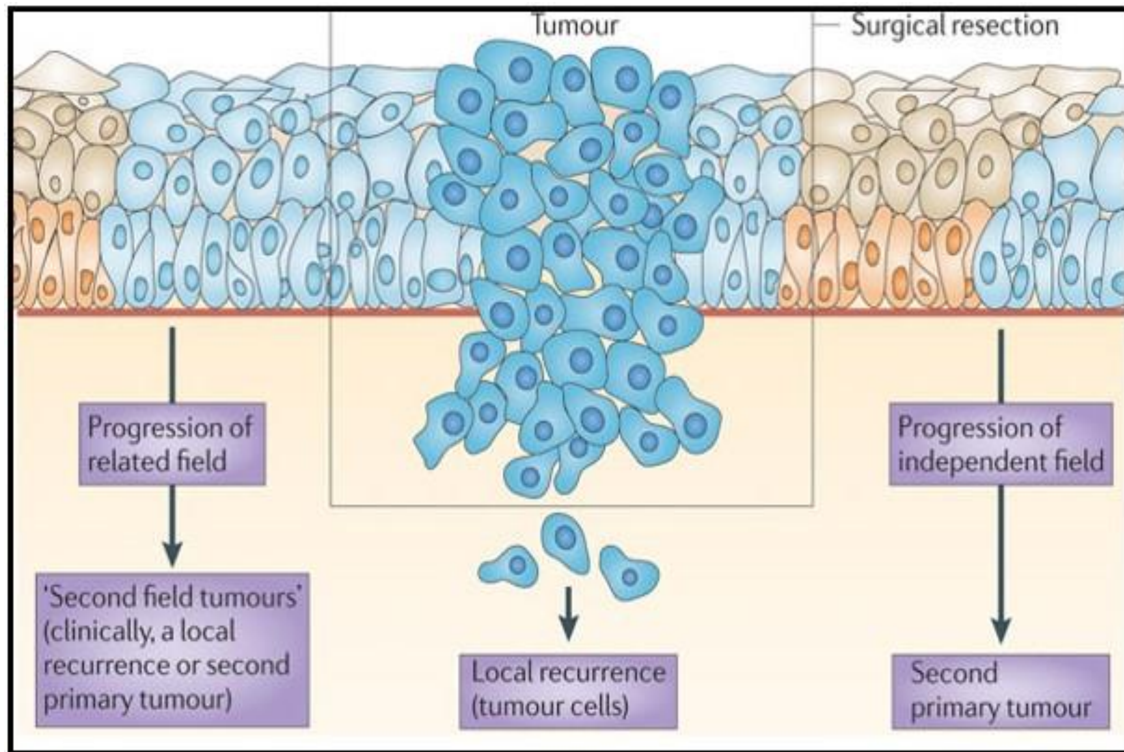

Fig 2A: Pathogenesis of second field tumors, local recurrence and second primary tumor

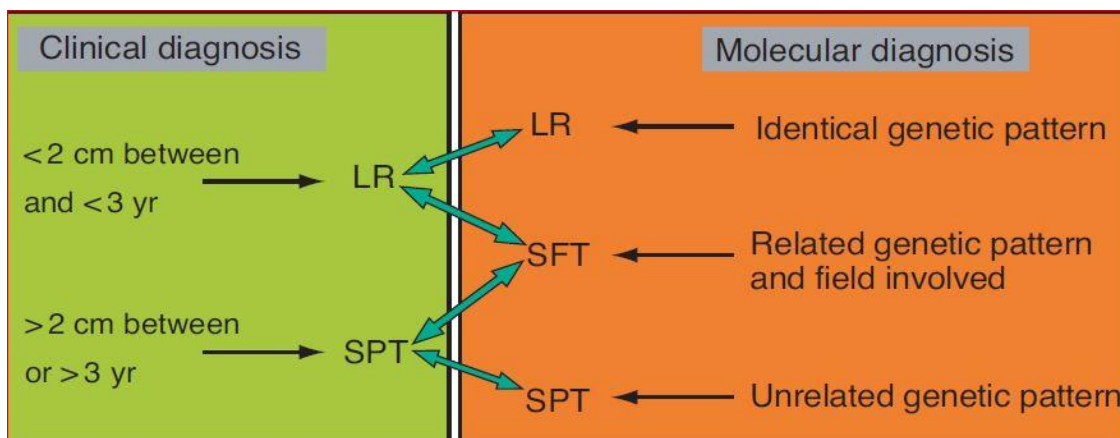

Fig 2B: Diagnosis of second field tumors, local recurrence and second primary tumor (Classification of secondary cancer after removal of a primary head and neck squamous cell carcinoma. The new classification (on the right) is based on molecular information on the relationship between the first and secondary tumors. Abbreviations: LR, local recurrence; SFT, second field tumor; SPT, second primary tumor)

Table 1: Intermediate end point markers

Histology	Dysplasia
	Carcinoma in situ
Differentiation antigens	Cytokeratins
	Secretory products
	Telomerase
	Retinoic acid receptor
Proliferation indices	PCNA
	Ki 67
Indices for genomic instability	Aneuploidy
	Microsatelite markers
	DNA adducts
	Micro nuclei
Tumour suppressor /oncogenes	P53
	P16
	Cyclin D1
	Fhit
	Ras
	C-jun
Growth factors/receptors	EGF
	EGFR
	VEGF
	CD34
	TGF α
Genetic studies	Chromosomal anomalies/ Abberations
	Loss of heterozygosity
	DNA sequence analysis
	Gene profiling
	Mitochondrial genome damage

Chemoprevention

Local therapy by excising established cancer is insufficient to prevent further disease, and usually, it is not feasible to remove all areas demonstrating molecular alterations surgically. Thus, chemoprevention is an available alternative which could render the mucosa less sensitive to DNA alterations.

Several compounds are being utilized, like CoX-2 inhibitors and most importantly 13-cis-retinoic acid which has been known to play a role in differentiation, development, and growth of epithelial cells. Further, it has also demonstrated a good response when used in the management of oral premalignant lesions.[11]

Use of field mapping biopsies

Thomson et al. used multiple biopsies to histologically map the extent of field cancerization in patients with multifocal disease, termed as field mapping biopsies. They identified 70 lesions in 16 patients, and most patients presented with 3–5 distinct lesions which were diagnosed histopathologically as hyperkeratosis and mild to moderate dysplasia. Interventional CO_2 laser surgery was utilized in these areas, and they believe that it is an efficacious, low morbidity treatment which is effective in eliminating precancer, recurrence, and malignant transformation.[6]

Interaction of minimal residual cancer and surgical wounds

This has been observed locally at the site of tumor resection as well as at the sites of surgical access to the body cavities remote from it, but usually recurrences occur locally

in up to 50% of cases. Initially, the tumor cells may get trapped in the provisional wound matrix composed of fibrin and other substrates at the surgical site. Further migration of adjacent cancer cells to the wound may be directed by chemokines and pro migratory factors (growth factors and extracellular matrix degradation products). Once they get concentrated in the wound environment, they may activate epithelial–mesenchymal transition. Growth factors and wound associated angiogenesis may promote local tumor formation. This aspect opens a whole new avenue in the identification of molecular substrates guiding tumor cell migration within a permissive environment and requires further research.[11]

Stroma in field cancerization

Ge et al. have proposed that stroma could contribute to field cancerization by developing genomic and epigenomic alterations in a similar fashion to epithelial cells and can subsequently affect the neighboring epithelial cells, though the alterations themselves do not affect the stroma. Further, these stromal cells can expand at the expense of the normal component and constitute an area of altered microenvironment. Additionally, the stromal component being more mobile can easily move to the surrounding submucosa compartment contributing to the development of SPT.[2]

Patients diagnosed with early stages of OSCC have a good prognosis; however, local recurrence and the development of second primary tumors (SPT) are important factors that can influence the survival rate. [5]

The realization that many, if not all, HNSCCs are preceded by genetically defined precursor lesions opens new possibilities for early diagnosis and prevention. This would be particularly valuable if a subgroup of lesions could be defined with a very high risk for progression. Patients with such high-risk fields in surgical margins should theoretically be followed by lifelong surveillance at regular intervals. Moreover, this technique could indicate a more conservative approach to adjuvant radiotherapy as far as the primary site is concerned. Current adjuvant treatment modalities (surgery, chemotherapy, and/or radiotherapy) are very effective in eradicating tumor cells, but these may not be the treatments of choice for relatively large fields of preneoplastic cells.[7]

The concept of an SFT offers a new opportunity for cancer chemoprevention. When a set of molecular markers has been validated, chemoprevention trials can start in patients having a high risk for an SFT. Clinical trials of this type have an important advantage: approximately where the lesion will develop is known, and the disease process can be followed by taking samples in a noninvasive way, that is, by the brushing of cells. [8]

Patients who have been treated for HNSCC are not only at risk for an SFT but also for an independent, new tumor, an SPT. Patients at risk for an SPT constitute a different patient group from the prevention point of view. Investigations of the tissue at risk are impractical because it is hard to predict precisely where a second primary will

develop; this can be at more remote sites such as the lungs. For this type of patient, it may also be harder to define a risk profile than for the patient at risk for an SFT. With respect to chemoprevention, it may be that patients at risk for an SPT could profit from a more systemic approach.[8]

The concept of SFT may also be applicable to other cancer types. In other organ systems, genetically altered cells have been detected in normal epithelium in association with an increased risk for developing multiple tumors. Fields have been described in the lung, skin, esophagus, colon, breast, and bladder. Thus it seems that the way is open for a chemo preventive approach for the prevention of an SFT in other patient groups as well.[8]

Future perspective

Chemoprevention and cessation of smoking and alcohol may prevent the development of second primary tumors if they arise independently, but they are of no benefit if multiple primary tumors are due to migration of already transformed clone of cells However, as continuous use of these risk factors may impact the development of new tumors, chemoprevention and cessation of habit should be advocated as it does have a role in the prevention of the development of malignancy. [2]

Definitive therapy for genetically altered fields can be targeted by genetic ablation of the altered clonal population, repair of genetic damage in the affected cells, or continuous treatment with chemo preventive agents, however; extensive research is warranted in this area. Knowledge of genetic alteration may provide basis for gene-based therapy for preneoplastic lesions.[2]

Conclusion

HNSCCs are often preceded by large preneoplastic lesions. This may also be true for a significant number of other tumor types. Once a carcinoma has been removed by the physician, the presence of a remaining field with a large number of preneoplastic cells is likely to increase the risk for an SFT at that particular or an adjacent site. Detection and monitoring of these fields at risk and the development of a targeted molecular intervention may have profound implications for cancer prevention. Field cancerization is a perplexing dilemma for clinicians and surgeons alike. Furthermore, it significantly affects the morbidity and mortality of oral cancer patients. The solution to this problem seems to be remote; lifelong surveillance for high-risk patients at regular intervals still remains the mainstay in the anticipation of more precise targeted treatments comprising of surgery, radiotherapy, chemotherapy, and perhaps gene therapy or oncolytic viral therapy that can be developed in patients with evidence of field change.

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