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Content

Review

An overview on the Infection Control Protocols for Dental clinics in the Pandemic Era

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Abstract

According to the surveys carried out Dental hygienists, Dental assistants and Dentists are at the highest risk of developing Covid 19. This is attributed to the fact that Dentistry is a branch pertaining to directly dealing with the mouth which is one of the highest reservoirs of microorganisms. Almost all the clinical procedures carried out in Dentistry are aerosols generating which puts both the dental personnel and the patients being treated at risk. The dental aerosols due to its small size and composition have the ability to linger in the air for a prolonged time which elevates the health risk.

This article will provide an overview of prevalent guidelines and current practices, recommend correct use of PPE in the dental setting, and discuss various technologies which aid in air management thereby ensuring the safety of the entire dental team and patients during the covid-19 outbreak.

Keywords: Covid-19, Infection Control, Aerosols, PPE.

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INTRODUCTION

As of June 2021 Coronavirus Disease-19 (COVID-19), has infected more than 181 million individuals worldwide and has caused 3.9 million deaths.[1,2] According to the surveys carried out Dental hygienists, Dental assistants and Dentists are at the highest risk of developing COVID-19.[3,4] This is attributed to the fact that Dentistry is a branch pertaining to directly dealing with the mouth which is one of the highest reservoirs of microorganisms. Almost all the clinical procedures carried out in Dentistry are aerosols generating which puts both the dental personnel and the patients being treated at risk.[5] The dental aerosols due to its small size and composition have the ability to linger in the air for a prolonged time which elevates the health risk.[6]

A large number of pathogens are localized in the oral cavity, which can be transmitted in different ways during dental procedures, usually by means of air/water syringe and high-speed instruments.[7,8] The procedures that are recommended to minimize the dissemination of aerosol includes use of personal protective equipment such as gloves, masks, protective eye wears, face shield and use of high-power suction during dental procedures.
With the current pandemic situation of COVID-19, dentists are in a state of psychological distress and fear while working under such an unprecedented situation. The field of dentistry is experiencing a stage where it is needed to amplify and improvise the way of practice. Though there are plethora of articles on infection control and management, dentists worldwide are still indecisive on how to go about and what technology to choose to equip their clinics for better infection control. This article will provide an overview of prevalent guidelines and current practices, recommend correct use of PPE in the dental setting, and discuss various technologies which aid in air management thereby ensuring the safety of the entire dental team and patients.

Protocols to be followed before the patient enters a dental operatory.

- Thorough screening and triage

- Ensure the patient is wearing mouth mask.
- Check the temperature.
- Make sure everyone always adheres to the infection control etiquettes by posting visual alert signs, posters etc.[9]
- Provide supplies for respiratory hygiene and cough etiquette, including Alcohol-based hand rub (ABHR) with at least 60% alcohol, tissues etc.
- Install physical barriers at reception areas to limit close contact between triage personnel and potentially infectious patients.
- Remove toys, magazines, and other frequently touched objects from the waiting room that cannot be regularly cleaned and disinfected, this limits ways that you can contract or spread the virus while in the office.
- Along with the routine history taking, focus on patient's past or current illness, travel history and details of contacts with any COVID-19 positive cases and document it and refer the patients for appropriate care if there is a suspected positive case.
- In the waiting area ensure the waiting room is adequately ventilated. 60 L/s per patient is considered as adequately ventilated: in rooms with normal ventilation.[10]
- Distance of 1meter should be maintained in between the waiting patients throughout.
- Make sure the patients sanitize their hands with ABHRs and if hands are visibly soiled then with water and soap.[11]

Protocols for the Dentists and other Dental personnel

During routine dental practice, guidelines by CDC (Centers for Disease Control) is usually followed as "Standard precautions of infection control". The standard precautionary measures should be amplified by implementation of additional precautions to ensure the standards of infection control are adhered, to prevent any potential COVID-19 transmission. The dentists and other dental personnel should seek appropriate knowledge and training on correct use of PPE (Personal Protective Equipment), management of potential COVID-19 carriers and air and surface management of their operatory to avert any contamination.[12]

Every clinic should have a preparedness model for the provision of oral health case during this pandemic. Bordani M and Donnelly L has described a 'Preparedness model' which can aid in guiding individuals to make sense of the situation at hand by looking at available information. This concept helps in understanding the threats posed by COVID-19 by utilizing different perspectives of oral health care providers, administrators, and patients.[13] Therefore, it enhances the readiness to deal with emergencies and handling the special needs patients in dental offices in this pandemic.

Before entering a patient room or care area:

- Perform hand hygiene (wash your hands with soap and water for at least 20 seconds or use a hand sanitizer).
- Put on a clean gown or protective clothing that covers personal clothing and skin likely to become soiled with blood, saliva, or other potentially infectious materials.
- Put on a surgical mask or respirator and secure it properly.
- Put on eye protection (goggles or a face shield that covers the front and sides of the face).
- Put on clean non-sterile gloves.
- Enter the patient room or care area.

After completion of dental care:

- Remove gloves.
- Remove gown or protective clothing and discard the gown in a dedicated container for waste or linen.
- Exit the patient room or care area.
- Perform hand hygiene (wash your hands with soap and water for at least 20 seconds or use a hand sanitizer).
- Remove eye protection by carefully grabbing the strap and pulling upwards and away from head. Do not touch the front of the eye protection.
- Remove and discard surgical mask or respirator and do not touch the front of the respirator or mask.

- Perform hand hygiene.

Protocols for Aerosol Generating Procedures:

Dental drilling procedures aerosolize saliva and products of drilling, producing particles small enough to penetrate deep into the lungs.[14] Procedures that commonly generate lot of aerosols should be avoided, alternative non-aerosol techniques if available should be utilized or if necessary, should be performed very cautiously.

It is common to use water coolant while performing many dental procedures, The water coolant when combined with bodily fluids such as blood and saliva generates bioaerosols that are contaminated with micro-organisms and have the potential to remain in the air for considerable duration of time, which can be inhaled by the dental personnel or other patients potentially causing disease transmission.[15] Hence, we need to take measures to ensure the aerosol generating procedures are accomplished with appropriate care. In case aerosol generating procedures cannot be avoided and must be performed, the following protocols may help in limiting the dissemination of aerosols.

- Airborne infection isolation room should be utilized for any Aerosol generating procedures.
- Dental Health Care professional (DHCP) in the room should wear an N95 or equivalent or higher-level respirator, as well as eye, gloves, and a gown.
- Only the concerned operator, assistant and patient should be allowed in the room and no visitors should be allowed to accompany.
- Clean and disinfect treatment room surfaces promptly after the completion of the treatment.
- Limit transport and movement of the patient outside of the room to medically essential purposes.
- Patients should be encouraged to wear a facemask or cloth face covering to contain secretions during transport.

- Consider scheduling the possible suspected or confirmed COVID-19 positive patient at the end of the day.
- Do not schedule any other patients at that time.
- When calculating daily volumes of patients consider the time required to clean and disinfect the clinic between patients and follow the Guidelines for Infection Control in Dental Health-Care Settings of 2003.[16]
- To clean and disinfect the dental operatory after a patient with suspected or confirmed COVID-19, DHCP should delay entry into the operatory until a sufficient time has elapsed for enough air changes to remove potentially infectious particles.[17]

Current Guidelines for PPE

FACEMASKS/RESPIRATORS: Cloth masks are not considered PPE and should be avoided during care of patients with suspected or confirmed COVID-19 case and any procedures that warrant use of respirator or appropriate face mask. For any type of facemask, appropriate use and management are essential to ensure they are as effective as possible. [18]

WHO provides the following guidance on the correct use of masks

- Perform hand hygiene before putting on the mask.
- Inspect the mask for tears or holes, and do not use a damaged mask.
- Place the mask carefully, ensuring it covers the mouth and nose, adjust to the nose bridge and tie it securely to minimize any gaps between the face and the mask. If using ear loops, ensure these do not cross over as this widens the gap between the face and the mask.
- Avoid touching the mask while wearing it. If the mask is accidently touched, perform hand hygiene.
- Remove the mask using the appropriate technique. Do not touch the front of the mask, but rather untie it from behind.
- Replace the mask as soon as it becomes damp with a new clean, dry mask.

- Either discard the mask or place it in a clean plastic resealable bag where it is kept until it can be washed and cleaned. Do not store the mask around the arm or wrist or pull it down to rest around the chin or neck.
- Perform hand hygiene immediately afterward discarding a mask.
- Do not re-use single-use mask.
- Discard single-use masks after each use and properly dispose of them immediately upon removal.
- Do not remove the mask to speak.
- Do not share your mask with others.
- Wash fabric masks in soap or detergent and preferably hot water (at least 60°C/140°F) at least once a day. If it is not possible to wash the masks in hot water, then wash the mask in soap/detergent and room temperature water, followed by boiling the mask for 1 minute.
- Ensure proper design of face shields to cover the sides of the face and below the chin.[9]

Protocols for Eye Protection, Gloves and Gowns

EYE PROTECTION

- Wear an eye protection (i.e., goggles or a face shield that covers the front and sides of the face) upon entry to the patient room or care area.
- Avoid eyewear (e.g., safety glasses, trauma glasses) with gaps between glasses and the face likely do not protect eyes from aerosols.
- Ensure that eye protection is compatible with the respirator, and they do not hinder the functions of one another.
- Remove eye protection after leaving the patient room or care area, unless implementing extended use.
- Reusable eye protection (e.g., goggles) must be cleaned and disinfected according to manufacturer's reprocessing instructions prior to re-use. Disposable eye protection should be

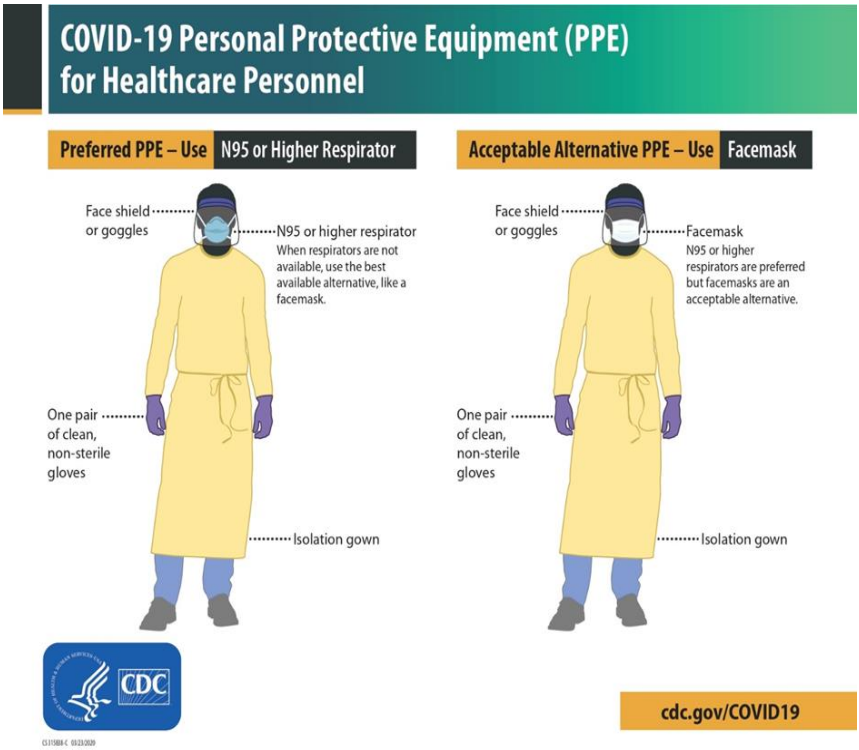
discarded after use unless following protocols for extended use or reuse.[19]

GLOVES

- Put on clean, non-sterile gloves upon entry into the patient room or care area.
- Change gloves if they become torn or heavily contaminated.
- Remove and discard gloves before leaving the patient room or care area, and immediately perform hand hygiene.
- Double gloving is not recommended when providing care to patients with suspected or confirmed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection.[19]

GOWNS

- Put on a clean isolation gown upon entry into the patient room or area. Change the gown if it becomes soiled. Remove and discard the gown in a dedicated container for waste or linen before leaving the patient room or care area. Disposable gowns should be discarded after use. Reusable (i.e., washable or cloth) gowns should be laundered after each use.
- In general, HCP caring for patients with suspected or confirmed SARS-CoV-2 infection should not wear more than one isolation gown at a time.[19]



Picture 1- Personal Protective equipment

Picture 2- Protocols for wearing and removing masks

PROTOCOLS FOR HANDLING OF CONTAMINATED LAUNDRY OR PPE

- Contaminated laundry should be handled as little as possible with a minimum of agitation.

- They should be bagged or containerized and should not be sorted or rinsed in the location where it was used.
- They should be transported in clearly labelled or colour coded bags.
- If there is risk of leakage from the bag due to wet laundry, use leakproof bags.
- Regular washing cycles can be used.
- Personnel handling the laundry should be geared up in appropriate PPE. [20]

VENTILATION SYSTEM

The World Health Organisation (WHO) has advised medical personnel to consider taking 'airborne precautions'. It is important to protect dentists and patients and reduce the amount of spatter produced during dental procedures. In order to prevent airborne transmission, dental offices should be sufficiently ventilated, with great emphasis placed upon removing bioaerosols. [5]

To facilitate natural ventilation, WHO recommends the use of negative pressure room with a minimum of 12 air changes per hour or at least 160L/s per patient. Mechanical ventilation should commence before treating the next patient. For room with natural ventilation, 60L/s per patient is considered adequate ventilation. [21]

AIR AND SURFACE MANAGEMENT

Engineering controls through mechanical ventilation and air filtration are considered a higher level of precaution than PPE and are important mechanisms to reduce the risks of airborne disease transmission in an indoor environment such as the dental treatment rooms. The US CDC guidance recommends that dental offices consider improving the building mechanical ventilation systems and/or adding a portable air cleaner (PAC) to minimize potential risks associated with aerosols in dental offices.[22]

The commonly used devices are high-volume evacuator (HVA) and high efficiency particulate arrestor (HEPA) filters. HVA is

the easiest way to remove the dental aerosols. The device is held at approximately 6-15mm from the active ultrasonic tip. It is effective in removing air at a rate of up to 2.83m^3 per minute and it can potentially reduce contamination by 90%. [23] Assistants are needed in operating it. However modified versions are available which address this issue.

HEPA filters

A study by Ren YF et al (2021) on the effects of mechanical ventilation and portable air cleaner suggest that addition of PAC with a HEPA filter significantly reduced aerosol accumulation and accelerated aerosol removal. [22]

Although HEPA can remove 99.9% of the particles measuring 0.3µm in diameter, one disadvantage of HEPA is that it may retain the microorganisms which can proliferate and enter back into the filtered air. [24]

SURFACE MANAGEMENT

The contaminated fingers could sequentially transfer virus to up to seven clean surfaces. [25] In dentistry, conventional manual disinfection of medical device surfaces is used, and this needs a two-stage disinfection procedure which includes surface rehydration followed by disinfection, for effective inactivation of bacteria and viruses on dry surfaces. The most effective disinfectants are ethanol at strong concentration while sodium hypochlorite and hydrogen peroxide require a minimal concentration to be effective with a low impact on human health. Also, ethanol at 62% and 71% is similarly efficacious against coronavirus but can be used for small surfaces. Ethanol has been widely used for the decontamination of hands based on 80% ethanol or 75% 2-propanol, and these are sufficiently efficacious. For cleaning the workstation surfaces, sodium hypochlorite is suitable at a concentration of 0.05% with efficient and sufficient procedures and when used at a concentration of 0.1%, it is effective in 1 min. Also, hydrogen peroxide is effective with a low concentration of 0.5% and an action time of 1 min. It is used

for cleaning and disinfection implant drills because it preserves the drill structure after 50 cycles of decontamination. [26]

A study by Scarano Antonio (2020) concluded that the decontamination technique that best suits the needs of the dental clinic is peroxide and hypochlorous which can be sprayed via a device at high turbine speed with the ability of producing small aerosol particles, recommendable also for their low cost.[27]

Kampf et al (2020) revealed SARS and MERS can persist on inanimate surfaces up to 9 days. Certain disinfectants like 62% - 71% ethanol, 0.5% hydrogen peroxide and 0.1% sodium hypochlorite can be used in tackling them.[26]

Although germicidal agents have advantages against Covid-19, studies have shown Alcohol-based hand sanitizers can potentially cause dry skin, infection, and alcohol poisoning especially amongst children, along with other health problems. Possible risks with disinfectants should be known and appropriate measures have to be taken to ensure the associated risks are averted.[28]

CONCLUSION

Limiting or controlling aerosols in the dental operatory should be the main concern of dentists worldwide, so choosing the right technique and technology is very critical. Although, total eradication of aerosols during dental treatment is next to impossible, it is possible to minimize the risk of transmission by a strategic combination of various protective procedures along with the application of the universal barrier technique. These aerosols represent a potential route for disease transmission. This transmission can be minimized by incorporating several infection control measures into the routine precautions used during the patient care.

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Original Article

Screening of Antiangiogenic Potential of Fresh *Moringa oleifera* Leaves Extract Using Chick Chorioallantoic Membrane Assay

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Abstract

Background: Angiogenesis is the formation of new blood vessels that supports the progression of cancer. Angiogenesis inhibition will inhibit metastasis and growth of tumour cells. *Moringa oleifera* leaves extracts have been reported to possess therapeutic effects like anticancer, antimicrobial, anti-inflammatory and hypotensive effects. Chick chorioallantoic membrane is an extraembryonic membrane which consists of high density of capillary networks. This enables it to be used in this research to screen the antiangiogenic potential of fresh *Moringa oleifera* leaves extracts. **Materials and Methods:** Preliminary phytochemical screenings were carried out on extracts. Fresh fertile chicken eggs were divided into different treatment groups. Sample treatment was given to the eggs on day six of incubation. Pre- and post- treatment images of the chorioallantoic membrane were taken using stereomicroscope. Percentage of blood vessels increased/ reduced after treatment were determined at the end of 24 and 48 hours of treatment. **Results:** Preliminary phytochemical screening of methanolic extract of *Moringa oleifera* leaves showed the presence of alkaloid, flavonoid, tannins, and steroid. The screening also revealed that aqueous *Moringa oleifera* leaves extracts contained alkaloid, flavonoid, saponins, tannins and steroid. Statistical analysis revealed that the antiangiogenic effect was increased with increased methanolic leaves extracts concentration ($p<0.05$). The analysis also found

that the antiangiogenic effect was not significantly increased with the increased in aqueous leaves extracts concentration ($p>0.05$). **Conclusion:** Methanolic and aqueous extracts of *Moringa oleifera* contain various phytochemicals that inhibits angiogenesis.

Keywords: Antiangiogenesis, CAM assay, *Moringa oleifera*.

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Introduction

Cancer cell undergoes rapid and uncontrolled cell division that has the ability to metastasise to different parts of the body. Tumour cells stimulate angiogenesis by releasing angiogenic growth factors like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). These factors induce endothelial sprouting and formation of new blood vessels. In few conditions, an imbalance of angiogenic and anti-angiogenic factors, results in uncontrolled growth of blood vessels that lead to development of cancer. [1-3]

Currently, patients show more interest towards natural medicine for prevention and treatment of ailments. [1,4] Exploration of natural reserves for their therapeutic potential might lead to more effective, safe and affordable treatment when compared with conventional therapy. Extensive research on medicinal plants is being conducted all over the world to meet the increasing demand for safe therapy. [5]

Moringa oleifera is a tree of Moringaceae plant family and rich in phytochemicals [6] with less reported toxicity.[7] Anti-cancer properties of *M. oleifera* leaves have been established by few research studies. However, anti-angiogenic potential of *M.*

oleifera is not reported till date. Hence, this research study is focussed on determining the angiogenesis inhibition property of the *M. oleifera* fresh leaves.

Chick choriallantoic membrane (CAM) is an extraembryonic membrane formed between day 4 and 5 of embryonic development of chick. The membrane is rich in vascular network of blood vessels. [8] During the embryonic development stage of days 11 and 12, the vascular system of CAM becomes highly angiogenic and new blood vessels are continuously formed. During this period CAM is highly responsive to proangiogenic and antiangiogenic stimuli. [2] CAM contains different extracellular matrix proteins which include type 1 collagen, fibronectin and laminin. [2,10] The presence of these proteins will create and mimic cancer cell environments. Also, CAM is an immunocompromised vascular tissue that is suitable for screening proangiogenic and antiangiogenic properties of compounds. Hence, in this research study, CAM assay was adapted to explore the anti-angiogenic potential of *M. oleifera* [9].

Materials and Methods

Plant extraction: Fresh *M. oleifera* leaves were collected and dried under shade. Dried leaves were coarsely powdered and approximately 30 g of the powder was boiled with methanol and distilled water separately for 6 hours in water baths. The filtrates were then concentrated and dried to get the final extracts.

Qualitative phytochemical screening : Preliminary screening for phytochemicals was carried out based on the standard procedure. [11,12] Extracts were dissolved in 5 mL of solvents and the solution was used for qualitative analysis of alkaloids, flavonoids, tannins, saponins and steroids.

CAM assay: Fresh fertile chicken eggs were collected from a nearby hatchery and the eggs were cleaned to remove the dirt sticking to the shell. They were divided into six groups for treatment with phosphate buffer saline (PBS), standard and

different concentrations of extracts. Eggs were incubated for 5 days at 37 - 37.5ºC and 55 - 60% humidity. On day 5, candling of eggs was done to check on viability and embryo growth. Eggs that showed development of embryo were taken to the process of window making on the same day. A window of 2 x 2 cm was made by removing the shell on each egg after displacing the CAM layer carefully. The eggs were sealed with airtight paraffin and were further incubated for another 24 hours.

On day six whatman filter paper discs soaked in sample solutions were placed onto the CAM according to the treatment groups. The eggs were further incubated for 24 and 48 hours after the treatment. Pre- and post-treatment CAM were observed under stereomicroscope and the images were taken and analysed manually by counting the number of blood vessels. Blood vessels that branched out from the main branch were considered for the analysis. Percentage reduction/ increase in blood vessels was calculated using the formula:

$$\%\ \text{change in blood vessels} = \frac{\text{Difference in number of blood vessels after treatment}}{\text{Number of blood vessels before treatment}} \times 100\%$$

Statistical analysis : Statistical analysis was carried out by using IBM SPSS v.26 software. One-way Anova was chosen to determine the statistical significance between different treatment groups. Tukey post-hoc test was carried out to determine the significant difference between each treatment group.

Results

Fresh leaves of *M. oleifera* were dried in shade for 1 week, extracted and their yield and physicochemical characteristics are reported in Table 1.

Table 1: Yield and Physical characteristics of *M. oleifera* leaves extracts

	Methanolic extract	**Aqueous extract**

Yield (%)	2.52	19.60
Colour	Greenish dark	Brown
Consistency	Sticky and oily	Powder
Odour	Characteristic odour	Characteristic odour

Aqueous and methanol extracts were screened for phytochemicals and are reported in Table 2. The screening was done to identify the groups of phytochemicals present in the extracts.

Table 2: Qualitative Phytochemical Screening of *M. oleifera* leaves extracts

Phytochemials (Tests)	**Methanolic**	**Aqueous**
Alkaloid (Dragendorff's Test)	+	+
Flavonoid (Sodium Hydroxide Test)	+	+
Saponins (Foam Test)	-	+
Tannins (Ferric Chloride Test)	+	+
Steroids (Salkowski Test)	+	+

+ = Presence - = Absence

Photographic images of CAM layer of each sample before and after application of the study compound were captured. The region was assessed again after 24 and 48 hours of sample application. The change in the number of blood vessels is reported in Table 3.

Table 3: Percentage change in blood vessels on the CAM region of interest

Groups	% change in blood vessels (Mean ± SD)	
	Post-24 hours	Post-48 hours
Negative control	42.48 ± 4.74*	65.85 ± 7.04*
Positive control	42.39 ± 5.43#	57.19 ± 5.29#
50% methanolic extract	21.73 ± 4.45#	52.94 ± 6.58#
100% methanolic extract	64.20 ± 6.25#	89.07 ± 6.66#
50% aqueous extract	25.57 ± 5.39#	52.70 ± 7.19#
100% aqueous extract	38.22 ± 4.77#	66.64 ± 6.49#

* Percentage increase in blood vessels.

Percentage decrease in blood vessels.

Statistical analysis between treatment groups and their significance values are presented as pictographic presentation in Figure 1 and 2 below.

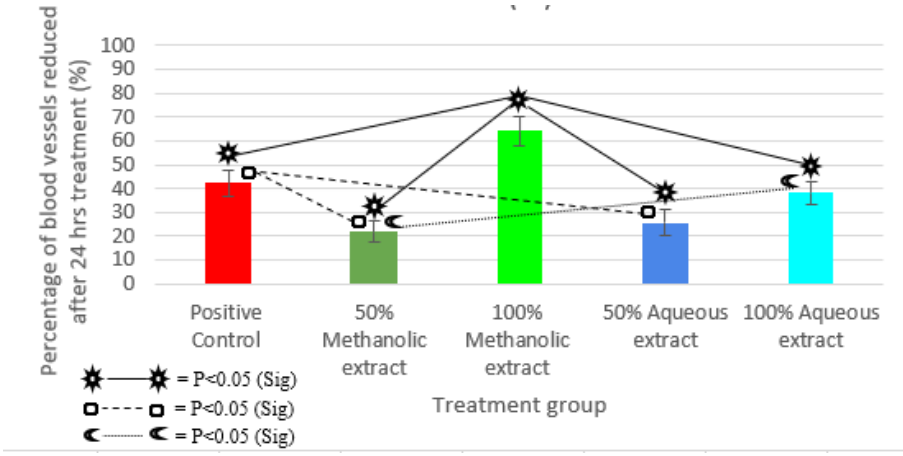

Figure 1: Comparison of percentage reduction in blood vessels after 24 hours of treatment

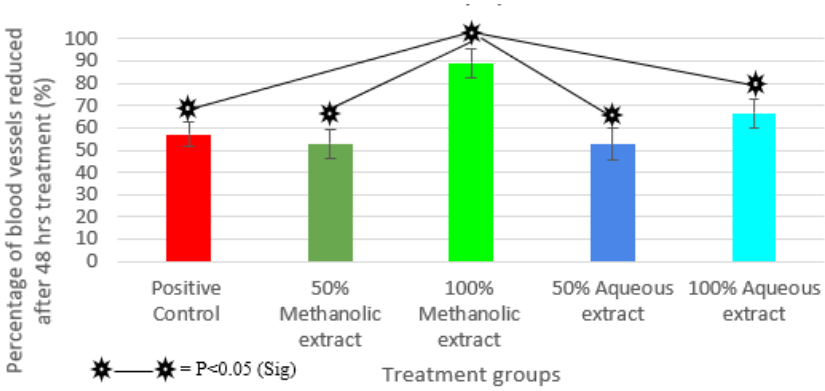

Figure 2: Comparison of percentage reduction in blood vessels after 48 hours of treatment

Discussion

Phytochemicals present in *M. oleifera* fresh leaves were extracted by decoction method of extraction using methanol and distilled water. The percentage yield and physical characteristics of the completely dried extracts are presented in Table 1. The aqueous extracts yield (19.60%) was found to be higher than methanolic extracts (2.52%) which explains that the leaves of *M. oleifera* are rich in water soluble components. Methanolic extract was sticky and oily after complete drying. This could be due to the presence of steroids or any other oily constituents that are easily soluble in methanol.

Preliminary qualitative analysis revealed the presence of various phytochemicals. Both the extracts contained alkaloids, flavonoids, tannins and steroids. Additionally, aqueous extract also has saponins which are absent in the methanolic extract. These phytochemicals have already been studied by various researchers and have proven pharmacological benefits.[12-14] The most common flavonoid found in the leaves of *M. oleifera* include kaempferol and quercetin [14]. Flavonoids have been reported to possess antiangiogenic, antioxidant and antiproliferative

properties. Angiogenesis inhibition will prevent neo angiogenic processes and cause rapidly multiplying cancer cells deficient of oxygen and nutrients which will eventually lead to death of cancer cells by inducing apoptosis.[15,16]

Studies have proven that *M. oleifera* possess anticancer properties against cell lines of colorectal cancer and breast adenocarcinoma.[17,18] However, the antiangiogenic effect of the leaves has not been proven yet. Hence in this study CAM assay was carried out using chicken eggs to screen for antiangiogenic *M. oleifera* leaves extracts. Fertilised and incubated chicken eggs were divided into 6 groups of negative control, positive control, 50% methanolic extract, 100% methanolic extract, 50% aqueous extract and 100% aqueous extract. PBS was used as solvent to dissolve the standard drug and extract samples. PBS helps to moisturise cells, balance the pH and prevents shrivelling and rupturing of cells as it is non-toxic and isotonic to the cells. [19] Hence, negative control group was treated with PBS of pH 7.4 to nullify the solvent effect in the study. Sunitinib, tyrosine-kinase inhibitor is widely used clinically to treat various gastrointestinal tumours. It specifically inhibits VEGF receptor, PDGF receptor and proto-oncogenes [20] with the IC_{50} of 0.01 µM.[21] Hence, sunitinib was used as a standard drug to compare the antiangiogenic effect of extracts. Samples of negative control group showed an increase in blood vessels of about 42.48% after 24-hours of treatment and 65.85% after 48 hours of treatment (Table 3). This confirms that PBS did not show any effect on the process of angiogenesis, and the embryo development was not altered by PBS. However, 33% of negative control samples faced a survivability problem after making a window on the shells which may be due to exposure to the dust during the process of window making or contamination.

The antiangiogenic effects of the treatment groups were determined and percentage reduction in number of blood vessels after 24 and 48 hours of treatments were calculated and are tabulated in Table 3.

Sunitinib showed an average reduction of 42.39% and 57.19% after 24 hours and 48 hours of treatment, respectively. Out of four sample treatment groups the highest percentage reduction of blood vessels was observed in 100% methanolic leaf extract after 24- and 48-hours of treatment. An average of about 64.20% and 89.07% reduction of blood vessels was seen in 100% methanolic extract post- 24 and 48 hours of treatment. Whereas 50% methanolic extract showed less inhibition than 50% aqueous extract after 24 hours. After 48 hours of treatment, both 50% aqueous and methanolic extract groups showed similar inhibition of blood vessels. Aqueous extract of 100% concentration exerted weaker antiangiogenesis after 24-hours, whereas, after 48 hours it showed stronger antiangiogenic potential when compared with the standard sunitinib.

Based on this research findings antiangiogenic potential of *M. oleifera* fresh leaves extract can be sequenced as 100% methanolic extract > sunitinib > 100% aqueous extract > 50% aqueous extract > 50% methanolic extract post-24 hours treatment. Antiangiogenic potential post 48 hours treatment can be sequenced as 100% methanolic extract > 100% aqueous extract > sunitinib > 50% methanolic extract group > 50% aqueous extract.

Statistical analysis by one-way ANOVA showed that the treatment groups post-24 and post-48 hours of treatment displayed significant ($p<0.05$) data. Post-hoc test was also carried out to determine the significance between treatment study groups. Significance data is presented in Figure 1 and Figure 2 which shows that the study groups possess a comparable antiangiogenic effect with the standard agent. [22,23]

The objectives of this research were successfully achieved as various phytochemicals had been extracted out from the fresh *M. oleifera* leaves using methanol and distilled water. Studies have shown that the anticancer effect of *M. oleifera* leaves could mostly be due to the presence of phytochemicals like isothiocyanate, niazimicin and quercetin.[24, 25] Through this research, it is shown that *M. oleifera* fresh leaves extracts possess

antiangiogenic properties and an attempt to fill the research gap by starting another path in understanding the anticancer mechanism has been initiated.

Conclusion

This study is preliminary research that proves antiangiogenic potential of fresh *M. oleifera* leaves extracts. Methanolic and aqueous extracts contain various phytochemicals that need to be isolated and elucidated for its structure. It is concluded that methanolic leaves extract shows higher antiangiogenic effect than aqueous extract. However, more studies are needed to further evaluate the phytochemicals and antiangiogenic effects of *Moringa oleifera* leaves by overcoming the limitation of this study.

Limitations: Separation, isolation and quantification of phytochemicals was not done in the study. Another limitation is, in the CAM assay possibility for elimination of inflammatory triggers was not carried out. Hence, these inflammatory triggers could have influenced the process of angiogenesis. Advanced software can be used to quantify the antiangiogenic effects in the CAM like MountainsSPIP 8 software and Wimasis Image Analysis.

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Original article

Assessment of IOTN application among dental undergraduates-A pilot study

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Abstract

Background: Various oral health problems can be affected with the presence of malocclusion such as compromising on dentofacial esthetics, speech, mandibular function, and psychological wellbeing of the individual. The degree of severity of malocclusion needs to be evaluated beforehand to plan orthodontic treatment accurately. This study was done to assess the application of dental health component and aesthetic component of IOTN among dental undergraduates. **Materials and Methods:** The sample consisted of 40 BDS students (24 females and 16 males) belonging to 2012-13 batch in their final year with a mean age of 24±2 years for grading the study models and photographs from the orthodontic department patient record library. The cast and photographs were examined for the dental health component (DHC) and esthetic component (EC) at faculty of dentistry, SEGi university. **Results:** The mean measurements of overjet, crossbite, displacement and overbite were 3.85mm, 4.09mm, 6.63mm and 4.34mm respectively of the students as against 3.50mm, 4.50mm, 7.00mm and 4.00mm of the control. The mean measurements were almost like the control value in overbite, crossbite and displacement. However, there was some variation with the measurement of overjet. 75% of students were able to get similar results as the control in the dental health component of IOTN while only 25% managed to get similar results with the control with the aesthetic component. **Conclusion:** Therefore, this suggests more emphasis is required in training before the skill demonstration of recording dental

health component parameters and aesthetic component appreciation which can bias the overall grading and referral.

key words: Aesthetic component (AC), Boley Gauge, Dental health component (DHC), Epidemiology, Index of orthodontic treatment need (IOTN), Undergraduates.

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Introduction

Oral health envisions myriad of factors, one among them being structural relation in achieving balance, efficiency, and functional harmony. Patients altered with these relation and occlusion mostly do not realize that they have problem and others feel that they need treatment which they cannot afford it or obtain it.[1,2] The perceived and demand need also varies with social and cultural requirements.[3] The degree of severity of malocclusion needs to be evaluated beforehand to refer and plan the orthodontic treatment accurately.[4] Many orthodontic indices were developed to determine and systematically categorize the degree of malocclusion such as Summers' Occlusal index[5], the Treatment Priority index[6], the Need for Orthodontic index[7], Index of Outcome, Complexity and Need (ICON)[8] and the Index of Orthodontic Treatment Needs (IOTN).

IOTN is a versatile index which is applicable directly, chair side on the patient with tabulated specific individualized incremental progressive parameters to examine, as well as this same can also be done indirectly with study models and clinical photographs for grading. It is also crafted for epidemiological survey purpose with five parameters and utilized in both direct and indirect method with the help of records.[9-13] These methodologies can be applied

for both dental health component (DHC) and esthetic component (EC) by general dental practitioners, specialist, and or by trained personel.[14]

Index of Orthodontic Treatment Need (IOTN) is a method of defining the severity of occlusal traits which may cause a threat to the life expectancy of the dentition, temporomandibular joint and associated function.[15] It is useful as a method for planning, contracting and monitoring NHS orthodontic service as shown in a surveys by UK dental public health consultant.[16] The utility of IOTN is even complimented by the British orthodontists as 'quick', 'simple' and 'easy to use' and, make available the treatment beyond the socioeconomic barriers.[17-19] Therefore the reliability of application among younger generation undergraduates was randomly measured in this study.

Materials and Methods

This study was conducted at faculty of dentistry, SEGi university, Malaysia. The sample consisted of total 40 students in their final year of their BDS program of 2012-13 batch. After the ethical committee approval all the students were introduced to the IOTN module with tutorials and hands-on practical sessions indirectly on patient study models and clinical intraoral photographs at secondary care. The epidemiological module involving missing, overjet, crossbite, displacement and overbite (MOCDO) was selected for the study purpose. The total sample was subdivided into 4 groups each containing 10 students. IOTN grading proforma, one patient study model casts, digital Boley gauge, and intraoral photograph-front view of the same patient were utilized for the study.

One patient pretreatment record was selected randomly from the record library of orthodontic department and duplicated into four similar sets to challenge the skills. This was pre calibrated by two skilled supervisors to alleviate the inter examiner differences to set the accuracy levels which acted as a control for both DHC and AC. These four sets of records were double blinded and were

subjected to student's evaluation with a lottery method for the first time for visual appreciation/ psychological distraction that they were of different patients. The sample was evaluated at two different time points within a 4-week interval between each procedure to assess intra examiner differences for accuracy and reproducibility. First decimal was considered and rounded-off to the nearest whole number for ease in data processing.

Statistical Analysis

Data was analyzed using Chi-Square test and independent t-test SPSS (version 22) software for the mean and standard deviation of the students against the supervisor's (control) values for dental health parameters like missing, overjet, crossbite, displacement, overbite, dental health component grading and the aesthetic component grading. Significance level was set at $p<0.05$.

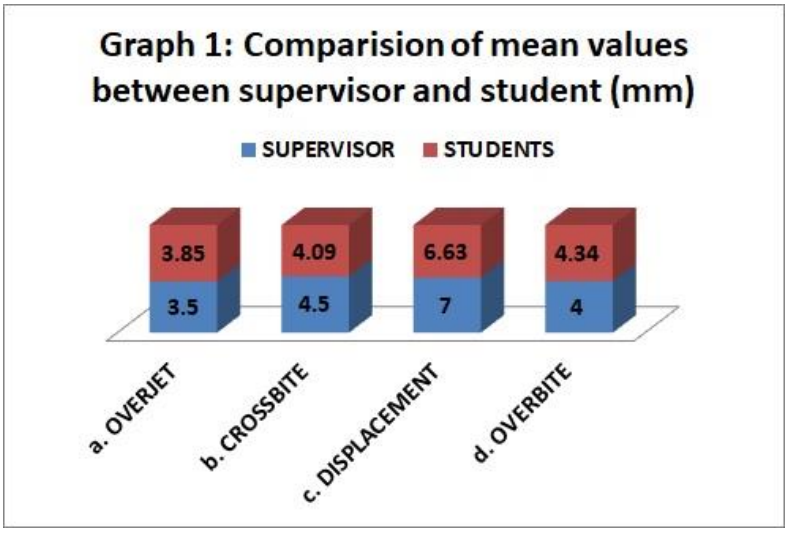
RESULTS

The discrete data (Figure 1 (a-f)) furnished the following details. The intra examiner differences were not as significant as majority reproduced the values with a standard deviation of ±0.2-0.4 mm ($p<0.001$). Missing was identified by 90 percent of students. There were differences among the students with the parameter estimation.

Overjet (Figure 1a) was mapped over a range between 3 to 6 mm with a mean of 3.85 mm (Graph 1a). Most of them, 14 students measured 4 mm with the next highest being 3 mm of overjet by 13 students compared to 3.5 mm of the control. Only 4 students were coinciding with the supervisors. Crossbite was measured between 0 to 6 mm (Figure 1b) ranges with a mean of 4.09 mm (Graph 1b). 18 students measured 4 mm and others deviating between 0 and 6 mm compared to 4.5 mm of the control value marked by only 1 student. The displacement was mapped over a range between 2.5 to 10 mm (Figure 1c) with a mean of 6.63 mm (Graph 1c). Majority (11 students) measured 9 mm with the next highest being 7 mm by 7 students which was also the control value. Overbite was mapped over a range between 2 to 7 mm

(Figure 1d) with a mean of 4.34 mm (Graph 1d). Many (11 students) mapped 11 mm with the next highest being 4 mm by 8 students, the later matching supervisor value.

Dental health component (Figure 1e) was graded at 4 by 30 students making it 75% matching with the supervisor value (Graph 2b) followed by 9 and 1 students respectively at 3 and 1 grades. However, contrasting ratings were picked up against the control value 6. 25% matched (Graph 2a) the aesthetic component (Figure 1f) and the rest varied between 2 and 7 ratings.



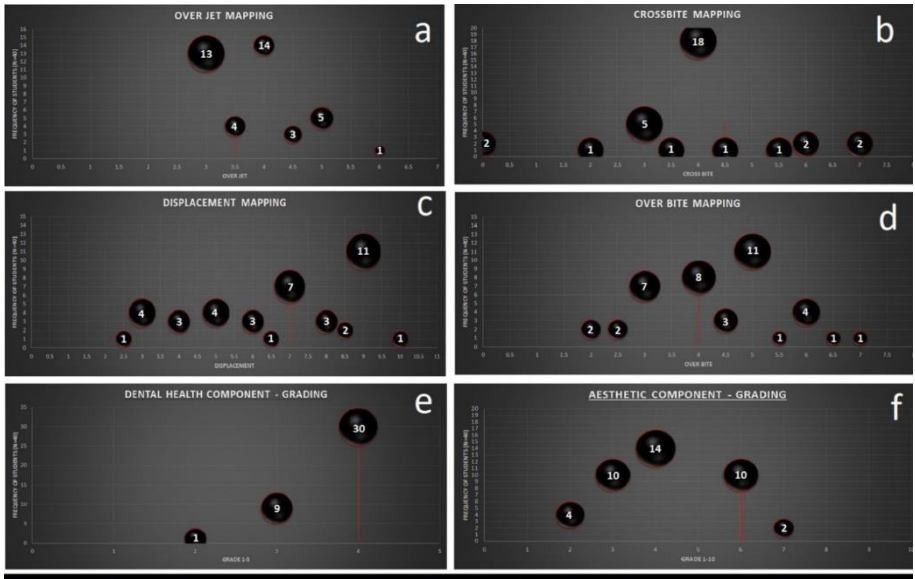
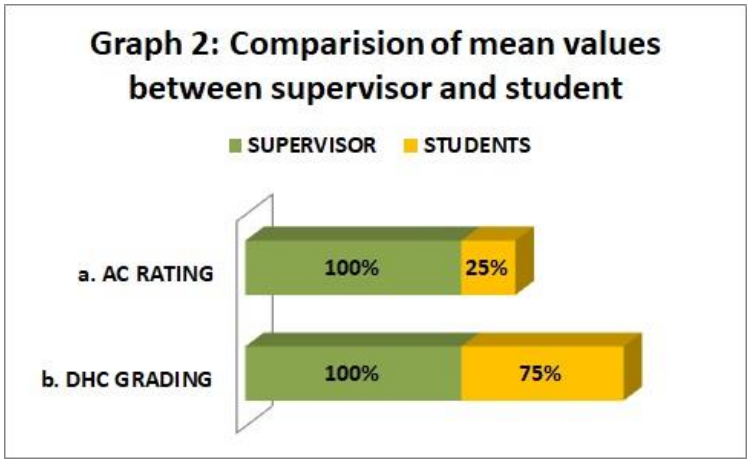


Figure 1 (a-f): Mapping of overjet (a), crossbite (b), displacement (c) overbite (d), DHC grading (e), AC rating (f).



DISCUSSION

This solitary model methodology pilot study is unique with a sample size of 40 and 1 patient record to examine, that was randomly selected and examined to test the specificity horizontally. Jawad Z et al[13] also did similar study with different model including the frequency of IOTN use in primary or secondary care along with training intervals and the accuracy of use. The IOTN components like DHC and AC had significant difference in calibrating and grading in this study which were similar to early studies conducted by Popat H et al[20] and Loke ST[21] in general with 30 models each and 70 and 13 sample size.

Dental health components such as overjet had significant difference ($p \leq 0.005$) with the mean of students being 3.85 compared to that of control i.e. 3.50 and a standard deviation of 0.76. Crossbite, displacement and overbite were insignificant ($p \geq 0.005$) i.e. *p values* were *0.066, 0.29 and 0.09* with mean and standard deviation comparison between student and control being 4.09:4.50±1.40; 6.63:7.00±2.26 and 4.34:4.00±1.24 respectively (Graph 1 a-d). This results were in agreement with Jawad Z et al[13] and Puri A[23] stating the frequency of use of IOTN as an important factor concurrently with registrant group like dental foundation trainees and place of work like primary care, secondary care and both stating weak performance.

Aesthetic component showed significant difference ($p=0.0$) with control value. Only 25% of students rated similar, with 75% (Graph 2a) being unable to subjectively perceive which was quiet different with the analytical dental health component i.e. 75% of students were approximately ($p=0.003$) correlating to the control value with the rest 25% being varied to the normal range (Graph 2b). This result was in accordance with Jawad Z et al[13] where dental foundation trainees and general dental practitioners who completed their training between 1 to 5 years performed weak than other registrants. Gilmour ASM et. al[22] also confirmed in their study that 35.5% did the assessment on their own with limited confidence, slowly followed by 32.4% with own on

following advice. In their study they confirmed only 19% performed IOTN assessment on own with confidence.

Conclusions

This study envisions that more emphasis must be given to enhance students' skills in recording and perceiving the IOTN components which can influence grading decision making, apart from regular clinical examination. Every student is unique and their cognitive and psychomotor skill applications also vary from individual to individual in spite of thorough training. Training in future may need to focus on additional assessment diagnostic and measurement skills in DHC and AC in order to have better objectivity and agreement before subjecting these individuals for epidemiological surveys and individual clinical assessment for quality referral.

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Case report

Atypical Orthodontic induced external apical root resorption: A Case report

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Abstract

Orthodontic induced external apical root resorption is one of the idiopathic phenomenon's as an effect, force generated through mechanotherapy as one of the cause and the biological tissues with their diversified variations as witness. It is also sub classified into iatrogenic as a result of indeterminate application of orthodontic forces with subconscious appreciation of the existing conditions. Numerous factors were identified to be related to this irreversible pathologic condition, but none were proven scientifically. Genetics and salivary markers proved the reliability with time. Different assessment methods were also identified to clinically diagnose it both subjectively and objectively. Mostly it is identified through routine radiographic stage records or certain prediction radiographs for root resorption probability assessment. This case report discusses one such encounter which was experienced after stage 2 mechanics involving quite a few teeth. Considering the biotype of the individual and tooth morphology the treatment was terminated and recovery measures were briefed to uplift the self-esteem of the individual.

KEY WORDS: Genetics, Malocclusion, Open bite, Orthodontics, Root resorption.

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INTRODUCTION

External apical root resorption (EARR) is one of the complications of orthodontic treatment besides other idiopathic causes and conditions, leading to permanent shortening of tooth root structure involving one or more teeth, generally maxillary central and lateral incisors being affected.[1] The degree of severity can be manifested proportionally with the type of force and magnitude generated by orthodontic appliances and classified as pathological in nature.[2,3] More than a third of individuals treated with fixed appliances evidenced greater than 3 mm of root length resorption, whereas 2% to 5% witnessed up to 5 mm whereby compromising the function and lifespan of the tooth involved.[4,5]

Its numerous ways of occurrence and its clinical manifestations also remained highly variable suggesting new insights for additional diagnostic tools and markers. Routine pretreatment radiographic assessment, random intraoral periapical radiographs (IOPAR) proved efficient in that they were prospective by ruling out the contribution of different root anomalies as well as identification of EARR during the course of treatment with minimal compromise.[6-8]

The present case report discusses one such unconditional occurrence in a class II malocclusion out of exemplary precautions thereby compromising the objective fulfillment and leading to discharge of treatment.

CASE REPORT

Clinical examination

A 19-year-old female patient presented with inability to bite with her front teeth and some speech difficulty. She had unremarkable medical history. Clinically, she was thin biotype with symmetrical extraoral face, convex profile and increased vertical proportions. Her lips were potentially incompetent with acute nasolabial angle; shallow mentolabial sulcus and simple

tongue thrust habit on swallow. This was further complicated by increased overjet of 11mm, hyperdivergent jaw bases with anterior openbite by 1 mm, narrow arches, palatally erupted 14, upper midline shift towards right, cuspal class II relation on right and full tooth class II on left side, class II division 1 incisor relation and circum-oral muscular hyperactivity on swallow (Figure 1: a-d). The Temporomandibular joints function was asymptomatic with evident jaw deviation on maximum opening and spontaneous reduction to normal on closure.

Radiographic examination

Orthopantomogram (OPG) revealed certain teeth with pipette-shaped, pointed apical third root contours with others dilacerated and also rectangular morphology (Figure 2a). The skeletal relation was class II contributed by severe prognathic maxilla and mild retrognathic mandible. The maxillary dentition had a neutral compensation with protrusion while mandibular teeth had unfavorable compensation with both inclination and protrusion (Figure 2b).

Treatment plan

Establishing her treatment prognosis with average outcome, camouflage treatment was planned with patient's consent and disobedience for surgical approach in achieving optimal structural balance and functional efficiency with esthetic harmony. Damon MBT self-ligating 0.022X0.028" prescription was bonded from second molar to second molar in both the arches. Damon system was elected for her by assuming its passive self-ligation with low friction will reduce the resistance between the root surface and bone thereby minimizing the incidence of root resorption. Symmetric extractions of 14 and 24 were done to restore overjet with some compromise by 2-3mm along with non-extraction in the opposing arch. Subsequently, little more space generation was planned by widening of arches as well as molar distalization of the upper left molars to establish cuspal class II molar relation and simultaneously correcting the midline, and

also for bilateral intrusion of posterior teeth to restore optimal vertical proportion. For this, 18 and 28 were also included into therapeutic extractions alongside the premolars. Infra zygomatic cortical mini screw assistance was considered to reinforce the anchorage.

Stage I treatment of aligning and leveling was done for 13 months with Critical type A anchorage over 0.014", 0.016 and 0.014X0.025" thermal Nickel Titanium (T-NiTi) archwires followed by 0.017X0.025", 0.019X0.025" thermal T-NiTi ending with 0.019X0.025" stainless steel (SS) arch wires. The duration between the appointments was also delayed due to multiple times bracket and buccal tube displacements along with archwire changes to maintain optimal conditions to avoid any root resorption. Stage II objective of space closure was carried out for 11 months on a gentle reverse curve of Spee with active-tie backs, reinforced with intermaxillary class II elastics of 2 ounce force to control the vertical relation of incisors. Overjet was effectively addressed and a stage II OPG taken to proceed further with molar distalization, Intrusion of posterior teeth and midline correction with infra zygomatic cortical mini-implants assistance. The OPG and certain periapical radiographs revealed multiple teeth EARR both in the maxillary anterior and posterior teeth as well as in certain mandibular teeth (Figure 3) compromising further treatment execution and discharge all other procedures. The most affected being maxillary incisors and premolars followed by canines, molars (Figure 4) and least mandibular, as no major mechanics was involved. Seventy percent of the objectives were fulfilled by the end of radiographic root resorption encounter. Clinically the intraoral picture was health and absolute without any signs of EARR (Figure 5) which was occurring within the socket. The appliance was debonded on patient's request and education given on for further maintenance and support over managing of the incidence.

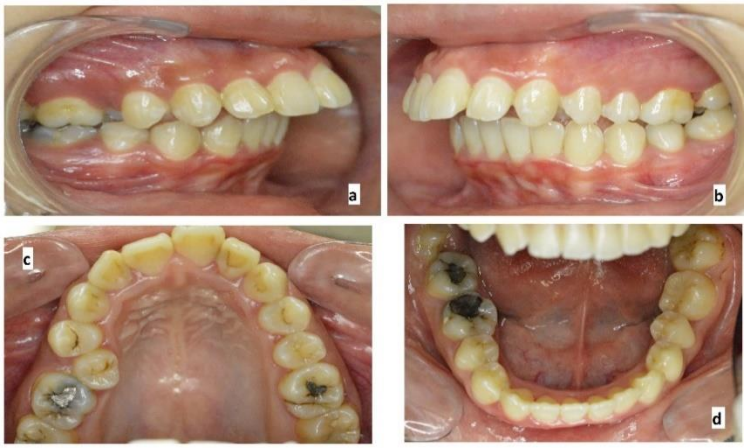

Figure 1: Pretreatment Intraoral photographs (a-d).

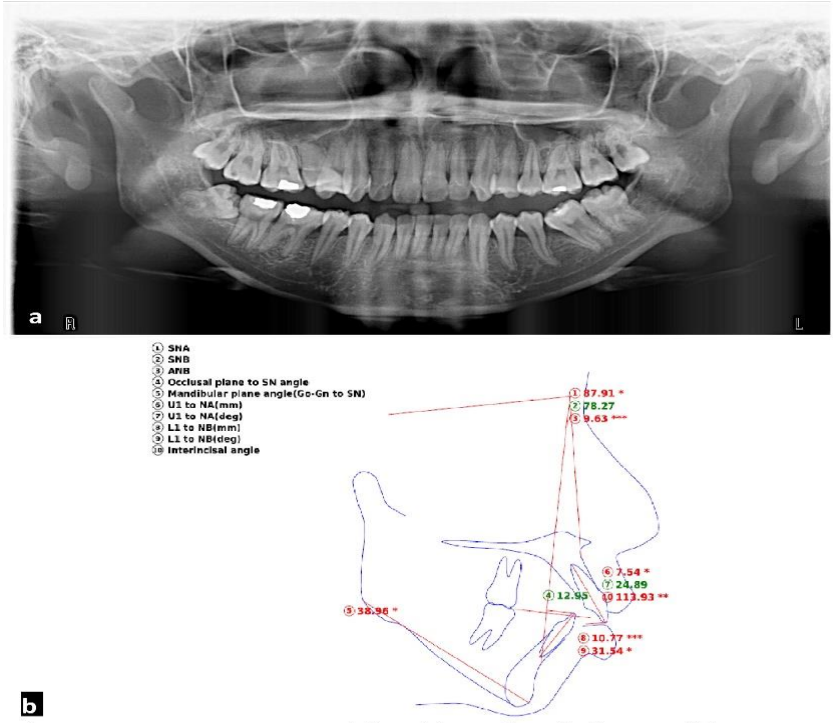

Figure 2: Pretreatment opg (a) and lateral cephalogram (b).

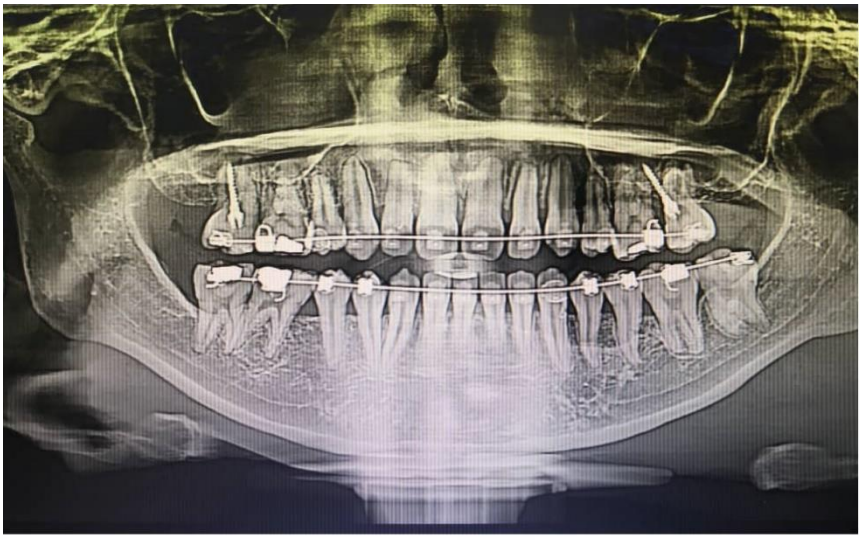

Figure 3: Mid treatment OPG

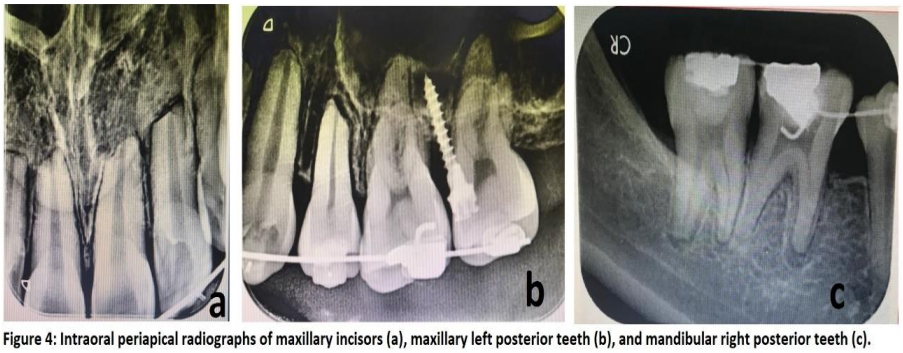

Figure 4: Intraoral periapical radiographs of maxillary incisors (a), maxillary left posterior teeth (b), and mandibular right posterior teeth (c).

Figure 5: Mid treatment Intraoral photographs (a-e).

DISCUSSION

Orthodontic induced external apical root resorption (OIERR) is a globally prevalent undesirable pathological condition without any discrimination even today, that is widely documented by researchers.[9] It's genetic predisposition, ever since published by Newman et al (1975) with family clustering of inexplicable inheritance pattern among its diversity, was later supported with one of such findings by Al-Qawasmi RA (2003)[10] relating to proinflamatory cytokines like IL-1A and IL-1B on IL-1 gene cluster on human chromosome 2q13 substantiating the clinical perception that there is more to root resorption than amount of force or type of appliance used. In accordance with the radiographic finding of thin, pointed, barrel shaped and dilacerated root morphology as a predisposing factor and radiographic marker in prediction of EARR in this individual, Katona TR (2006)[8] and Antônio Geraldo de O (2011)[11] also found

greater correlation of root resorption with similar findings after the orthodontic treatment.

Among the types of malocclusions, class II is mostly reported for orthodontic intervention alongside the complexity. As the growth spurt was completed camouflage treatment was elected by dental compensation of skeletal malrelation. Janson G (2016)[12] found incidence of OIERR in both nonextraction and extraction treatment approaches ranging from mild to severe with clinically insignificant difference in results which was correlating in this condition. Visible changes can be witnessed as early as within 6 months during aligning and leveling phase or by the end of space closure phase through stage radiographs. About 4.1% of patients had an average resorption of at least 1.5 mm of the 4 maxillary incisors, and about 15.5% had 1 maxillary incisor or more with resorption of at least 2.0 mm from 3 to 9 months after initiation of fixed appliance therapy in their study. Although teeth with long, narrow, and deviated roots are at increased risk of resorption during this early stage, the explained variance of these risk factors is less than 25%.[13]

Gingival crevicular fluid markers and salivary markers are the evolving areas in this field to act much earlier than the radiographic findings.[14] Over time many adjunctive therapies with devices were innovated to overcome such collateral encounters like acceleDent[15], micro-osteoperforation[16] (Propel), low-level laser therapy[17] (LLLT), low-intensity pulsed ultrasound[18] (LIPUS), etc. to minimize the intensity of root resorption and hasten tooth movement by improving the bone remodeling. However root resorption even with these aids proved not promising with variable results in the literature review.[19,20] . In one of the study by El-Bialy et al. (2004)[21], revealed the healing effects of LIPUS induced by OIEARR through ultrasound, suggesting more intervention studies in this area. Gay G et al.[22] in their study on Invisalign aligner therapy, being advanced in the field of orthodontic therapy, reported root resorption with an

average of less than 10 percent of their original root length in almost every individual.

CONCLUSION

In spite of even considering majority of factors, the root resorption became inevitable in this condition, instituting that it is a consideration of fact beyond mechanical factors and variable tooth morphology. More emphasis should be shadowed on delivering the mechanical forces at optimal thresholds to rule out the inadvertent mechanotransduction as one of the major cause and invariable understanding the role of other factors in its occurrence.

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