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Assessment of knowledge, attitude, and practice of tissue preservation among dentists practicing at Navi Mumbai and Mumbai: A questionnaire-based survey

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Abstract---Background: In histopathology laboratory various steps are carried to obtain a histopathological, cytological, or molecular diagnosis. Two major go hand in hand in laboratories, one is research activities conducted in laboratories which has an enormous importance second is diagnostic procedures. Any diagnosis given by pathologist majorly depends upon the visual interpretation. Hence these procedures are supposed to be followed dutifully. Fixation and prefixation is one of the steps which helps to sustain the tissue vitality and if performed well fixation preserves pathological and molecular tissue archives. Material and methods: A self-designed questionnaire were administered to 202 dentists practicing in Navi Mumbai and Navi Mumbai, consisting of 21 questions stating about their demographic data, professional aspects and knowledge, attitude and practice about preservation of tissue samples. Statistical analysis: Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted. Comparison of frequencies of categories of variables with groups was done using chi square test. Results: The response rate was high as participants were aware of biopsy, fixatives and tissue preservation still there were some obligatory findings, like use of saline, saliva and other material as a fixative, dentist willing to keep formalin at clinic as a fixative material were not aware of biomedical waste management of fixative. Conclusion: The importance of tissue preservation must be accentuated among undergraduate, post graduate students. Lectures, webinars and continuing dental education programs should be conducted to sensitize the topic.

Keywords---histotechnical procedure, artefact, histological diagnosis.

Introduction

Diagnosis reported by pathologists are interpretation of slides by visual evaluation of cells and tissue morphology. Any stained tissue section when comes, across the pathologist sight it is mandatory to obtain a high-quality stained tissue section for an ease of diagnosis. In routine dental practice, dentists come across substantial number of pathologies. It is rightly stated by American Academy of Oral & Maxillofacial pathologist that any abnormal tissue must be sent for microscopic evaluation and diagnosis. (1) Therefore, dental practitioner should be aware of handling of tissue specimen and associated complications.

One of the steps in histotechnical process is fixation which has a focused goal of getting the cell/tissue in a "life like manner". Basically, fixation aims to stop the degeneration of cells/tissue which starts as soon as tissue is deprived of blood supply. Fixation also helps in delaying of autolysis and decomposition of body fluids. It is to stop putrefaction of tissue. (2) As tissue specimen reaching the histopathology laboratory must be in proper preservative or fixative material. Hence the knowledge of fixatives used in clinics and hospitals becomes obligatory amongst all clinicians and surgeons.

Artefact has been defined as “An artificial structure or a tissue alteration on a prepared microscopic slide as a result of an extraneous factor”. Classification of artefacts depending upon aetiology is as follows; during surgery, during fixation and transport, Tissue processing artifacts (3). In present literature we came across articles which suggests that improper fixation leads to degeneration of tissue. Artifacts like autolysis, separation of epithelium and connective tissue, formalin pigments, pigments of mercury, shrinkage of cellular components, teaming artifacts etc. (4), In recent studies they mentioned that fixation procedure if not conducted in an optimal manner it may lead to brown-yellow pigments, shrinkage, diffusion artifacts. (5) The present study aims to understand the knowledge, awareness, and practice of tissue preservation among dentist at Mumbai and Navi Mumbai.

Material and Methods

This is a novel study of 21-point questionnaire tool. (Table 1) The study was piloted in homogenous group of dentists and validated from expert. The reliability of questionnaire tool is done by performing Chronbach's alpha coefficient test, which was 0.749, Hence study questionnaire tool was accepted. In present study after completing the pilot study, sample size estimated which was 202.

Sr. No	Questions	Options
1.	Age	
2.	Gender	Female Male Other
3.	Education	BDS MDS PhD
4.	Affiliation	Institution Dental College Medical College Private Clinic
5.	If MDS which speciality	Oral Medicine Oral pathology Oral Surgery Periodontology Conservative Dentistry & Endodontics Prosthodontics Public health Dentistry Orthodontics
6.	Practicing location	Mumbai Navi Mumbai
7.	Are biopsies done in your clinic/Institute/Dental College	Yes No
8.	If yes, then who performs the biopsy?	General Dentist Oral Surgeon General Surgeon

		Oral Pathologist Other
9.	Number of biopsies per month:	1-5 6-10 11-50 More than 51
10.	Assessment method of biopsies	General Laboratory General Pathologist Oral Pathologist Oral Pathologist from Institution None of the above
11.	Time duration from excision from preservation:	Immediately after excision 5-10 minutes after excision 1-2 hours from excision 1-2 days after excision
12.	Are you aware of different fixatives?	Yes Partly Yes Uncertain Not at all None of the above
13.	Which type of fixative container do you use?	Glass blubs Amber coloured bottle Plastic bottles Company provided blubs None of the above
14.	Which fixative agent do you use at your clinic?	Formalin Toluidine blue/ Lugol's solution 1 percent Saline Equal mixture of Saline and spirit Saliva None of the above Other
15.	Have you ever used Saliva, Spirit, Mixture of saline and spirit, saline or toluidine blue a fixative?	Yes Partly Yes Uncertain Not at all None of the above
16.	Do you think formalin bulb should be kept in clinics, institutions, and hospitals?	Strongly agree Agree Uncertain Disagree Strongly Disagree
17.	Frequency of change in formalin at clinic should be?	Do not use formalin at all Every day Once a week Once a month Once a six month

18.	Are you aware of biomedical measures taken to dispose of formalin/fixation waste?	Yes Partly Yes Uncertain Not at all None of the above
19.	Do you think that proper precautions should be taken for tissue preservation?	Strongly agree Agree Uncertain Disagree Strongly Disagree
20.	Do you think awareness for proper fixative techniques will help avoiding artefacts and subsequent better visual interpretation for pathologist?	Strongly agree Agree Uncertain Disagree Strongly Disagree
21.	Do you think awareness for tissue preservation through webinars, CDE and seminars should be conducted?	Strongly agree Agree Uncertain Disagree Strongly Disagree

Exclusion criteria was dentists who have never conducted biopsy by themselves or by consultant. The study tool is physically distributed and collected filled questionnaire tool from 202 dental surgeons. Data measurement was done using Likert scale. Few questions were given specific relevant choices. The modifying data in the study considered were age, gender, educational qualification, and subject speciality to which the participant belonged.

Results

202 Dentists were approached to participate in study, 103 were GDP (general dental practitioners) and 99 were speciality dental surgeons. (Table 2) 142 Male and 60 females participated with age ranging from 24 to 61 and mean age was 36.37.) With regards to affiliation 143 were practicing individually at private clinic, 47 were attached to dental college and remaining 12 were working in some institutions. Whereas 127 participants were practicing in Mumbai and 75 participants were practicing in Navi Mumbai.

Speciality	Frequency	Percent
Conservative Dentistry & endodontics	18	8.9
Nil	103	51.0
OMDR	3	1.5
OMFS	14	6.9
Oral Pathologist	9	4.5
Orthodontics	12	5.9
Pedodontics	5	2.5

Periodontology	18	8.9
Prosthodontics	17	8.4
Public health dentistry	3	1.5
Total	202	100.0

Table 2. Distribution as per speciality

We asked whether biopsies are conducted in respective clinics/Institutions where participants are practicing. 104 answered partly yes, 90 said yes and 8 were uncertain about biopsy procedure employed at clinic/Institution/Dental college (Table 3).

Biopsies Performed at Dental Clinic/ Dental College/ Institution	Frequency	Percent
NONE OF THE ABOVE	0	0
NOT AT ALL	0	0
PARTLY YES	104	51.4
UNCERTAIN	8	4.0
YES	90	44.6
Total	202	100.0

Table 3: Distribution as per biopsies performed in Dental Clinic/Institution/Dental College

Further we asked about provided biopsy procedure carried by respective personnel ,98 dentists replied procedure is done by Oral Surgeon, 73 said it is done by Oral Pathologists, 29 said other specialty dentists and 2 GDP did biopsy procedure at their clinic by themselves. (Table 4)

Biopsies performed by	Frequency	Percent
General dentist	2	1.0
Oral pathologist	73	36.1
Oral Surgeon	98	48.5
Other	29	14.4
Total	202	100.0

Table 4. Distribution as per Biopsies performed by

We asked about the frequency of biopsies performed at clinic, 177 (87.6%) replied 1-5, 13(6.4%) dentists said 6-10 and 12 (5.9%) dentists replied 11-50 biopsies are conducted per month. (Table5).

Biopsy Number per month	Frequency	Percent
1 TO 5	177	87.6
11 TO 50	12	5.9

6 TO 10	13	6.4
Total	202	100.0

Table 5: Distribution as per biopsy numbers per month

Assessment of tissue done by	Frequency	Percent
General Laboratory	1	.5
General pathologist	17	8.4
None of the above	3	1.5
Oral pathologist	146	72.3
Oral Pathologist from Institution	35	17.3
Total	202	100.0

Table 6. Distribution as per assessment of biopsied tissue done by

Next question was focused on assessment of biopsied tissue, 146(72.30%) said they prefer sending tissue to Oral Pathologist, 35(17.3%) replied to oral pathologists from institutions, 17(8.4%) dentists replied to general pathologist, only one (0.5%) dentist answered general laboratory and 3(1.5%) said they don't say biopsied to any of the mentioned options. (Table 6)

Next question asked to assess awareness was time duration taken for transfer of tissue after excision, 114(56.4%) replied immediately after excision, 43(21.3%) said 5-10 minutes after excision and 14(6.9%) replied after 1-2 hours and 31(15.3%) answered time duration taken for transport of tissue into fixative should be done after 1 to days after excision. (Table 7).

Duration of transfer of tissue to fixative/preservative after excision	Frequency	Percent
1 TO 2 DAYS	31	15.3
1 TO 2 HOURS	14	6.9
5 TO 10 MINUTES AFTER EXCISION	43	21.3
IMMEDIATELY AFTER EXCISION	114	56.4
Total	202	100.0

Table 7. Distribution done by time duration of transfer of biopsied tissue into fixative/preservative

To assess practice, we asked about different fixatives used, 80(39.6%) said they have knowledge, 89 (44.1%) answered partly they know about different fixatives, 19 (9.4%) said they are uncertain about it and 14(6.9%) don't have knowledge at all. (Table 8).

Knowledge of different fixatives	Frequency	Percent
not at all	14	6.9
partly yes	89	44.1
uncertain	19	9.4

yes	80	39.6
Total	202	100.0

Table 8: Distribution as per knowledge of different fixatives

Containers used for tissue preservation	Frequency	Percent
AMBER COLOURED BOTTLE	61	30.2
COMPANY PROVIDED BULBS	21	10.4
GLASS BULBS	71	35.2
NONE OF THE ABOVE	10	5.0
PLASTIC BOTTELS	39	19.3
Total	202	100.0

Table 9: Distribution as per container used for tissue preservation

Further we asked about the type of fixative container used by dentists, 71(35.2%) answered Glass bulbs, 61(30.2%) said they use amber coloured bottles, 21(10.4%) replied company provided bulbs and 39 9(19. %) said they use plastic bottles and 10 (5%) replied they don't use any of the mentioned type of container for preservation of tissue. (Table 9)

To assess awareness of fixative agents we asked about the type of fixative agent used, 137(16.8) said they use formalin, 39(19.3%) said they saline, 14(7%) said they use equal mixture of saline and spirit, 4(2%) use TOULIDINE BLUE /LUGOLS SOLUTION 1% and 8(4%) said they don't use any of the mentioned agent as a fixative. (Table 10)

Fixative used at Dental Clinic/ Institution/ Dental College	Frequency	Percent
EQUAL MIXTURE OF SALINE AND SPIRIT	14	7.0
FORMALIN	137	67.8
NONE OF THE ABOVE	8	4.0
SALINE	39	19.3
TOULIDINE BLUE /LUGOLS SOLUTION 1%	4	2.0
Total	202	100.0

Table 10: Distribution as per fixative agent used at Dental Clinic/Institution/Dental College

To know dentists used alternatives of formalin we asked have they used saliva, saline, saline plus spirit or Toluidine blue/ Lugols solution, 8(4%0 replied partly yes, 85(42.1%) said yes,6(3%) answered they are uncertain about it, 98(48.5%) answered not at all and 5(2.5%) said none of the above. (Table 11).

Alternative Fixatives used	Frequency	Percent
None of the above	5	2.5
Not at all	98	48.5

partly yes	8	4.0
uncertain	6	3.0
yes	85	42.1
Total	202	100.0

Table 11: Distribution as per alternative fixative used

Subsequently we asked about dentist's opinion with regards to keeping formalin bulbs at clinic/ institute, 66(32.7%) strongly agreed, 87 (43.1%) agreed, 32(15.8%) were uncertain about it and 17(8.4%) disagreed completely. We asked about the practice of change of formalin bulb, we found that 71(35.2%), 53 (26.2%) said once in week, 52 (25.7%) said once in six months, 21(10.4%) answered that don't use formalin at all, 4(2%) said formalin bulb should be changed every day, whereas only one dentist said formalin bulb shouldn't change at all. (Table 12)

Duration of change of formalin	Frequency	Percent
DO NOT USE FORMALIN AT ALL	21	10.4
EVERY DAY	4	2.0
NOT AT ALL	1	.5
ONCE IN 6 MONTHS	52	25.7
ONCE IN A MONTH	71	35.2
ONCE IN A WEEK	53	26.2
Total	202	100.0

Table 12. Distribution done as per change in formalin bulb

In last part, we asked about awareness of biomedical waste management, 75(37.1%) answered yes. 43(21.3%) said partly they are aware, 51(25.1%) answered that they are uncertain about the biomedical waste management of fixative agents, 25(12 %) answered not at all and remaining 8(4%) said none of the above. We asked opinion about proper precaution should be taken or not while tissue preservation, 114(55.9%) strongly agreed, 79(39.1%) agreed, 7(3.5%) were uncertain and 2(1%) disagreed to take proper precautions to preserve tissue. (Table 13)

Knowledge of biomedical waste management	Frequency	Percent
None of the above	8	4.0
not at all	25	12.4
partly yes	43	21.3
uncertain	51	25.2
yes	75	37.1
Total	202	100.0

Table 13: Distribution as per knowledge of biomedical waste management of fixative agent

We asked opinion about relation of proper precaution and its relationship with pathologists' interpretation, 119(58.9%) strongly agreed, 64(31.7%) agreed, 13(6.4%) were uncertain and 6(3%) disagreed that there is any correlation. Last question we asked about the interest to gain knowledge about tissue preservation through CDE programs, webinars and conferences, for which 138(67.3%) said yes, 54(27.7%) answered partly yes, 6(3%) was uncertain and 4(2%) were not at all interested to learn about tissue preservation. Succeeding, we tried comparing qualification and use of alternative fixatives, (Table 14) Chi square value for this comparison was 10.364 and p value was .110. We didn't observe any serious difference among GDPs and speciality dental Surgeons and fixative agent used.

	Fixative used at dental college/Institute/ Private clinic	EDUCATION	EDUCATION	Total
		BDS	MDS	
EQUAL MIXTURE OF SALINE AND SPIRIT	Count	5	9	14
	% Within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	35.8%	64.2%	100.0%
	% Within EDUCATION	35.7%	64.2%	2.0%
FORMALIN	Count	65	72	137
	% Within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	47.4%	52.6%	100.0%
	% Within EDUCATION	63.1%	72.7%	67.8%
NONE OF THE ABOVE	Count	4	4	8
	% Within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	50.0%	50.0%	100.0%
	% Within EDUCATION	3.9%	4.0%	4.0%
SALINE	Count	27	12	39
	% Within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	70.8%	30.2%	100.0%
	% Within EDUCATION	25.2%	11.1%	18.3%
TOULIDINE BLUE /LUGOLS SOLUTION 1%	Count	2	2	4
	% within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	50.0%	50.0%	100.0%

Total	% Within EDUCATION	1.9%	2.0%	2.0%
	Count	103	99	202
	% Within FORM OF FIXATIVE AGENT USED IN YOUR CLINIC	51.0%	49.0%	100.0%
	% Within EDUCATION	100.0%	100.0%	100.0%

Table 14: Comparison between education of dentist and willingness to keep formalin at Clinic/ workstation

Discussion

Histopathological diagnosis is all about microscopic examination, observation, and interpretation. Interference in histotechnical steps causes faulty slides which may lead to a misinterpretation or delayed diagnosis. Where fixation is one of the important steps. As mentioned in results our findings were in accordance with previous studies where general dental professionals less than 30% dental professionals have ever conducted a biopsy and 10% said they routinely conduct the biopsy, most of them said that they would rather refer the patient to respective specialist. (6). In another study they found that 33.34% dentist referred biopsy to respective specialist and 31.34% GDP's referred patient to higher centre, where 14.93% dentist said they perform biopsy by themselves at their clinic. (7) Anandani et al found that 11.3% dentists perform biopsies by their own, 50.8% refer to specialist and 31.3% dentists refer patient to a higher centre. (8) E Anuradha Sunil et al stated that they found that only 11% of dentist perform biopsies and almost GDPs preferred sending patients to higher centre and the numbers were too high that is 98%. (9). Savage et al stated that biopsy performed by personnel represented 10% GDP and 14 % specialist routinely participated in performing biopsy, They also mentioned about the specimen amount of received specimen was maximum by oral medicine and radiologist, (10)

In present literature in Wan et al article they found GDP came across biopsy procedure mostly once in a year or once in five years whereas specialist said they come across biopsy procedure once in a month or once in week. The dentists who biopsied tissue every week, were oral medicine specialist and oral surgeons. (10) Anandani et al 50.8% dentist found the tissue supposed to be biopsied in a month but 83.8 % dentist either referred or performed the biopsy. (8) Mugud et al stated that 68.65% dentists perform biopsy at least once a year which was not relatable with our study (7)

Majority of dental surgeon sent tissue for assessment to an Oral Pathologist, . E Anuradha et al found that 59% dentists preferred sending biopsy for assessment to a general pathologist and 23% dentists performing biopsies sent biopsy for assessment to a dental college. (9) Jornet et al found that 69% of dentists send tissue for analysis where 31% said they don't send tissue for analysis, and 27% said sometimes they prefer sending biopsy for histopathological analysis. (11) Anandani et al observed that 37% dentists always send biopsied tissue for

analysis and 46.3% dentist preferred sending biopsied tissue for analysis when they suspect premalignancy or malignancy. (8)

Majority in our study replied that tissue should be transferred to fixative immediately after excision. Massoumeh Zargaran et mentioned that delayed fixation can lead to tissue shrinkage and fragmentation of nuclei. (12) Rastogi et al mention altered features due to delayed fixation like shrinkage of tissue, cytoplasmic clustering, loss of morphology of nuclei and nucleoli. (13)

In our study we found maximum dentists were aware of formalin as fixative whereas some used alternatives. Murgod et al found that 49.26% dentists think that before sending biopsy for assessment it should be preserved in formalin as a preservative, 31.34% preferred thought saline, and 13.43 % alcohol and 5.97 % didn't give any response. In actual 32.60 % practiced preserving tissue in formalin, 56.40 said they preserve tissue using saline and 11 % preferred alcohol. (7) Anandani et al found that 52.60% GDPs preferred using formalin, 23%used saline and 17% dentists used alcohol. Our study too proved that dentists are being totally aware of fixative material still use other material at clinic as a fixative. (8)

Our study suggested most of the dentists used glass bulbs for preservation of tissue. The royal college of pathologist of Australia suggested that fixative container should be large enough to contain specimen without any damage or distortion and should allow fixative to penetrate from all sides. Container should be kept closed (14). Slaoui et al suggested containers with appropriate lids, should be relatable to the sample size, preferable large neck plastic containers should be used as it has a benefit of reusing. (15) College of American Pathologists guidelines states that correct labelled container should be used which should be impermeable, rigid, unbreakable, and non-reactive to the fixative. (16)

We asked the same question omitting formalin in question have they used Saliva, Spirit, Mixture of saline and spirit, saline, or toluidine blue as a fixative at their clinic and we found that 42.1% dentist answered yes, this could lead any practitioner to medicolegal issues. We asked about keeping formalin in clinic, majority were willing to keep it although they must understand about the proper technique to handle it and what all are the hazards effects. New devices and containers are available in market which have advanced techniques where sample is placed in container and lid is sealed formalin will be realized in the container only when pressure is applied on lid. (17)

It becomes mandatory when dentists keep specimen container with formalin, it must be freshly prepared. We strongly believe biopsies should be properly planned and dentists should ask laboratory assistant/ attendant or delivery boy to provide freshly prepared 10% neutral buffered formalin. We asked about awareness of biomedical waste management. It could be hazards if formalin gets spill or splash it causes health hazards. Proper specimen handling must be trained to dentist to avoid any effects of formalin to patients, dentists, dental assistants. As formalin is an oncogenic ingredient (18), it must be handled by trained histopathology technicians, delivery boys or laboratory attendants. Then

an appropriate treatment of chemical waste management can be dealt by histopathology laboratory authorities by following municipal rules and regulations of bio medical waste management. (19)

About proper preservation maximum dentists agreed that proper preservation of tissue must be done. Need of proper specimen handling and preservation should be acknowledged. Dentists are also aware of problems faced by pathologists are crucial and proper preservation technique can help pathologists' visual interpretation. We assessed the interest of dentists about training of tissue preservative dentists were interested. Murgod et al stated 95.52% said yes to gain knowledge about lesions and biopsy procedures. (7) Succeeding we found there is no correlation of education and use of alternative fixatives.

Conclusion

If any procedure helps in subsequent, reproducible, and long term histological and cytological archives and present evidence, it becomes mandatory to assure a proper preservation method. A basic change has to be incorporated in system that is using correct preservative and following its biomedical waste management. In histopathological processes formalin is very important chemical reagent which can't be replaced by alternatives which are studied in all these years. Hence there is a hope for innovations to replace formalin.

The negligence of clinicians towards the fixatives could be due to availability issues, miscommunication, or lack of communication with laboratory professional, or lack of awareness about damage caused to a tissue and subsequent diagnosis. Our study doesn't focus on dentists' knowledge, but to incorporate an ethical and medicolegally correct tissue handling. Hence, we suggest proper standardized operative procedure has to be employed.

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