Indian Dental Association
Madras Branch

E - MIDAS JOURNAL

"An Official Journal of IDA - Madras Branch"

Chennai/Volume:4/Issue:1/Pages:1-20/March 2017
www.idamadras.com

eISSN 2454 - 8928

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President's Message

Dr. A P Maheshwar
President
IDA Madras Branch

Dear Members,

Greetings from IDA Madras Branch!

IDA Madras Branch is the first branch to have been formed, even before independence. We strive to give the best of information and updates to all our members.

I take this opportunity to invite our members to send interesting articles and case reviews. I would like to inform everyone that the National Students Conference is to be hosted by our branch this year. Further updates will follow.

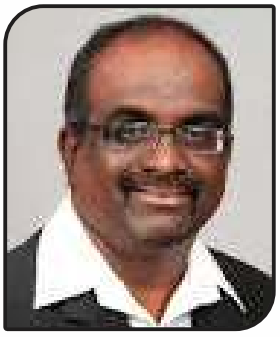
My hearty congratulations to the young and vibrant editorial team led by Dr. C. K. Dilip Kumar, our Editor. I wish the team many more journals to follow.

Jai Hind !!Jai IDA!!

Dr. A P Maheshwar

e-MIDAS Chennai/Volume:4/Issue:1/Pages1-20/March 2017

Secretary's Message



Dr. H. Thamizhchelvan
Hon. Branch Secretary
IDA - Madras Branch
Hon. Secretary National CDH
IDA (Head Office)

BE A HERO

ALWAYS SAY "I HAVE NO FEAR" SAID SWAMI VIVEKANANDA

We are in an innovative world of medicine where the materials and armamentarium changes every day. It is the duty of dental students and surgeons have to update themselves.

We IDA Madras Branch lead with innovative ideas and close the gap between latest dentistry and day to day practices.

At this moment I am Happy to say that IDA Madras Branch has won the following awards from IDA Head Office.

1. IDA Runners Up Trophy Award - Best Local Branch Secretary
2. Dr. Ramakanth Venson Award - Student Activity
3. Dr. J.M.Rao Trophy Award - Student Membership

I am to proud to share that I have been elected as Hon. Secretary National IDA CDH.

Regards

Dr. H. Thamizhchelvan

Letter from the Editor

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

I hope the year 2016 ended with peace for everyone inspite the natural calamities like Vardha and a nationwide financial chaos caused by demonetization which shook the entire nation, nevertheless let this new year 2017 herald new beginnings for all of us. With the commencement of the New Year I am happy to congratulate our branch and its members for receiving 2 National Awards; 'Dr. Ramakanth Venson Award' for Student Activity & 'Dr. J. M.Rao Trophy Award' for Student Membership.

I also extend an earnest applaud for our branch secretary for receiving 'IDA Runners Up Trophy award' for Best Local Branch Secretary. The editorial team requests all the members and readers to support our branch journal by sending manuscripts to emidasjournal@gmail.com or log on to www.idamadras.com

Dr. C.K.Dilip Kumar

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Original

Tobacco Utilisation and It's Effects on Oral Health – A Study Amongst Migrant Labourers in Kancheepuram District of Tamil Nadu

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Abstract

Introduction: The Indian nation is currently reaping the fruits of the economic liberalisation policies of the nineties and the subsequent information technology boom. Across the country, cities are witnessing large scale construction projects as India heads into the future. Caught in all this maelstrom is the migrant labourer. Amongst this population, tobacco use is rampant not just as part of culture, it also happens to be the answer to their other frequent companions – boredom, loneliness and worry. **Method**: A random sampling amongst fifteen workers threw up surprising numbers with regard to tobacco habits and precancerous lesions. The significant figures persuaded us to attempt a larger study within this population. 252 migrant workers underwent oral screening at various camp sites in one of the sub – urban zones of Chennai City. **Results**: Within the study population, the prevalence of tobacco habit was 57%. 21% of the whole sample had some form of oral pre – cancerous lesion. An eighteen year old girl had oral sub mucous fibrosis. Tobacco is a leading harbinger of death to a greater proportion of those hailing from low socio – economic back grounds. Poor access to medical facilities for early diagnosis and care fuelled by illiteracy tends to fan the trend. **Conclusion**: These migrant labourers are changing the face of the Indian nation but what about themselves? Are they being forgotten by the nation? This study is a small effort towards understanding and highlighting one of the ways in which India is being ravaged by tobacco.

Key Words: Migrant Labourers, Tobacco, Oral pre – Cancerous Lesions, Leukoplakia, India.

Introduction

The Indian sub – continent is home to the largest democracy. India is reaping the fruits of the economic liberalisation of the nineties and the subsequent information technology boom. Across the nation, cities are witnessing multiple - large scale construction projects as India heads into the future. When one pauses and looks a little bit deeper, there appears to be more to the scene – it's not all boom.

Caught in this maelstrom is a crowd that have gone un noticed as they neither fall in the rural or urban geography owing to their highly mobile nature of employment – The Migrant Labourer. Regardless of the duration of their stay, labour migrants face myriad challenges at their destinations in a country that is dizzying in its diversity of languages and cultures. Among the challenges: restricted access to basic needs such as identity documentation, social entitlements, housing, financial services and emotional needs as they are far from home and hearth. Most tend to resort to tobacco for solace. Tobacco happens to be the answer to their frequent companions - boredom, loneliness and worry.

The use of tobacco and areca nut in various forms is very popular and is deeply ingrained in many Indian socio-cultural and religious activities. However, tobacco is a leading harbinger of death to a greater proportion of those hailing from low socio – economic back grounds. Poor access to medical facilities for early diagnosis and care fuelled by illiteracy tends to fan the trend. Tobacco is also one of India's most pressing challenges.

Aim

The aim of the study was to document the prevalence of tobacco use amongst migrant labourers and to assess the effect of tobacco habits on their oral health. The study was undertaken considering the recent surge in migrant population within India. There is also lack of literature on the use of tobacco in the aforementioned population. No literature is available on the prevalence of oral pre – cancerous lesion in this people group. The study also aims to make recommendations on the establishment of protocols for creating awareness, prevention, early diagnosis and treatment within this subset of the population.

Methodology

The study was carried out from the Department of Oral & Maxillofacial Surgery at SRM Kattankulathur Dental College in liaison with the Department of Community and Preventive Medicine, SRM University. It was a cross – sectional study carried out between April – June 2015.

Face to face interview with individual migrant labourers was followed up with a brief oral screening at migrant worker camp sites. The information gathered was documented on a prepared proforma. The total sample size was 252. Those willing for follow up were followed up at the parent institution or referred elsewhere. All of them were counselled on the adverse effects associated with the use of tobacco.

Results

The number of females in this study group was less than 1/5th of the total sample size. The greatest

proportion (63%) of workers belonged to the third decade of life. The mean age was 30.52 years. The greatest percentage of workers hailed from the eastern state of West Bengal. Illiteracy amongst the workers was rampant at 40.48%. Surprisingly however, 45% of them were skilled labourers.

57% of those studied practised some form of tobacco habit (Fig. 1 & 2). Amongst those less than 20 years of age, there was almost an equal proportion of tobacco and non – tobacco users unlike other age groups (Fig. 3). A greater proportion of tobacco users used smokeless tobacco ie. 59% (Fig. 4). The various types of smokeless tobacco used included paan, khaini, tambakku and gutkha (Fig. 5).

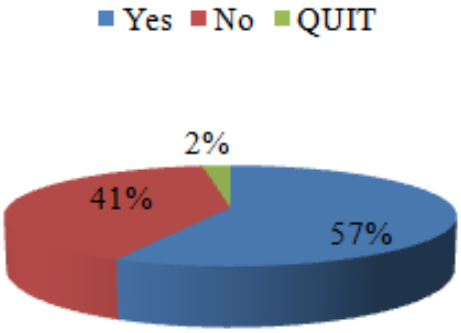


Fig. 1

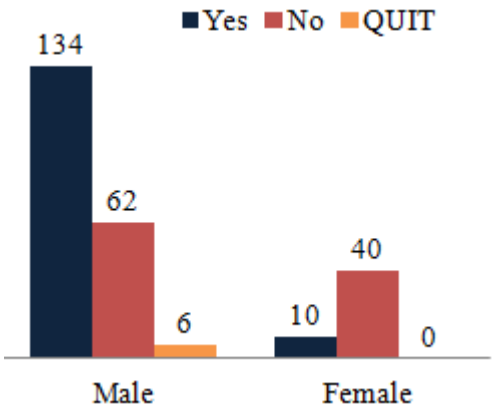
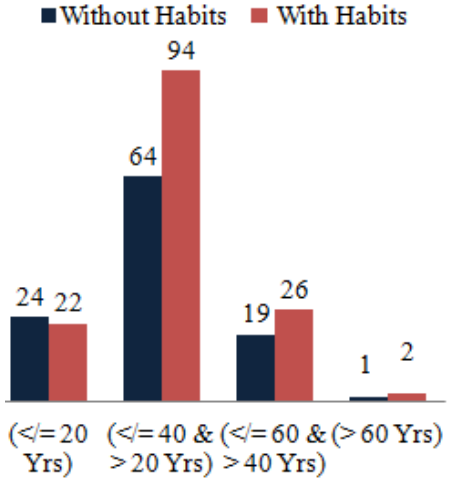


Fig. 2



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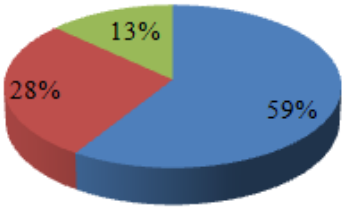

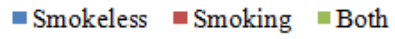

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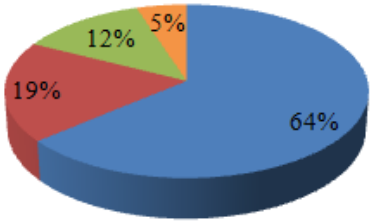

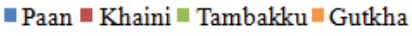

Fig. 5

52 individuals of the total sample size of 252 exhibited some form of precancerous lesion or condition. 4 of them had more than one kind of lesion. Leukoplakia was the most common lesion that was noted. Others included tobacco pouch keratosis, oral sub mucous fibrosis, smoker's palate, speckled leukoplakia etc (Fig. 6). (Fig. 7) depicts graphically the relationship between age and the presence of precancerous lesion in the studied population.

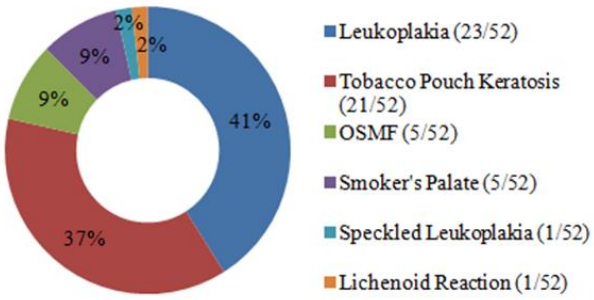

Fig. 6

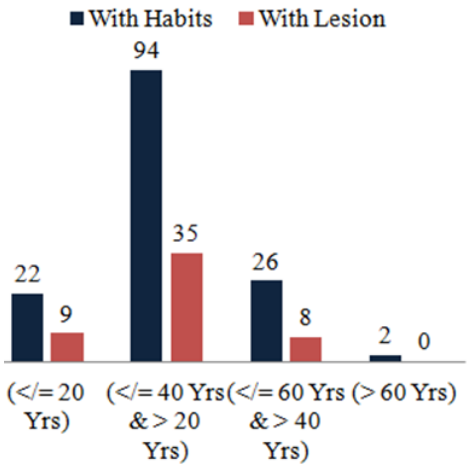

Fig. 7

For 232 out of 252 members, it was their first meeting with a dentist. However, 70.24% of them had no evidence of any dental caries. Oral hygiene was excellent in 18% of them and very poor in 21% of them.

Discussion

Tobacco is a legal drug that ultimately results in adding owe to its user. The World Health Organisation attributes 6 million global deaths each year to tobacco[1]. Studies reveal that rates of smoking have levelled off or are declining in the developed world[2,3]. The scenario is different in developing economies where a study carried out in 2002[4] reported smoking to be on the rise at 3.4% a year. Yet another burden borne by these developing nations is the higher percentage of use of smokeless forms of tobacco. In South and South East Asia, one third of the tobacco is consumed in the smokeless form[5,6]. Smokeless tobacco contains over 30 carcinogens including tobacco – specific nitrosamines, arsenic, beryllium, cadmium, nickel, chromium, nitrites, nitrates etc[7]. Tobacco use is deadly in any form or disguise.

India ranks 2nd in total tobacco consumption across the globe and is also the 3rd largest tobacco producer[8]. Smoked forms of tobacco in India range from the humble beedi and chutta to cigarettes, cigars, chillum (tobacco in a clay pipe), hookah etc. Smokeless forms include khaini, gutkha, paan masala, mawa, mishri, gudakhu etc. Tobacco is deeply ingrained in various facets of Indian society. Tobacco in its various forms is often part of most religious and socio cultural rituals. In the North Eastern part of the country, guests are customarily welcomed with some form of smokeless tobacco. Failure to do so is considered to be disrespectful. Traditional teachings handed down over the ages and followed by many women tend to suggest tobacco to be of medicinal value. Mishri is a roasted form of tobacco that is used as toothpowder by some communities. Gudakhu (tobacco & sugar molasses) preparations are directly applied to gums as part of addiction.

The report of Tobacco control published by the Indian health ministry in 2004 attributed 8 – 9 lakh deaths each year in the country to tobacco. Oral cancer ranks among the top three types of cancer in the country[9]. Beedi smoking has been indicted to have a 42% higher incidence of oral cancer as compared to cigarettes[10]. Apart from this smoking tobacco can result in lung cancer, cardiovascular and respiratory diseases to name but a few. The use of smokeless forms of tobacco has been associated with cancers of the lip, oral cavity, pharynx, digestive, respiratory and intrathoracic organs. 50% of oral cancer in India can be attributed to the use of smokeless tobacco[11].

Migrant workers largely hail from poor socio – economic backgrounds and tend to migrate to urban regions in search of higher pay. Most of them are illiterate or are limited with only a primary level of education. Metropolitan cities across the nation are enjoying growth and therefore provide a lot of opportunities to this subset of the population. However, most of the workers are part of large scale constructions; in addition a limited number of them are also involved in jobs such as housekeeping and other menial jobs. Many of them end up being exploited.

Legislations such as the Interstate Migrant Workmen Act (1979) are in place to empower and support the migrant worker. However, awareness regarding this is limited both within the general public and the migrant population. Many migrants—especially those who relocate to a place where the local language and culture is different from that of their region of origin—also face harassment and political exclusion; some of the many reasons that they turn to tobacco for solace.
A few studies on tobacco habits have been carried out in this subset of the population[12, 13, 14, 15]. However, in the South of the country, there was no study that was available. South India hosts some of the major metropolitan cities of the country such as Chennai, Bangalore, Kochi etc. Large scale construction projects, meagre jobs and cheap labour is supported by the migrant population. This study was carried out in Kacheepuram district a suburban zone of the Chennai Metropolis.

With regard to age distribution, the findings of this study were in concordance with the Payal et al[14] study carried out in Mumbai. No literature was available for comparison in the local vicinity. There were very few females when compared to males – this gender bias can probably be attributed to the nature of the profession and the socio cultural limitations. Iliteracy in the present study stood at 41% much higher than that depicted in existing literature. The payal et al[14] study in Mumbai revealed 32.8% of illiteracy whereas the Chintul Shah et al8 study in Ahmedabad suggested a further lower figure of 20%.

However, when it came to the crux of the matter – the overall prevalence level of tobacco usage (57%) was lesser compared to national levels. The study carried out in Ahmedabad8 reported the prevalence level of habits in their population to be as high as 70%, whereas the study in Mumbai14 revealed a prevalence rate of 68%. The greater proportion (60%) of tobacco users fell within 20 – 49 years of age. An almost equal (58%) fell within 40 – 60 years of age. In the study[14] amongst construction workers in Mumbai, greater percentage of tobacco usage was noted in those above 40 years of age. The greatest proportion of smokeless

tobacco was in the form of paan (64%) where as in the Ahmedabad15 study khaini (50%) took precedence.

Oral pre cancerous lesions were identified in 21% of those surveyed in our study. A cross – sectional study conducted amongst sub – urban population of Chennai in 2006[16], revealed only 4.1% of the study subjects to have some form of oral pre cancerous lesion. However, Similar to the present study, leukoplakia was the most frequently encountered. An article published in 2011, on oral pre cancerous lesions in a northern Indian city suggested prevalence of only 2.7%[17]. Yet another study[18] carried out in rural India, in Belgaum reported prevalence rates of tobacco habits of around 28%, much lesser than amongst the migrant population. The prevalence of oral lesion was only 12.4%. This comparison with rural and urban trends reveal the increased susceptibility of the migrant population to the adverse effects of tobacco. The Gupta study[19] carried out in the 1980's suggested malignant transformation rate of leukoplakia to be around 7%. However, recent studies[20,21] from the Far East point toward a higher rate of around 17%.

To add a note on their general oral health, even though for 92% of those examined it was their first dental examination, caries was evident in only in 30% of them. This is in contrast with the urban population of India. A study[22] carried out in Gwalior revealed 60% prevalence of dental caries in the population. However, in general poor periodontal health was rampant amongst the tobacco users in the cohort.

Access to good health care is sadly lacking for this subset of the population. They are limited by illiteracy and other socio cultural barriers such as language etc. Illiteracy and ignorance fuel fear of the unknown and most of them avoid hospitals. Many of those surveyed realised they had a lesion in their mouth only during the screening. Surprisingly, we noted that the response to counselling was good. They were receptive and were willing to make an effort to quit the habit.

Sadly, most of them reach the doctor at advanced stages of malignancy leading to loss of life as cure is impossible. Expensive treatment in terminal stages exhausts the resources of the family. In addition, families end up losing the principal bread winner of the family.

Data from Cancer Aid Society reveals that there are 1.3 billion tobacco users across the globe; 84% of these belong to developing countries and transitional economies like India. The salient point to highlight from our cohort include:-

- Prevalence of habit: 57%
- Prevalence of Pre – cancerous lesion: 21%
- 41% of those less than 20 years of age with habits have lesions
- 18 year old girl had oral sub mucous fibrosis

Tobacco is a consumer product that kills. Tobacco control in India took a step backwards in 2015 when the introduction of health warnings of over 85% on tobacco packs – up from the existing 40% was withheld. A study was jointly commissioned by India's Ministry of Health and the World Health Organisation on the economic burden of tobacco in India in the year 2011. It revealed that the economic burden amounted to 1.16% of India's Gross Domestic Product and 12 % more than India's combined state and central government spending on health care[23].

Intervention programs to create awareness amongst those especially of low socio – economic status is essential. Regular health education sessions, screening camps, counselling sessions on habit awareness can aid stem the rampage of tobacco in India. Employers of the migrant workers can be brought into the loop and they can aid by playing active roles in encouraging tobacco cessation. The need of the hour is a comprehensive health model that caters to the specific health needs of this forgotten sub set of our population. A creative supportive environment that helps increase their risk perception is essential.

With over a billion inhabitants, the burden of oral cancer in India is tremendous. This study revealed an increased predisposition of the young to pre oral pre cancerous lesions. This work is a small effort towards understanding the way tobacco is ravaging the Indian nation. The onus is on the scientific community to highlight the effects of the tobacco plague, so that further harm to the nation and its population can be restricted. The migrant workers are changing the face of India – but what about themselves? The greatest medicine of all is to teach people how not to need it.

Limitations

The gender ratio is skewed understandably due to socio – cultural issues. Data on alcohol consumption would have provided a more comprehensive report taking into consideration the literature on symbiosis of alcohol and tobacco in initiation of oral pre cancerous lesions. A more thorough follow up may have increased the benefit to the cohort. Further in depth studies are the need of the hour.

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Review

Diagnostic Revolution in Forensic DNA Analysis - A Review

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Abstract

Forensic DNA or DNA profiles has played major role in criminal justice system. Newer development in the field of nanotechnology has been of increasing interest in recent years. This paper reviews the evolution of DNA fingerprinting in the field of forensics to a point where trace amount of 'Junk DNA' can be used in 'Point of Care' diagnostics, thus increasing the practice of forensic odontology. A point of care device, microfluidics (lab on a chip) is becoming a cutting edge diagnostic technology in forensic analysis as it is designed to access advanced laboratory facilities within a bio-chip.

Key Words: DNA fingerprinting, forensic DNA, microfluidics, lab on chip, point of care diagnostics.

Introduction

Forensics play a main role in identifying an individual. There are many circumstances where an individual identity is of primary concern as in road accidents, mass disaster, crime scenes, sexual assaults etc.

Various approaches such as lip prints, bite marks, DNA profiling have been made to identify individual.(1) Of all traditional methods of identification. DNA profiling has come a long way from conventional fingerprints and has revolutionised the aspect of forensic odontology.(2) DNA analysis is considered greatest forensic tool since no one can alter their DNA sequence and it is hard to prevent leaving one's DNA at the scene.

STR (Short Tandem Repeats) are the simple sequences which repeats on DNA. The number of repeats in STR is highly variable among individuals. STR has many benefits such as it can be easy amplifiable, smaller size, easily separable, lower mutation rate etc. Therefore, with techniques like STR, we can characterise individual at fundamental level of DNA and variation between at this level can be used to discriminate between individuals.

Microfluidic forensic DNA analysis system is relatively new field which is aiming at truly automated chip to deliver DNA fingerprinting in minutes contrary to conventional DNA profiling which is time consuming and require technical expertise.

This review highlights the technique and concept that are vital to the successful outcome of the investigation and thus increase the practise of forensic odontology at ease.

DNA Fingerprinting

DNA testing is now an essential part of crime laboratory's armamentarium as they provide the best biometric information yielding identity. "Every contact leaves a trace". DNA typing was introduced into forensic science in the mid 1980's arising from discoveries made in biomedical research. This has become such an effective tool in forensic biology that almost all of the traditional techniques have fallen into disuse.

The first hours of investigation is considered the golden hour of investigation. For these reasons, there is strong need for relevant information becoming available as quickly as possible. But conventional forensic DNA analysis takes time and requires technical expertise. There are three to four different steps that a sample has to go through and minimum of three instruments are involved before any data is generated, each step taking at least 30 minutes to run. There are situations in which it is important to quickly and positively identify an individual. In this regard, systems utilised must be fast, portable and easy to maintain.

Three generations of DNA testing have been discovered over past decade. They include Restriction Fragment Length Polymorphisms (RFLP). Ray white, an American geneticist, identified regions of DNA that did not code for proteins but were highly variable between individuals. The strands of DNA were cut at specific location based on size and DNA fragments of defined lengths were produced, calling the variations restriction fragment length polymorphisms.(3) DNA readout was in the form of gel electrophoresis (Autoradiogram).

With this discovery, methods for mapping human genome were described and found that RFLP could be used to develop patterns of restricted DNA that were more or less specific to an individual.

Process is extremely laborious and time consuming. Relatively large amount of sample is required and takes 2 – 3 months to generate results.

DQ ALPHA(4)

This is the first PCR based test. This test uses test strips, checks for the presence or absence of blue dots in the strips. The intensity of blue dots differ and each

intensity of this blue denotes the presence a particular type of DNA sample.

Even though this technique has got some advantages like faster results (around half a day), relatively less sample required and much sensitive method, the discriminative power has not been satisfactory.

Short Tandem Repeats (STR)

Automated STR test is the 3rd generation DNA test. Eukaryote genome is largely made of coding and noncoding regions. The DNA in the noncoding regions referred to as 'Junk DNA' containing certain untranscribable or untranslatable DNA base sequences also appears to be useful. These base sequences shows sequence repeats and are termed satellite DNA. If the Repeats range between 2- 4 nucleotide sequence, it is termed microsatellite DNA or Short tandem Repeats (STR). Short tandem repeats which are sometimes referred to as microsatellite or simple sequence repeats, are accordion like stretches of DNA containing core repeat units of between 2 and 7 nucleotides in length that are tandemly repeated from approximately a half dozen to several dozen times.[5] These repeat sequence varies from person to person. These nucleotide variations help in making DNA fingerprinting of an individual and also understand DNA polymorphisms.

STR analysis is the current method of choice in forensic DNA analysis as it yields results that are nearly equivalent to individualisation. It is worth noting that these core STR loci occur in between genes in which a high degree of variability is tolerated and are thus not directly responsible for physical traits such as hair color or eye color or genetic diseases.[5]

Although human genome contains thousands upon thousands of STR markers, only a small core set of loci have been selected for use in forensic DNA and human identity testing. Millions of STR profiles are generated worldwide performing various forms of human identity testing, including DNA data basing, parentage testing. The conversion of sized DNA fragments to genotypes is the standardization between all forensic DNA laboratories for comparing data and is essential for laboratories utilising Combined DNA Index System (CODIS) database to compare profiles. Current forensic DNA typing kits uses a set of universal core loci established by Federal Bureau of Investigation (FBI) that have been catalogued in their CODIS database system. These universal 13 core loci contain highly polymorphic tetra nucleotide repeats that can be used to discriminate between individuals for forensic identification.

The short size and number of available STRs allowed scientists to amplify and analyse 3 or more loci simultaneously (multiplexing). Use of multiple loci enables a high power of discrimination in a single test without consuming much DNA.

STR are highly polymorphic ,require less sample material, much less than what can be seen through naked eye, generates very fast results, few hours to generate, discriminating power is high, had been initially discovered and was popular in 1990. PCR is widely used for STR profiling of DNA samples.

Other DNA tests include mitochondrial DNA test which is maternally inherited, sensitive, not discriminating and likely to be unique. Y–STR test is used for male DNA profile, when mixed samples are used.

In order to obtain results from the data present, various techniques have been used. They include

Polymerase Chain Reaction (PCR)

PCR is technique for replicating DNA in the lab (molecular xeroxing). In 1983, Kary Mullis developed the technique known as PCR which ultimately revolutionised molecular biology, including forensic DNA analysis.[6] Through PCR, forensic DNA analysis essentially became more rapid and sensitive.

Currently DNA sequencing and fragment sizing instruments are difficult to use and require highly trained operators. Although advancements to methods in forensic biology have streamlined the analysis process, the community still continues to research to develop faster methods.

Microfluidics or Lab on Chip (LOC)

One proposed alternative to lab analysis is the microfluidic total analysis system. A newer generation of point of care technology called lab on chip platform seeks to integrate and automate all the complexities of lab procedure into device, the size of computer chip. Ideally devices are used that can collect a sample from the crime scene, perform sample work up (Lysis and Extraction of DNA) and amplify and analyse the DNA.[7] The fully integrated system in development would essentially eliminate all manual processing by minimising the work to simply introducing a sample and a single button pressed. As a result, only minimal training could be required and any untrained personnel could operate it.

Microfluidics also lends itself well to automation, so researchers can make platforms that are very simple to use. All a person potentially has to do is take a sample, stick it into a machine, press the 'go' button, and wait for the instrument to spit out a DNA fingerprint. In many ways it is the same old chemistry but in a miniaturised form that now seamlessly integrates all

three or four of the processes onto one device and closes it off so that it is not susceptible to contamination.

An ideal microfluidic device should have ruggedization, should have capacity to handle various sample types and should have increased sensitivity and specificity superior to those performed conventionally using normal techniques and robotic instrumentation.

PCR steps uses the block thermocycler traditionally to cycle between the various temperatures, whereas researchers like Landers use infrared heating in microfluidics, which is much faster.[8] This shows that time saving comes in almost every step of the microfluidic process.

Microfluidic devices developed for point of care and clinical applications for cell lysis, sample work up, PCR and detection / analysers create opportunities which can also be applied within the forensic field. This self-containment means that human hands are taken out of the picture and reduces the potential for contamination, a crucial issue in forensic analysis.

The advantages of microfluidic DNA integrated device provides a degree of control over volumes, flow rates, surface area and composition and temperature control that cannot be obtained using conventional macro scale tools such as pipettes, centrifuge and mixers.[9] Single use biochips would minimise risk of contamination, need for cleaning and reprocessing of bio chips, makes it portable, can be carried out in resource limited areas, generate actional data in real time and cost effective. Secondly miniaturization allows for automation of parallel processes at high throughput and at a scale previously achievable only with large laboratory robots.

But the lack of awareness among health professionals like dentist, leads to its limited utilisation with subsequent lower production.

STR in Microfluidics

STR typing process is broadly used in molecular forensic analysis.[5] Conventional technique require experienced lab technicians to carry out the protocol. Although highly successful and reliable, current methodologies require 8-10 hours to complete under routine conditions, uses large sample volumes, costly reagents, and are labor-intensive.

The goal is to develop a rapid system capable of separations on short channel microfluidic devices with the required resolution needed for microsatellite analysis in forensic DNA samples.

To simplify the implementation of this process, it is desirable to use microfluidic technology and system engineering to build a platform that involves fully integrated disposal catridges which can be used by minimally trained operators and increase throughput capabilities with greater reproducibility of the test.[10]

The entire process from sample collection to obtaining a STR profile takes approximately 4 hrs. The instrument is loaded with a DNA processing cartridge which incorporates on-board pumps and valves which direct the delivery of sample and reagents to the various reaction chambers to allow DNA purification, amplification of the DNA by PCR. Following multiplex PCR amplification, DNA samples containing the length variant STR alleles are typically separated by capillary electrophoresis and genotyped by comparison to an allelic ladder supplied with the commercial kit.[5]

In order to overcome the need for quantification, a silica filter or beads can be used, which have a specific binding capacity to prevent an excess of DNA available for the amplification reaction. Utilizing a single, integrated and disposable microfluidic chip, the multi-step sample processing and analysis that consumes 8-10 hours for conventional forensic STR analysis, can be carried out in less than 45 minutes.[11] A fully integrated instrument that will provide sample in to results out forensic DNA analysis will dramatically reduce the costs (including labor, space and validation) of establishing and operating a forensic DNA lab.

Conclusion

These smart microfluidic platform will have a major impact on artificial intelligence and biological computations. The portability and ease-of-use microfluidic platforms certainly seems poised to broaden up the applications of DNA fingerprinting beyond forensic crime-solving such as for infectious disease detection or for other types of measurements based on molecular tests by just changing the cartridge and the assay chemistry and run the system. Effective DNA databases are being constructed and existing core loci have played and will continue to play a vital role in human identity testing.

Although there are many more details to work out to improve the efficiency of microfluidic systems, researchers hope that the time invested in validating microfluidic measurements will pay off in the long run. This technological revolution in forensic science could ultimately lead to a paradigm shift in which a new role of the forensic expert emerges as developer and custodian of integrated forensic platforms. Many more exciting scientific and technological advances are still on the horizon, there is no doubt that the future

landscape of forensic DNA analysis will look very different from what we see today.

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Review

Tumors of Fibrous Tissue Origin

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Abstract

Soft tissue tumors are enormously vast and yet relatively undiscovered. Tumors and tumor like lesions of soft tissue continues to be a challenge to both the surgeons and pathologists due to their diversity in biological behavior and histogenesis. Soft tissue tumors are mesenchymal proliferations that occur in the extraskeletal , non-epithelial tissues of the body ,excluding the viscera, coverings of the brain, and lymphoreticular system. Benign tumors are more common than malignant ones in ratio of 100:1. Hematoxylin & Eosin sections act as a useful diagnostic technique in the initial diagnosis of tumors. The goals of the review is to introduce the most common soft tissue entities of fibrous tissue origin and provide with a basic knowledge regarding the diversity of soft tissue tumors.

Key Words: Soft Tissue Tumors, Collagen, Fibroblasts, Histiocytes, Immunohistochemistry.

Introduction

Soft tissue lesions range from nonneoplastic conditions to benign and malignant tumors. Majority of soft tissue tumors are benign, with a very high cure rate after surgical excision. Malignant mesenchymal neoplasms amount to less than 1% of the overall human burden of malignant tumors but they are life threatening and may pose a significant diagnostic and therapeutic challenge since there are more than 50 histological subtypes of Soft tissue tumors, which are often associated with unique clinical, prognostic and therapeutic features. Fibroblastic /myofibroblastic tumors represent a very large subset of mesenchymal tumors.[1] Many lesions in this category contain cells with both fibroblastic and myofibroblastic features. As a dental practitioner, understanding of soft tissue neoplasms has increased significantly and it is important to gain plenty insight regarding common neoplasms of fibrous tissue origin

Common Tumors in Oral Cavity

FIBROMA (Irritational fibroma, traumatic fibroma, Focal fibrous hyperplasia, Fibrous nodule, fibromatosis fibroma)

Most common reactive hyperplasia of fibrous connective tissue in oral cavity occurs in response to local irritation or trauma.[2] Buccal mucosa is the most common location, along the bite line as a consequence of trauma from biting the cheek. Gingival fibromas represent fibrous maturation of a preexisting pyogenic granuloma. Smooth-surfaced elevated pink nodule that is similar in color to the surrounding mucosa. In rare cases, the surface may appear white due to hyperkeratosis or ulcerated due to continued irritation. Fibromas are sessile or pedunculated. Asymptomatic and common in 4th to 6th decades of life. Common in females. Epithelium is stratified often demonstrates atrophy of the rete ridges because of the underlying fibrous mass with collagenized and fibrous connective tissue in bundles of interlacing collagenous fibers interspersed with varying numbers of fibroblasts or fibrocytes. Collagen bundles may be arranged in a radiating, circular, or haphazard fashion and small blood vessels with chronic inflammation mostly of lymphocytes and plasma cells. CD 34, Vimentin can be used as IHC markers.[3] Conservative surgical excision is the treatment of choice with extremely rare recurrence.

Fibromatosis

Broad group of fibrous proliferations that have a biologic behavior and histopathologic pattern that is intermediate between those of benign fibrous lesions and fibro sarcoma. It is a firm painless mass with no gender predilection. Common oral site is submandibular region. If lesion occurs in children, it's termed as juvenile fibromatosis.[2] If lesion occurs within the bone it's termed as desmoplastic fibroma with mild resorption of underlying bone. Histopathologic Features consists of Characteristic proliferation of spindle-shaped cells fibroblasts that are arranged in streaming fascicles and are associated with variable amount of collagen. Lesion is poorly circumscribed and infiltrates the adjacent tissues. Vimentin, Smooth muscle actin, CD34, Desmin as markers.[4] Wide excision is the treatment. 23% recurrence rate has been reported for oral and para oral fibromatosis, but a higher recurrence rate.[2]

Myofibroma

Rare benign spindle cell neoplasm originates from myofibroblasts. It is a solitary exophytic mass with a diameter of 0.3–5.0 cm which occurs at any age. Most of the lesions are asymptomatic and exhibits rapid enlargement. Common oral location is the mandible, followed by the lips, cheek and tongue. Multicentric myofibromatosis primarily affects neonates and infants who may have tumors of the skin, subcutaneous tissue, muscle, bone and viscera. Intrabony tumors create radiolucent defects that usually tend to be poorly defined. Mandibular lesions are well defined unilocular or multilocular. Histopathology of myofibroma consists of interlacing

bundles of spindle cells with tapered ends and blunted nuclei with eosinophilic cytoplasm that resembled smooth muscle. Scattered mitoses are not uncommon. Centrally, the lesion is often more vascular with a hemangiopericytoma-like appearance. Surgical excision is the treatment of choice with a small percentage of recurrence after treatment. Lesions in Viscera or vital organs in infants can act more aggressively and sometimes proves to be fatal.[2] Tumors cells are positive for smooth muscle actin, muscle- specific actin for immunohistochemistry.

Fibrosarcoma

Most common soft tissue sarcoma. Tumor of mesenchymal cell origin that is composed of malignant fibroblasts in a collagenous background. Common in the extremities, only 10% occur in the head and neck region. Primary fibrosarcoma is central, arising within the medullary canal, or peripheral, arising from the periosteum that produces variable amounts of collagen. Secondary fibrosarcoma aggressive tumor with poorer prognosis, arises from a preexisting lesion or after radiotherapy to an area of bone or soft tissue. Tumor associated with genetic mutation from the translocation t (12;15)(p13;q25) giving rise to ETV6-NTRK3 (ETS variant gene 6; neurotrophic tyrosine kinase receptor type 3) gene fusion.[5]

Slow-growing masses that reach considerable size before they produce pain. Commonly in patients of fourth decade of life. Histopath features consists of fascicles of spindle-shaped fibroblasts that classically form a "herringbone" pattern. Fibroblasts are plump with pale eosinophilic cytoplasm and deeply staining spindled nuclei with tapered ends. Cellular and nuclear pleomorphism along with more frequent mitotic activity is noted. Moderate amount of mature collagen may be produced, with areas of hyalinization surgical excision including a wide margin of adjacent normal tissue is the treatment.

Recurrence is noted in about half of cases, and survival rates range from 40% to 70%. IHC Positivity for smooth muscle actin, desmin, S100 protein, and CD34, vimentin. vimentin and negativity for S-100 protein, CD 68, cytokeratin cocktail, HMB-45, CD34, pan actin HHF 35, desmin, smooth muscle actin and epithelial membrane antigen (EMA).[6]

Fibrous Histiocytoma

(Dermatofibroma, sclerosing hemangioma, fibroxanthoma & nodular subepidermal fibrosis)

A benign but diverse group of neoplasms which exhibit both fibroblastic and histiocytic differentiation. Small, firm nodule occur in middle-aged and older adults Characterized by a submucosal, Cellular aggregation of spindle-shaped, fibroblast like cells with relatively pale, oval nuclei. spindled cells may be arranged randomly but usually large areas with tumor cells streaming in interlacing fascicles from a central nidus and intersecting with cells from adjacent aggregates, imparting a storiform or crisscross pattern. lesional stroma is occasionally very densely fibrotic or hyalinized. Scattered rounded foamy histiocytic cells and touton-type multinucleated giant cells are also present. Chronic inflammatory cells, especially lymphocytes, are usually scattered throughout the tumor in small numbers. Few lesions occasionally show areas of calcification or bone metaplasia or present clear granular, balloon, or signet-ring cells.[7] Local surgical excision is the treatment. 5–10% of cases recurring locally. CD34 and Factor xiiia positivity in IHC.[8]

Malignant Fibrous Histiocytoma

(Pleomorphic sarcoma)

Sarcoma with both fibroblastic and histiocytic features. A tumor of older age group. The most common complaint is an expanding mass that may or may not be painful or ulcerated. Histopathalogical features consist of Spindled fibroblast like cells which tend to be arranged in short woven fascicles or bundles. Spindle cells may be long and thin with minimal atypia, but there are usually areas with plump cells containing enlarged, hyperchromatic and irregular nuclei. Rounded, polygonal and irregularly shaped histiocyte like cells may dominate some areas of the lesion. Chronic inflammatory cells often scattered sparsely throughout the tumor including lymphocytes and plasma cells. An aggressive tumor that is usually treated by radical surgical resection. 40% of patients have local recurrences approximately. Vimentin, CD68, CD38, CD34, SMA is positivity for IHC.[7]

Conclusion

Soft-tissue tumors are tumor like lesions which are encountered often in dental practice. These tumors can occur anywhere in the body but few are locally aggressive and tend to recur in head and neck region due to the proximity of vital structures. Hence, it is important to diagnose soft tissue neoplasms by evaluating clinically, histologically or by using IHC markers which help in establishing the cell origin because treatment is challenging in few lesions like fibrosarcoma, malignant fibrous histocytoma due to its aggressive clinical behavior and propensity for local infiltration with a tendency for recurrence. So, it is important to be aware of few soft tissue tumors of fibrous origin with the ultimate goal of improving patient management and outcome with close follow-up.

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Case Report

Single Step Apexification using Mineral Trioxide Aggregate - A Case Report

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Abstract

Traumatic dental injuries to teeth with an immature apex results in pulpal necrosis and an open apex that pose a significant challenge for complete debridement, disinfection and three dimensional obturation of the root canal system. Apexification is a procedure to promote an artificial apical barrier across an open apex in a non-vital tooth to accommodate the filling materials within the confines of the root canal. Mineral Trioxide Aggregate (MTA) has emerged as a promising material owing to its biocompatibility and superior sealing ability. This is a report of two cases with immature apex treated successfully using MTA as an apical plug.

Key Words: Mineral Trioxide Aggregate, Apexification, Single Step Apexification.

Introduction

Traumatic dental injuries are more common in maxillary anterior teeth with immature apex that leads to pulp necrosis [1]. Studies have estimated that majority of dental trauma occurred before the age of 12 (86%) [2]. Injuries involving the immature permanent teeth renders the dental pulp non vital and if this occurs prior to the complete root formation and apical closure, it may affect the development of the roots of the involved teeth causing apical resorption or open apex [3] Immature teeth with open apices have thin dentinal walls that are prone for cervical root fractures thereby reducing the overall prognosis of the teeth [2][4].

The success of an endodontic treatment largely depends on proper apical and coronal seal [5]. Teeth with open apex often pose a challenge due to the lack of adequate apical seal during obturation. Historically the management of immature open apex in non-vital teeth was confined to custom fitting the filling materials, paste fills and apical surgery. Many authors have described the use of custom made gutta percha cones, however it is not advisable since the apical diameter is wider than the coronal diameter which makes condensation of gutta percha impossible [6].

Apexification is defined as 'a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp [7] Calcium hydroxide has been the material of choice for apexification [6] It involved repeated changes of intra canal material over the course of 5-20 months [8]. Calcific barrier against the open apex was induced. Although calcium hydroxide has shown clinical success, it had its own drawbacks. This therapy required multiple appointments and a high level of patient compliance[9]. Furthermore, the lack of an appropriate coronal seal makes the tooth susceptible to re-infection [10]. During calcium hydroxide dressings at regular intervals, repeated use of intra canal irrigants like NaOCl and organic solvents like EDTA resulted in furthermore weakening of the dentin making the tooth more susceptible to fractures [11].

Morse et al (A) defined one visit apexification as a non-surgical condensation of a biocompatible material into the apical end of the root canal. Recently, various materials namely tricalcium phosphate, calcium hydroxide, freeze dried bone and free-dried dentin were proposed for apexification procedure and favorable outcomes have been reported in literatures[6]. Mineral trioxide Aggregate (MTA) introduced by Torabenajad in 1993 gained importance as a material of choice for single step apexification due to its superior properties of biocompatibility and adequate sealing ability that has shown to create a successful artificial calcific barrier against the immature open apices[9]. This article presents two cases with immature open apeices that was managed by single step apexification using MTA as an apical plug

Case Report

A 30 year old male patient reported to the Dept. of Conservative Dentistry and Endodontics, Sri Venkateswara Dental College and Hospital, Chennai with the chief complaint of fractured and discolored upper front tooth. Patient's history of presenting illness revealed that he had a traumatic incidence at the age of 8 years. The patient remained asymptomatic since then. Patient also gave a history of treatment for the same tooth three years back after which he failed to report for the subsequent appointments. Medical history of the patient was non-contributory. Intraoral examination revealed Ellis and Davey's class IV fracture with discoloration in left upper central incisor 21. Intraoral periapical radiograph revealed blunderbuss canal and periapical radiolucency in 21 (Fig.1). The concerned tooth did not respond to the pulp sensibility tests using cold test (Roeko Endo-

Frost, Coltene, Germany) and Electric Pulp testing (Digitest, Confident, Bangalore, India). Clinical examination, Radiographic examination and vitality tests led to the diagnosis of attempted root canal treatment in 21 with periapical pathology.

Fig 1- Pre-operative Radiograph

A nonsurgical method of root canal treatment with single step apexification using MTA was planned with the consent from the patient.

In the first visit the tooth was anesthetized using 2% Lignocaine with 1:80,000 adrenaline (Lignox, Indoco Remedies Ltd., India). Endodontic access cavity was refined using endo access bur (Maillefer, Dentsply, Ballaigues, Switzerland). Canal patency was checked with number 10 K-file (Mani, Inc.; Tochigi, Japan). Canal was copiously irrigated using 3% Sodium hypochlorite (Prime Dental Products, Thane, India). Working length was determined radiographically using Ingle's method. Chemo-mechanical preparation was carried out using 80size K-file (Mani, Inc.; Tochigi, Japan) in a circumferential filing motion to remove intracanal debris and necrotic pulp tissue. Root canal debridement was done using irrigants 3% NaoCl and Normal saline (Baxter, India pvt. Ltd., Tamilnadu, India) at regular intervals. The canal was dried using paper points and calcium hydroxide intracanal medicament (RC Cal, Prime dental products, Thane, India) was placed. The access cavity was temporized using Zinc oxide Eugenol (Deepak Enterprises, Mumbai, India). Patient was recalled after 1 week.

At the recall visit, the access cavity was reopened and the calcium hydroxide paste was removed using 3% NaOCl and saline. The canal was dried using paper points. Mineral Trioxide Aggregate (MTA) [Angelus Industria de produtos odontologicos, Londrina, Brazil] was mixed according to the manufacturer's instructions to a wet sandy consistency. MTA mix was carried into the root canal and condensed into the apical portion using hand pluggers of appropriate size. Increments of MTA were then placed and condensed using the same technique till adequate thickness of 3-4 mm at the apical end was achieved. Moist cotton was then placed and the tooth was temporized. Patient was recalled after 2 days.

In the next visit, the set of MTA was confirmed clinically as a hard stop in the canal using a hand plugger and radiographically by an intraoral periapical radiograph (Fig.2).

Fig 2 - Apical Plug of MTA

Following this, the root canals were dried with paper points (Maillefer, Dentsply, Ballaigues, Switzerland) and obturated with 2% gutta-percha (Maillefer, Dentsply, Ballaigues, Switzerland) and AH plus resin sealer (Maillefer, Dentsply, Ballaigues, Switzerland) by cold lateral condensation technique. A postoperative radiograph was taken to confirm the same (Fig.3)

Fig 3 - Obturation Radiograph

The access cavity was sealed with glass ionomer cement (GC Gold label, Japan). In subsequent appointments the core was built up using composite resin (Denfil, Vericomco. LTD, Korea) following which crown preparation was done. Impressions were recorded with Elastomeric impression material (Aquasil, Denstply, Germany). Casts were made and sent to laboratory for fabrication of final prosthesis. Final prosthesis of porcelain Fused metal (PFM) crown was then cemented using luting type glass ionomer cement (GC Corporation, Tokyo, Japan). Patient was reviewed after 3 months for clinical and radiographic healing (Fig.4). Patient was found to be asymptomatic.

Fig 4- Post-operative Radiograph

Discussion

Endodontic treatment of teeth with open apex is always a challenge to the endodontist. An immature open apex has a wide apical diameter that poses significant difficulty in canal debridement, disinfection and obtaining a three dimensional obturation. For a successful endodontic treatment in a tooth with an immature apex, cleaning and disinfecting the canal is more important than shaping the canals. Over instrumentation could weaken the tooth since the canals of an immature teeth has thin dentinal walls.

Literature search revealed a number of options for the management of a traumatized vital immature open apex that includes various revascularization protocols with subsequent follow up for apical closure and permanent prosthetic restoration of the tooth, multiple visits with calcium hydroxide apexification, single step apexification using MTA or extraction followed by subsequent replacement with removable, fixed or implant prosthesis [12].

Though the clinical success rates of calcium hydroxide apexification are high, the risk of reinfection and root fracture has made the clinician to resort to other methods. With the advent of Mineral Trioxide Aggregate, a better alternative to $Ca(OH)_2$ was identified. MTA is a powder consisting of fine hydrophilic particles that binds in the presence of moisture. It consists of tricalcium silicate, tricalcium oxide and silicate oxide. It has superior properties of biocompatibility, low solubility, adequate apical sealing ability and hydrophilic nature. The pH of MTA is 12.5 which is similar to that of $Ca(OH)_2$ which might impart some antimicrobial properties[13] Because of its superior biological properties, MTA has proven to be the material of choice for creating an artificial apical plug in an open apex tooth[14]. The adequate thickness of the apical plug of MTA varied from 3mm to 5mm. MTA barrier of 5mm is significantly stronger and shows less leakage than 2mm barrier [15].

Regeneration is the ideal desirable outcome for any restorative procedure. Though novel method of using MTA for single step apexification reduces the patient's treatment time yet there is always a search for materials that would successfully regenerate the odontogenic tissues. This technique using MTA reduces the radiation exposure, chances for root fracture and susceptibility to re-infection. The choice of treatment regimen for teeth with open apices always depends on the individual case and operator experience and familiarity with handling the various materials [16].

Conclusion

Single visit apexification using MTA has been followed successfully over the last 10 years as a suitable alternative to Calcium Hydroxide. MTA has proved to be the future of endodontics and a new boon in an effective management of teeth with immature apex by achieving a good periradicular seal.

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