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Study of Frozen Section in Oral Cancer in Context to Metachromatic Stain

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ABSTRACT:-

Background–Oral cancer is a malignant lesion that appears on the lip or mouth cavity. Is occasionally described as a squamous cell carcinoma (OSCC), because it is in the dental area, 90% of malignancies histologically emerge from the squamous epithelial cells & oral cavity is basically lined by squamous lining. Oral cavity squamous cell carcinomas (OSCCs), responsible for 3-5 percent of all malignancies, are the eighth most prevalent cancers in the world out of 10. In order to reduce the possibility of recurrence to zero, diagnosis isusually lead by quickoperative removal of the malignancy with safer surgical or operative margins, which seems to be practically improbable. The perplexed oral cavity anatomy which make it problematic for the person who performs the operation to properly judge surgical borders of oral malignancies in a pinpoint manner and positive surgical boundaries compromise local and regional disease control, thus surgeon usually rely on frozen section or cryosections for proper assessment and identification of clear surgical margins. So frozen sectioning assessment of surgical boundaries is emerging as a very accurate identification tool in oral malignancies. Now the needful is to identify the active soft tissue boundaries of the tumour in the OT table itself, so that they can be removed properly. Frozen sections (cryosections) are a pathologica facility use for prompt micro anatomical examination and for specimen or disease diagnosis, typically used for oncological surgery and intra-operative rapid diagnosis.

Objectives–

1.To estimate the accuracy of frozen section in oral cancer in context to metachromatic stain

2.To compare the accuracy of frozen section in context to metachromatic stain by

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correlating with histopathological finding.

Methods -The study will be a prospective study carried out for a period of two years in Histopathology Department, Jawaharlal Nehru Medical College, DMIMS (DU), Sawangi, Wardha, Maharashtra.Minimum 45 cases will be studied for which correlation of utility of frozen section in context to metachromatic stain in suspected case of oral malignancy. Frozen section with metachromatic stain like toluidine blue will be clubbed together for diagnosis of oral malignancies in which clear surgical margins are very much important for future prognosis and well being.

Results – The results will be undertaken in SPSS software.

Conclusion – The conclusion will be based on the findings for study.

INTRODUCTION –

350,000 to 400,000 fresh cases of oral malignancies are perceived globally every year, making it one of the sizeable public health affairs to be concerned about. Varying incidences and incidents are observed in different countries and different topographical regions, but are most commonly seen in developing countries. 2% of malignant tumours or malignancies are oral and pharyngeal cancer. Oral cancer diagnosis and care will reduce the mortality risk associated with oral cancer treatment and ensure long-term survival[1].

Oral cavity squamous cell carcinomas (OSCCs), responsible for 3-5 percent of all malignancies, are the eighth most prevalent cancers in the world out of 10. Oral malignancies are related with a high risk of mortality and morbidity, often due to delay in diagnosis[2], with below the number of 50% of patients being asymptomatic in the initial stages and presents many symptoms in the later stages at the time of diagnosis[3].To improve the survival rate, early diagnosis and recognition of oral cancer are important and vital. There are currently a number of modern and evolving diagnostic aids and adjunctive strategies available[4]. 2% of malignant tumors or malignancies are oral and pharyngeal cancer. Oral cancer diagnosis and care will reduce the mortality risk associated with oral cancer treatment and ensure long-term survival[4]. Oral malignancies are related with a high risk of mortality and morbidity, often due to delay in diagnosis, with below the number of 50% of patients being asymptomatic in the initial stages and presents many symptoms in the later stages at the time of diagnosis.

For the need of reducing the risk of recurrence of the malignancies to null or equivalent, the medical identification is lead by instant operative excision of the tumors with secureoperative boundaries, which appears to be practically unfeasible.The needful is to pin point the active soft tissue boundaries of the tumour in the OT table itself so that the malignant surgical margins can be removed with precision and confidence. With a sensitivity of up to 88.8 percent and a sensitivity of 98.9 percent, the most widely used technique is frozen section biopsy[5,6]. Varying incidences and incidents are observed in different countries and different topographical regions, but are most commonly seen in developing countries. 2% of malignant tumours or malignancies are oral and pharyngeal cancer. Oral cancer diagnosis and care will

reduce the mortality risk associated with oral cancer treatment and ensure long-term survival[6]. Oral malignancies are related with a high risk of mortality and morbidity, often due to delay in diagnosis, with below the number of 50% of patients being asymptomatic in the initial stages and presents many symptoms in the later stages at the time of diagnosis[6].

Frozen section (cryosection) is a pathological procedure used to diagnose a specimen or disease and for rapid microscopic examination.It is usually used for oncologic surgery and rapid intraoperative patient diagnosis [7]. Layout of the frozen segment is always important to get a prompt diagnosis of the ongoing malignant condition during the course of surgery. Before closing the operation site, the person who performs surgery may want to know if the surgical border of his/her resection for a malignant process are free of any malignant focus, or if there is an foci of malignant process that needs to be detected and decided what to do next, or whether enough tissue has been collected to further strengthen the disease process. This can be done by using the Frozen Section. It should be marked that the Frozen Section approach is currently examining tissue morphology to draw a conclusion using modified H&E stains, but does not submit any other supporting strategies[8].

HISTORICAL PERSPECTTIVE OF FROZEN SECTION –

Lang first used the application of freezing in the nineteenth century to harden tissues. Later on, in 1818[9], De Riemer made a pioneering attempt to use the technique of the frozen section for histopathological diagnosis. Earlier segment on Frozen the methods were complex and difficult, requiring as much art as science[10] In 1949, compared to current processes in which cryostat is used, the procedure used by Hazard and Stevenson was fascinating. Then the freshly receivedtissue specimen is placed in 10 times its self volume in a fixative and heated to boiling point[11]. Then the fixed block is frozen between dry matter parts and cut at 10-15 µmthickness by a bladegenerally known as microtome. Meticulously thesectionis then transferred to a glass or porcelain beaker containing deminarelised water. The section is then followed bytransferring to a glass or porcelain jar containing carbolfuschin& toluidine blue solution through a glass rod. The segment is put on a clean glass slide after consecutive cleansing in a series of beakers filled withdeminarelised water, and right way coated with thirty (30) percent% sucrose or cane sugar and capsules. This chemical mixture will stay useable for an hour, and if the lip of the cover had been. The segment could be maintained for several days, rimmed withPermount or flexible colloidan[12].

CYROSTAT –

In 1959 it was developed asCryomicrotome, or generally known as a Cryostat. The Frozen segment technique has been revolutionized. A cooled box which contained the rotatory microtome is the cryostat. The tempereture in the Cryostat is maintained between -20 degrees Celsius and-30 degrees Celsius.The attending histopathology technician will start processing the tissue by freezing it with frozen aerosol sprays and position it on the cryostat for section cutting. Intra-cellular and inter-cellular water is frozen to create a hard block to allow the tissue of interest to be cut. The tissue pieces are sectioned and picked up on a glass slide, which is then ready for staining.

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The whole procedure will takes time of about 5-10 minutes. This duration is only to prepare the slides; the time taken by histopathologist to examine the slides under a microscope and arrive at a fruitful diagnosis is subjective. One research involving 700 laboratories worldwide found that 90% of frozen section block turnaround times (TAD) were within 20 minutes, estimated from the time histopathologist receive the
Cryo section specimens to the time histopathologist returned Frozen section diagnosis to surgeons[13]. The attending histopathology technician will start processing the tissue by freezing it with frozen aerosol sprays and position it on the cryostat for section cutting. Intra-cellular and inter-cellular water is frozen to create a hard block to allow the tissue of interest to be cut. The tissue pieces are sectioned and picked up on a glass slide, which is then ready for staining.

The procedures have been shown to increase over time periods, but the main issue occurs when physicians employ various techniques without providing sufficient knowledge of their feasibility and use. The use of toluidine blue, Vizliite Plus with Blue, Vizliite and Oroscoptic DA are the new methods, while biopsy remains the gold standard[14]

Toluidine blue is an readily usable, inexpensive, metachromatic colourant that is known to bind dividing cells to DNA. To stain cancerous and precancerous cells but excluding normal mucosa[15,16], has previously been identified. Due to its metachromatic property, it has many uses, including staining living tissue and a distinctive stain. To recognize dysplasia and carcinoma of the oral cavity, this pigment is commonly incorporated in vivid. Generally it is used to highlight factors such as granules of mast cell, mucin, and cartilage. It is also known as tolonium chloride because of the selectively stained elements of acid tissue, such as sulphates, carboxylates and radicals of phosphate[17].

Given this background we decided to test the stain toluidine blue for assessing tumor boundaries in operation theater or what is called intraoperative after the excision of mother lesion of oral cavity malignancies. Toluidine blue is a basic thiazine metachromatic dye with high phila for acidic part of the tissue material, thereby staining cells and tissues abundant in DNA and RNA. It has found its way in vast applications both as pivotal staining method in living tissues and as a special stain group owing to its metachromatic property. Toluidine blue has been used widely in histopathological laboratories to identify dysplasia and carcinoma/malignances of the oral cavity. This stain or dye has wide range of usage in the field of diagnosis and research.Toluidine blue is basically used in tissue for proper identification of components like mast cells granules, mucins, and cartilage for better study purpose.

RESEARCH GAP:
The present study is done to compare the outcome of frozen section study with conventional H&E sections of all oral malignant lesions in context with metachromatic stain.

METHODOLOGY:
STUDY DESIGN - It’s an analytical and prospective study

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PLACE OF STUDY – Department of Pathology, JNMC, Sawangi (Meghe), Wardha, Maharashtra.

DURATION – Study will be from 2020 to 2022 (2 years)

METHODS – Study of frozen section in oral cancer in context to metachromatic stain

SAMPLE SIZE – Minimum45 patients of suspected oral malignancy undergoing intraoperative surgery in OT complex of frozen section could be effectively used as the diagnostic step in suspected cases of malignancy of any organ.

MATERIALS REQUIRED FOR FROZEN SECTION.

The frozen section specimens, as per laboratory routine,

1) Cryostat (leica CM 1510 S)
2) Materials for Rapid Hand E staining (Harris hematoxylin , distilled water,scott's tap water, eosin, absolute alcohol, glacial acetic acid, xylene and dystyrene plasticizer xylene-DPX)
3) Metachromatic Stain (Toluidine Blue Dye Staining)
4) Specimens
5) Tissue freezing medium as optimum cutting temperature compound(OCT)
6) Compound light microscope
7) Glass slide and slide marker
8) Scalpel and blade
9) Normal saline.
10) Filter paper
11) Forceps and gloves

Materials required for paraffin embedded sections-

1) Automated tissue processor (leica TP 1020)
2) Rotatory microscope (leica RM 2125RT)
3) Compound light microscope
4) leukhart'smoulds
5) paraffin
6) tissue float bath
7) Hot plate
8) scalpel and blade
9) Glass blade
10) Cover slips
11) glass marker
12) Distyrene plasticizer in (DPX) mountant
13) Filter paper
14) Gauze piece

Methods:

In this present study, Toluidine Blue Dye and Rapid Hematoxylin (eosin method) will be for staining slides for frozen section

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Toluidine Blue Dye staining for frozen section[18]

1. One percent blue solution of toluidine was maintained. A senior resident irrigates the tumour surgical margins with regular normal saline, supplemented by one percent acetic acid, until the primary tumour was excised.
2. The surgical margins are then be gently gauze dried and painted with a cotton swab with the 1% toluidine blue solution.
3. The stain is left in place for thirty(30) seconds, followed by the tissue is again irrigated with regular normal saline (NaCl), after that by 1% acetic acid. The staining criterion will be separately identified by the resident histopathologist and recorded.
4. Surgical borders that which stained dark blue will be labelled as positive, and those that stained light blue will be labelled negative, or do not take the stain at all.

Rapid hematoxylin and eosin staining for frozen section[19]

1. All the resected specimen submitted for intraoperative frozen section has to be grossly examined and the representative area should be embedded in optimum cutting temperature compound(OCTC) and its frozen in cryostat at -20 degree celcius. The sections were cut and slides were made.
2. The sections are cut via cryostat and taken on glass slide
3. Sections are stained with hematoxylin for 1.5 minutes
4. washed with tap water shortly
5. After being immersed in acid alcohol for 1-2 second they were then rinsed with lithium carbonate, alkaline tap water or Scott's tap water
6. A 10-second stain with eosin
7. Rinsed in tap water
8. Clear, dehydrate and mount in DPX

STATISTICAL METHODS:

Sample size formula with desired error of margin[20]:

$n=Za/22\ p\ (1-p)/d^2$

Where,

Za/22p will be the level of Significance at 5% i.e. 95%

Confidence interval = 196

p = Prevalent

d = Desired error of margin

n = sample size

INCLUSION CRITERIA:

1) All cases of diagnosed oral carcinoma undergoing surgery would be treated as sample
2) Patients would be recruited irrespective of age, sex, race, and stage of the tumor.

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EXCLUSION CRITERIA:

1) Patients who have previous history of head and neck cancerous lesions.
2) Oropharyngeal tumours or prior care (surgery and/or radio/chemotherapy) for existing oral cancer.
3) Patients allergic to dye will be excluded from the study.

EXPECTED RESULTS:

The study will be conducted for a period of 2 years and all the observations will be depicted in a well tabulated master chart and conclusion will be drawn. These approximately 45 cases in our study will get correlated with different study group of a standard quality and whether correlation will be there or rarely not will get discussed.

DISCUSSION:

In our research/thesis, about 45 cases/patientsuffering from oral malignancieswill be corelatedwith multiple study groups of same discipline of a standard quality, so that correlation can be made out. In rare instances only cases will not be discussed.

MontasirJunaid et al [20] in 2012 done a comparative study of toluidine blue with frozen element in oral squamous cell carcinoma. The study included a group of 56 consenting patients with biopsy-proven OSCC, giving us a tumor margin of 280. Using toluidine blue staining and frozen section histopathology, margins were analysed. A receiver operator curve (ROC) was then applied to compare toluidine blue and frozen segment margin status evaluation. The findings showed that of the 280 margins tested, 11 were positive for toluidine blue staining, three were positive for frozen segment biopsy, and three were positive for final histopathology. Sensitivity and precision were 100 percent and 97 percent respectively for Toluidine blue staining. With a positive predictive value (PPV) of 27.2% and a negative predictive value (NPV) of 100%, the diagnostic accuracy of toluidine blue was found to be 97.1% The authors concluded that toluidine blue can be used as an efficient screening tool for determining intraoperative margins in resource-constrained settings and reducing the number of biopsies performed on the frozen portion.

Shukla A et al [21] in 2018 In detecting potentially malignant and malignant diseases of the oral cavity, the effectiveness of chemiluminescence and toluidine blue was compared. 42 patients were included in this prospective study with clinically evident premalignant lesions. Demographic data were collected and suspected lesions were investigated by light chemiluminescence (Vizilite) and local toluidine blue application (Mashberg's recommendation) was followed. Findings have been reported as positive or negative for each lesion under normal incandescent light. There was a biopsy and a histopathological study of the tissues. Similar studies were reported by Patton (22) and Kalmar (23). Agrawalet. al. reported on Touch Imprint Cytology(24).Jagtapet. al. reported on assessment of lymph node status in cases of metastatic malignancy by frozen section and imprint cytology (25). R elated studies were also reported by Kadashettiet. al (26), Sune et. al. (27), Gadbailet. al. (28).A number of related studies on oral carcinoma were reviewed (29-32).In the current research,

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the toluidine blue test was found to be moderately sensitive (63.33%) while the chemiluminescence test (Vizilite) was found to be extremely sensitive (90%); however, the specificity of the test was limited (90%) (50 percent).Therefore, the study concluded that both toluidine blue and Vizilite can be used as an adjunct to clear, traditional visual examination and for oral potentially malignant disorders in the screening protocol.

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