Original Article

Awareness, Attitude and Practice Regarding Disinfection and Handling of Extracted Teeth among the Students in a Dental College in India

Alok Kumar Sethi¹, Rajashree Samal², Banibrata Lahiri³, Asutosh Das¹, Gunjan Kumar⁴, Shibabrata Behera¹

Objective: The main objective of this study is to assess about the awareness, attitude, and practice regarding disinfection and handling of extracted teeth among the students of a dental college in Odisha, India.

Materials and Methods: The survey was done with a self-designed questionnaire distributed among the students of a dental college in Odisha, India. Data analysis was done using the Statistical Package for the Social Sciences Software (SPSS for Windows, Version 16.0, Released 2007, Chicago, SPSS Inc.) and the results obtained.

Results: Of the many methods available, the safest and practical methods to sterilize and disinfect the extracted human teeth could be autoclaving, submersion in 10% formalin and 5.25% sodium hypochlorite solutions. In addition, it was found that there is a significant need to educate the potential handlers of such extracted teeth about the latest statutory protocol and guidelines.

Conclusion: Most of the participants in the study were aware of the established safety protocols regarding how to handle the pathogenic specimens and were putting in practice those standardized guidelines. But still another good number of handlers need to change their casual attitude while following the safety guidelines so as to protect themselves, others and the environment from the pathogenic clinical specimens.

KEYWORDS: Awareness, disinfection, extracted human teeth, hepatitis B virus, human immunodeficiency virus, infection transmission

INTRODUCTION

Receiving right education through right approach goes a long way in honing one’s professional skills. In a professional setup, there could be many such techniques to learn. In the medical field, one of the best such methods is “learning by doing.” Both medical and dental students have to practice their technical and preclinical skills before they do it on the clinical patients so as to achieve greater accuracy and success. Dental procedures require working mostly on the teeth and associated structures. Extracted natural teeth have been used for a variety of dental teaching works such as learning the basics of cavity preparations and root canal treatment (RCT) in endodontics, preparing ground sections to study histological features, studying the properties of restorative materials to evaluate microleakage, and marginal integrity with bond strength evaluation to crown preparation techniques in prosthodontics. Furthermore, the extracted teeth have been used as milled “Natural Inlays” using the CELAY system.[1] Students have to practice on the artificial substitutes such as synthetic blocks or teeth (typodonts) which at times may not simulate the natural teeth leading to errors in the procedures done either routinely, during examinations or for further education and research. In addition, typodonts do have their limitations. Trainees

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_314_18

Address for correspondence: Dr. Alok Kumar Sethi,
Department of Oral and Maxillofacial Surgery,
Kalinga Institute of Dental Sciences, KIIT University,
Bhubaneswar - 751 024, Odisha, India.
E-mail: draloksethi@gmail.com

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How to cite this article: Sethi AK, Samal R, Lahiri B, Das A, Kumar G, Behera S. Awareness, attitude and practice regarding disinfection and handling of extracted teeth among the students in a dental college in India. J Int Soc Prevent Communit Dent 2018;8:488-94.
in order to get better clinical skills and acumen must practice on the extracted and to be discarded natural teeth which closely simulate real clinical situations. These extracted teeth can be the result of needful extractions as in painful pulpitis or in hyperdontia patients undergoing routine extractions.[2] However, these procured extracted human teeth which are being used by the trainees have been found as a potential source of infection. Many transmissible pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), or other bloodborne pathogens commonly Staphylococcus aureus might be present in the pulp or periradicular tissues attached to the extracted human tooth.[3,4] Working on such nonsterilized teeth poses a greater risk of exposure to those deadly microbial population and subsequent disease state.

Controlling infection at the source is critical in preventing its further transmission among the involved staff. Directives by the Americans with Disabilities Act and Centers for Disease Control (CDC) mandates thorough removal of all pathogenic organisms viable over the items used in the patient treatment purposes. Further, these are to be used with caution to prevent disease transmission. This study aims to find about the attitude, awareness, and practice about how the dental students handle the nearly contaminated extracted teeth which they have to use in bettering their clinical skills.

**Materials and Methods**

The survey was done by the close-ended questionnaire method after obtaining the needful Ethical Committee approval (KIMS/KIIT/IEC/83/2015). It consisted of 23 questions [Table 1]. The questionnaire was distributed at random among the 200 undergraduate and postgraduate students studying in a dental college at Bhubaneswar without any sexual predilection or bias [Table 2]. A pilot study was conducted with \( n = 20 \), before sample selection. The absolute precision was found to be 5% and the confidence level was attained to be 95%. The sample size was derived to be 200. Participation of the respondents was purely voluntarily with prior informed consent obtained. The questions included about general awareness, personal information, and attitude and awareness of handling the extracted teeth along with the sociodemographic aspects surrounding the concerned topic. Most appropriate answers as decided by the respondents were recorded and tabulated for statistical analysis.

Test–retest method was employed to validate the questionnaire. Senior, experienced practitioners were burdened with the task to validate the questions to be asked of the respondents on the basis of analysis scale. Some questions were modified to improve the clarity of understanding by the survey population. Validity of the questionnaire approached 80%. Validity of each question came out to be above 74% which was quite satisfactory and acceptable. The reliability of the questionnaire was assessed using the Cronbach’s alpha (\( \alpha \)). This made it an acceptable study. After the final assessment, the questionnaire consisted of 23 questions.

To score the knowledge and performance related questions, each correct response was given a score of one; each wrong response was given a score of two and no answer was given a score of zero [Table 3]. Other questions with many options were given a score of 1, 2, 3, 4, and 5, respectively.

All the responses received from the participants were recorded in the Microsoft Excel sheet. The descriptive analysis was done using frequencies and percentages. The independent \( t \)-test was done to compare the mean attitude and practice scores across genders and years of study. Statistical analysis was performed using the SPSS Inc. Released 2007 (SPSS for Windows, version 16.0. Chicago, IL, USA, SPSS Inc.) and statistical significance was defined at \( P \leq 0.05 \).

### Results

Of the 199 respondents, 82 (41%) were male and 117 (59%) were female (\( P = 0.012 \)) [Tables 2, 4, and 5]. Males take up stressful activities quite differently than the females. May be because of the hormonal built up and the ability to deal with the particular situation. Hence, error done under work pressure is less likely in males.[5] Among these, maximum respondents were 3\(^{rd}\)-year undergraduates (37.7%) and the least were 1\(^{st}\)-year Post Graduates (PGs) (1.5%) [Figure 1, Table 2, 4, and 5]. About 57.4% of all respondents practiced their preclinical work on the extracted teeth, while the rest opted for other options. Between the typodont and the extracted teeth, the latter was preferred more (59.3%). Working on the extracted teeth helps in getting the same tactile sensation as like working in the real case scenario.
| Questions                                                                 | n (%)     | P      |
|--------------------------------------------------------------------------|-----------|--------|
| Is preclinical work practiced on the extracted teeth?                    |           |        |
| a. Yes                                                                   | 115 (84)  | 0.009  |
| b. No                                                                    | 57.4 (39.6)|        |
| Which type of tooth is preferred more for doing preclinical work?         |           |        |
| a. Typodont                                                              | 81 (40.7) | 0.02   |
| b. Extracted tooth                                                       | 118 (59.3)|        |
| Collection of extracted teeth is done from                                |           |        |
| a. Institutions                                                          | 78 (39.1) | 0.04   |
| b. Private practitioners                                                  | 84 (42.2) |        |
| c. Quacks                                                                | 6 (3.01)  |        |
| d. Both institutions and private practitioners                            | 31 (15.6) |        |
| Do you think that the extracted human teeth should be considered as a potential source of infection? |           |        |
| a. Yes                                                                   | 115 (57.4)| 0.00   |
| b. No                                                                    | 79 (39.4) |        |
| a. Don’t know                                                            | 5 (3.2)   |        |
| Have you ever encountered any incidence where the extracted teeth were a potential source of infection? |           |        |
| a. Yes                                                                   | 17 (8.5)  | 0.00   |
| b. No                                                                    | 181 (91)  |        |
| c. Don’t know                                                            | 1 (0.5)   |        |
| Is there a chance of HBV transmission through extracted teeth being used for educational purpose? |           |        |
| a. Yes                                                                   | 87 (43.9) | 0.004  |
| b. No                                                                    | 50 (25.3) |        |
| c. Don’t know                                                            | 62 (30.8) |        |
| Is there a chance of HCV transmission through extracted teeth being used for educational purpose? |           |        |
| a. Yes                                                                   | 71 (36.2) | 0.09   |
| b. No                                                                    | 51 (26.1) |        |
| c. Don’t know                                                            | 77 (37.7) |        |
| Is there a chance of HIV transmission through extracted teeth being used for educational purpose? |           |        |
| a. Yes                                                                   | 89 (44.9) | 0.00   |
| b. No                                                                    | 67 (33.8) |        |
| c. Don’t know                                                            | 43 (21.2) |        |
| Is it necessary to have guidelines by institutions or regulatory bodies regarding handling of extracted teeth? |           |        |
| a. Yes                                                                   | 166 (83.3)| 0.00   |
| b. No                                                                    | 16 (8.1)  |        |
| c. Don’t know                                                            | 17 (8.6)  |        |
| Have you updated yourself about the guidelines issued by regulatory body regarding handling of extracted teeth? |           |        |
| a. Yes                                                                   | 33 (19.8) | 0.00   |
| b. No                                                                    | 166 (80.2)|        |
| Is it necessary to wear gloves while working on extracted teeth?         |           |        |
| a. Yes                                                                   | 180 (90.4)| 0.00   |
| b. No                                                                    | 12 (6.1)  |        |
| c. Don’t know                                                            | 7 (3.6)   |        |
| Is it necessary to wear mouth masks while working on extracted teeth?    |           |        |
| a. Yes                                                                   | 160 (80.3)| 0.00   |
| b. No                                                                    | 31 (16.6) |        |
| c. Don’t know                                                            | 8 (4.1)   |        |
| Is it necessary to wear apron while working on extracted teeth?          |           |        |
| a. Yes                                                                   | 137 (68.7)| 0.02   |
| b. No                                                                    | 42 (21.2) |        |
| c. Don’t know                                                            | 20 (10.1) |        |
| Is it necessary to wear safety glasses while working on extracted teeth?|           |        |
| a. Yes                                                                   | 118 (59.3)| 0.03   |
| b. No                                                                    | 43 (21.6) |        |
| c. Don’t know                                                            | 38 (19.1) |        |

Contd...
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Table 1: Contd...

| Questions                                                                 | n (%)  | P     |
|---------------------------------------------------------------------------|--------|-------|
| Are extracted teeth disinfected or sterilized before use?                 |        |       |
| a. Yes                                                                    | 159 (79.8) | 0.00  |
| b. No                                                                     | 26 (13.1)  |       |
| c. Don’t know                                                             | 15 (07.1)  |       |
| Is it necessary to wear gloves, mouth masks, or aprons while disinfecting or sterilizing extracted teeth? |        |       |
| a. Yes                                                                    | 169 (84.8) | 0.00  |
| b. No                                                                     | 14 (7.1)   |       |
| c. Don’t know                                                             | 16 (8.1)   |       |
| Is it necessary to preserve extracted teeth before use?                   |        |       |
| a. Yes                                                                    | 164 (82.8) | 0.00  |
| b. No                                                                     | 15 (7.5)    |       |
| c. Don’t know                                                             | 19 (8.5)    |       |
| Which method do you think is preferable for disinfection or sterilization of the extracted teeth? |        |       |
| a. Hydrogen peroxide                                                      | 87 (43.7)  | 0.00  |
| b. Boiling water                                                          | 29 (14.5)  |       |
| c. Sodium hypochlorite                                                    | 12 (6.1)   |       |
| d. Glutaraldehyde                                                         | 21 (10.4)  |       |
| e. Normal saline                                                          | 34 (16.9)  |       |
| f. Chlorhexidine                                                          | 6 (03.1)   |       |
| g. All of the above                                                      | 51 (25.5)  |       |
| Is it hazardous to use 10% formalin solution for storage or disinfection of extracted teeth? |        | 0.013 |
| a. Yes                                                                    | 52 (26.1)  |       |
| b. No                                                                     | 63 (31.7)  |       |
| c. Don’t know                                                             | 68 (34.2)  |       |
| After storage of extracted teeth in disinfectant medium have you ever noticed any change in its surface characteristics? |        | 0.015 |
| a. Yes                                                                    | 109 (54.8) |       |
| b. No                                                                     | 90 (45.2)  |       |
| Can extracted teeth be autoclaved before use?                             |        |       |
| a. Yes                                                                    | 37 (18.6)  | 0.00  |
| b. No                                                                     | 57 (28.6)  |       |
| c. Don’t know                                                             | 106 (53.3) |       |
| Can extracted teeth prefilled with silver amalgam restorations be autoclaved? |        |       |
| a. Yes                                                                    | 16 (8.0)   | 0.00  |
| b. No                                                                     | 75 (37.7)  |       |
| c. Don’t know                                                             | 108 (54.3) |       |
| How extracted teeth should be disposed of?                                |        |       |
| a. Yellow-colored bins                                                    | 64 (32.2)  | 0.00  |
| b. Red-colored bins                                                       | 68 (34.2)  |       |
| c. General garbage bins                                                   | 21 (10.6)  |       |
| d. Others                                                                 | 46 (23.1)  |       |

HBV=Hepatitis B virus, HIV=Human immunodeficiency virus, HCV=Hepatitis C virus

Most of the teeth were collected from the institutions as more patients were referred to the bigger setups. A small percentage of the sample population (3.2%) had no knowledge that the extracted teeth could be potential source of infection, whereas 39.4% regarded extracted teeth as safe to work on. About 91% of the respondents could not recall any untoward incident happening with the use of extracted teeth which could be infected. Nearly 36.2% and 44.9% of the people were sure that the teeth may transmit hepatitis C virus (HCV) and HIV, respectively. Large number of respondents (83.3%) felt the need for statutory guidelines to handle the extracted teeth. Not many (80.2%) had kept themselves updated about the latest guidelines for safety. Many agreed to the need for wearing gloves, mouth masks, safe glasses, and apron while working and also while sterilizing the teeth. About 82.8% felt the need to preserve the teeth before putting it to preclinical use. Hydrogen peroxide was more commonly used chemical agent to disinfect or sterilize the teeth rather than chlorhexidine solution.
DISCUSSION

The medical fraternity falls in the very high-risk category with increased chances of coming in contact with some of the deadliest of the microbial world. Disease transmission among individuals has been a great concern in both the medical and dental field with many latest trends and customs coming into practice. Those who know about and follow the universal precautions regarding the spread and control of the diseases may take precautionary measurements so as to contain it. But what about those who have just stepped into this field? Their knowledge regarding infection control may be limited. Dental students have to perform various preclinical procedures before they embark their clinical journey. Working on the extracted teeth is one such preclinical activity required in dentistry. Various bodily fluids come into direct contact with the tooth and may be harbored in the pulp, radicular, and periradicular tissues of the extracted teeth. These body fluids which include the blood and saliva are potential source of infection for HIV, HBV, and HCV along with other bloodborne pathogens. Occupational Safety and Health Administration regulations considers extracted human teeth used further either for research and teaching purposes as potential infective sources. Scholars may contact the pathogenic organisms through aerosols while working on them or experience unintentional injuries with the instruments during handling.

As extracted teeth are source of multiple infections, disinfection of such teeth becomes a crucial step before they can be used in the preclinical setups or elsewhere to prevent further dissemination of diseases. Disinfection could be any procedure that reduces significantly the microbial flora present over the surface of an object while sterilization procedure removes the spores also. Various methods have been used either to disinfect or sterilize the extracted teeth. Sterilizing agents which have been mentioned in the literature are chemical compounds such as sodium hypochlorite, absolute alcohol, sodium chloramine, glutaraldehyde solution, household bleach, normal saline, or by physical methods such as ethylene oxide and gamma radiation. A method as advocated by CDC is storage of extracted teeth in 1:10 ratio household bleach though it is not much successful. Ethylene oxide may reduce the Bacillus subtilis spores ranging from 20% to 36%.[6] Sterilization process should not affect or alter the physicochemical properties of the tooth structure, thereby making it unfit for the preclinical purposes. At the same time, it should be free of all the microbiota even from the deep pulpal space. Of the many new sterilization methods introduced recently, gamma irradiation though a bit expensive is very effective due to its penetrating effects and has no significant altering effects on the mechanical properties of the tooth structure. Sterilization of extracted teeth was evaluated by comparing gamma radiation to that with autoclaving, ethylene oxide, and

Table 2: Sociodemographic distribution of the samples

| Gender      | n (%)   | P (χ²) |
|-------------|---------|--------|
| Male        | 82 (41) | 0.012  |
| Female      | 117 (59)|        |
| Total       | 199     |        |

Year of study

| Year of study | n (%)   | P (χ²) |
|---------------|---------|--------|
| 3rd-year UG   | 75 (37.7)| 0.00   |
| 4th-year UG   | 65 (32.7)|        |
| Interns       | 48 (24.1)|        |
| 1st-year PG   | 3 (1.5)  |        |
| 2nd-year PG   | 8 (4.0)  |        |

UG=Undergraduate, PG=Postgraduate

Table 3: Scoring method

| Score | Correct answer | Wrong answer |
|-------|----------------|--------------|
| 0     | 1              | 2            |

Table 4: Comparison of mean attitude scores between year of study and genders using unpaired t-test

| Gender      | Mean score (SD) | t     | df | SE of difference | P    | Overall mean score |
|-------------|-----------------|-------|----|-----------------|------|-------------------|
| Male        | 3.1 (0.73)      | 1.54  | 197| 0.272           | 0.19 | 3.25              |
| Female      | 3.4 (1.21)      |       |    |                 |      |                    |
| Year of study |          | |    |                 |      |                    |
| 3rd-year UG | 1.7 (0.03)      | 1.97  | 197| 0.098           | 0.01 | 3.25              |
| 4th-year UG | 2.2 (0.3)       |       |    |                 |      |                    |
| Interns     | 3.7 (0.4)       |       |    |                 |      |                    |
| 1st-year PG | 4.3 (1.1)       |       |    |                 |      |                    |
| 2nd-year PG | 4.6 (1.5)       |       |    |                 |      |                    |

SD=Standard deviation, UG=Undergraduate, PG=Postgraduate, SE=Standard error

Table 5: Comparison of mean practice scores between year of study and genders using unpaired t-test

| Gender      | Mean score (SD) | t     | df | SE of difference | P    | Overall mean score |
|-------------|-----------------|-------|----|-----------------|------|-------------------|
| Male        | 3.14 (0.4)      | 1.7   | 197| 0.112           | 0.078| 3.54              |
| Female      | 3.94 (1.1)      |       |    |                 |      |                    |
| Year of study |          | |    |                 |      |                    |
| 3rd-year UG | 2.3 (0.43)      | 1.87  | 197| 0.45            | 0.04 | 3.54              |
| 4th-year UG | 2.8 (0.59)      |       |    |                 |      |                    |
| Interns     | 3.6 (1.2)       |       |    |                 |      |                    |
| 1st-year PG | 4.2 (1.62)      |       |    |                 |      |                    |
| 2nd-year PG | 4.8 (1.5)       |       |    |                 |      |                    |

SD=Standard deviation, UG=Undergraduate, PG=Postgraduate, SE=Standard error
dry heat. It was shown that gamma radiation sterilizes teeth and endodontic filling materials without altering the structure and function of dentin. For complete sterilization, a dose of 173 k-rad with the help of a Cesium radiation source was enough. Furthermore, no detectable changes in the irradiated teeth were found with gamma irradiation, but all other methods introduced some detectable changes.[14] In another study, it was showed that only autoclaving for 40 min at 240°F and 20 psi or soaking in 10% formalin solution for 1 week was almost 100% effective in preventing microbial growth.[12] It has also been showed that autoclaving at 121°C, 15 psi for 30 min and immersion in 10% formalin for 7 min is effective in disinfecting or sterilizing extracted human teeth. They reported that chemicals such as 2.6% sodium hypochlorite, 3% hydrogen peroxide, and boiling in water are not effective disinfectants of the teeth.[13]

The tooth cutting characteristics of the stored extracted teeth also have been studied. It has been found that autoclaving decreases the dentin hardness as noted by decreased microhardness readings than those found in the control group. Both the inorganic and the organic constituents of the teeth are affected by autoclaving. However, gamma irradiation causes no significant changes in the hardness levels.[14] Autoclaving can be one of the best sterilization methods for materials exposed to body fluids. However, teeth can be significantly damaged or altered by the sterilization process done in an autoclave. There is also concern about sterilization of extracted teeth with amalgam restorations in an autoclave as it may release mercury vapors in the air through autoclave exhaust and can cause residual mercury contamination. Thermal cycling may cause teeth with amalgam restorations to fracture due to differences in their coefficient of thermal expansion.[13] All this may suggest that autoclaving may not be a good option to sterilize extracted teeth that are going to be used for preclinical teaching.

Preclinical students have acceptable knowledge about different methods of sterilization which they use before working on the teeth. Some may take the effort to sterilize while others may choose to work directly unaware of the harm associated with it. Senior experienced participants showed that they were capable to some extent about handling of the possibly infected extracted teeth specimens. They were therefore less likely to cause spread of infection iatrogenically.[15] In a study by Kumar et al., 87.5% of the students were asked to sterilize the teeth before they could start working on it.[15] This shows that more number of students are concerned with safety and do take necessary precautions before they work with potentially hazardous things. Since most of the students have general pathology and microbiology in their syllabus, they are generally aware of the methods which can be employed to sterilize the teeth considering the fact that the extracted teeth can be a source of infection. They further accepted that such teeth should and must be sterilized before putting into another use. Extracted teeth are more commonly used in the conservative department and then in prosthodontics to learn preclinical steps as required in the subjects. Typodonts are also used but they do not simulate the exact clinical scenario as the extracted natural teeth which may lead to some incidental errors. Some procedures are preferably more convenient on typodonts such as crown cutting, veneers, or jacket preparations but other procedures such as practicing the intricacies of RCT may require actual extracted natural teeth. Moreover, typodonts remain an expensive option to work on. Government-aided institutes remain the common choice for procuring the extracted teeth because of highly subsidized treatment costs leading to more patient turnover there.

Of the many chemical compounds available commonly such as formalin, hydrogen peroxide, or bleach; still, they are not used by the students frequently. Hydrogen peroxide routinely being used in the clinical situations for other works is frequently used to sterilize the teeth though ineffective.[16,17] For effective disinfection of the teeth, 5.25% sodium hypochlorite and autoclaving could be useful.[18] 2% glutaraldehyde has not shown promising results in sterilization of the teeth.[19] Hashemipour et al. reported that formaldehyde is the only antiseptic solution that can achieve an effective antimicrobial concentration within the pulp space. In addition, the only disinfectant solution that penetrates the pulp chamber is 10% formalin. It can be considered as an effective antimicrobial solution.[20] The effects of formalin on the teeth also has been extensively studied. Obturated teeth when stored in formalin solution show less rate of apical microleakage which is quite significant when compared to storage in nonfixed specimens.[21] However, formalin is carcinogenic as reported in animal studies, thereby limiting its use to some needful areas. CDC recommends that the teeth used for educational and research purposes should be disinfected with sodium hypochlorite or liquid chemical germicides. However, treatment of extracted teeth with sodium hypochlorite can increase the porosity of human enamel by deproteinization.[22]

In addition to these, there are many commercially available compounds that may sterilize the extracted teeth but remain elusive due to cost factor and availability. It was also found that the students did use various other techniques such as using mouth masks, gloves, and aprons to protect themselves. This also reduced the chances of cross contamination among themselves. Guidelines given
by CDC (Centers for Disease Control and Prevention) on how to handle the extracted teeth mentions these as clinical specimens which contain human blood and are therefore potentially infective. These are to be handled with utmost precautions similar to that used for a biopsy specimen. Storage medium commonly available and to be used at least should be household bleach (sodium hypochlorite) in a ratio of 1:10 diluted with tap water. People coming in contact with such specimens should protect themselves adequately by using gloves, face masks, and eye shields. The working area should be thoroughly cleaned and decontaminated after every session. The organization should also take attention so as to minimize the life-threatening risks to the health-care workers who are exposed to such vulnerabilities. It may also identify and minimize the errors and make proper arrangements either for proper disposal or handling.[23]

**CONCLUSION**

In our study, we found that the knowledge, performance, and attitude of dental students in relation to sterilization or disinfection methods of extracted human teeth were quite satisfactory. However, there remain few shortcomings about different teaching methodologies leading to few lapses in the adequate sterilization of the teeth and then ethical disposal of the leftovers. Hence, a disciplined training and teaching methodology in this field is the need of the hour and would go a long way in protecting themselves while learning and also would cause minimal harm to the environment.

**FINANCIAL SUPPORT AND SPONSORSHIP**

Nil.

**CONFLICTS OF INTEREST**

There are no conflicts of interest.

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