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Knowledge About Histochemistry Of Oral Tissue Among Undergraduate Students

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ABSTRACT:

BACKGROUND: Histochemistry is an important technique that is used for the visualization of biological structures. It is concerned with the identification and distribution of various chemical components of tissues through the use of stains, indicators as well as microscopy.

AIM: This study aimed to assess the knowledge about the histochemistry of oral tissue among undergraduate students of private dental college.

MATERIALS AND METHODS: This is a questionnaire based cross sectional study conducted among UG students in a private dental college. The questionnaire survey was conducted among 111 dental students. The data was collected and the chi square test was used to analyze and comparative bar graphs were plotted and it is statistically significant only if the p value is less than 0.05.

RESULT: In our study, the majority of first year dental students(58%) have knowledge about the histochemistry of oral tissue and only 11% of second year students were aware about histochemistry of oral tissue. And 64% of students were about van gieson's stain and only 10% were aware that fibronectin helps in cell adhesion. The association between year of study and percentage of response yields a p value of 0.000 with Pearson chi square test. Hence, it is statistically significant.

CONCLUSION: According to the study, first year students have good knowledge on histochemistry of oral tissue and having more practical classes simultaneously with theoretical class will increase the knowledge about histochemistry of oral tissue among students.

INTRODUCTION:

Histochemistry is an important technique that is used for the visualization of biological structures. It is concerned with the identification and distribution of various chemical components of tissues through the use of stains, indicators as well microscopy(1). Histochemistry combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues and it is the qualitative and or quantitative assessment of chemical groups within cells and tissues. This is done without disturbing the tissue architecture(2).

The most common substances studied are the keratin using performic acid alcian blue, mucins and glycogen using PAS stain, proteoglycans using Alcian blue, glycoproteins using PAS stain, collagen fibers using Van Gieson's stain, reticular fibers using silver stains, elastic fibers using aldehyde fuchsin and fats using sudan black(3). The students should know the methods to delineate the substances in various pathological conditions in order to arrive at a diagnosis(4).Our team has extensive knowledge and research experience that has translate into high quality publications(5),(6),(7),(8),(9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24). Thus, the aim of this study is to assess the knowledge about histochemistry of oral tissue among undergraduate students of private dental college.

MATERIALS AND METHODS :

A descriptive cross-sectional study was done to analyse knowledge and attitude about histochemistry of oral tissue among undergraduate students of private dental college. The survey was conducted among 111 undergraduates. A self administrated questionnaire of 10 closed-ended was prepared and distributed among undergraduate students through online-based survey forms "Google form". The responses were collected, tabulated in excel sheet and analysed using SPSS(Statistical Package for the Social Sciences) and results were represented in a bar and pie graph. Chi square test was used to analyze and comparative bar graphs were plotted and it is statistically significant only if the p value is less than 0.05.

The questionnaire contains following questions,

- Are you aware of histochemistry of oral tissue ?
1. Yes
2. No

- Which the following stain is used for identification of heparin sulphate?
1. Tollen's reagent
2. Toluidine blue
3. Benedict's solution
4. None

- Which of the following proteins do you think helps in cell adhesion ?
1. Actin
2. Myosin
3. Keratin
4. Fibronectin

- PAS stain is used for identification of ?
1. Mucin
2. Laminin
3. Fibronectin
4. Osteonectin

- Which stain do you think is used to differentiate between muscle and collagen fibre?
1. PAS stain
2. Van gieson's stain
3. Silver stain
4. Aldehyde fuchsin stain

- Which is the most frequently studied enzyme in bone during resorption?
1. Alkaline phosphatase
2. Acid phosphate
3. Both A & B
4. None

- Which of the fixatives do you think is used for histochemical staining of nucleic acid?
1. Rossman's fluid
2. Carnoy's mixture
3. Paraformaldehyde
4. Urany lactate

- Which of the following stains do you think is used for study of lipids?
1. Alcian blue
2. Toluidine blue
3. PAS
4. Sudan black

- Which dyes do you think are used for demonstration of proteoglycan?
1. Toluidine blue
2. Azure A
3. Alcian blue
4. All of the above

RESULT

In our present study, a total of 111 respondents were included. Among them, 86% belonged to the age group of 18-20 and the rest 14% belonged to the age group of 21-23 **(figure 1)** and 52% were male and 48% were female **(figure 2)**. Majority were first year students(78%) followed by second year(11%) and third year students (11%) participated in this study **(figure 3)**. In this study, 59% of students were aware about the histochemistry of oral tissue and 31% were unaware **(figure 4)**. Majority(69%) of the population were aware of toluidine blue stains used for identification of heparan sulphate **(figure 5)**. About 64% of students responded had knowledge about van gieson's stain **(figure 8)** and whereas 43% of students were aware of PAS stain **(figure 7)**. Only 10% were aware that fibronectin is the cell adhesion molecule **(figure 6)**. Majority of 42% were aware that alkaline phosphatase is involved in bone resorption **(figure 9)**. Majority(57%) of the population were aware about fixatives used for nucleic acid staining **(figure 10)**. Only 30%(sudan black) of the population were aware about stains used in study of lipids **(figure 11)**. Only 15% of the population were aware that toluidine blue is used for the demonstration of proteoglycan **(figure 12)**. The association between year of study and percentage of response for awareness about histochemistry yields a p value of 0.000 Hence, it is statistically significant with Pearson chi square test where majority(58%) of first year students have adequate knowledge about histochemistry of oral tissue **(figure 13)**.

The association between year of study and response for the students who were aware that the protein helps in cell adhesion yields a p value of 0.000 and hence it is statistically significant the majority (10%) of first year students were aware that protein which helps in cell adhesion when compared to second year students **(figure 14)**. The association between year of study and response for the stain used in identification of heparan sulphate yields a p value of 0.000 and hence it is statistically significant. Majority (48%) of first year students were more aware that toluidine blue stain helps in identification of heparan sulphate when compared to second year students **(figure 15)**.
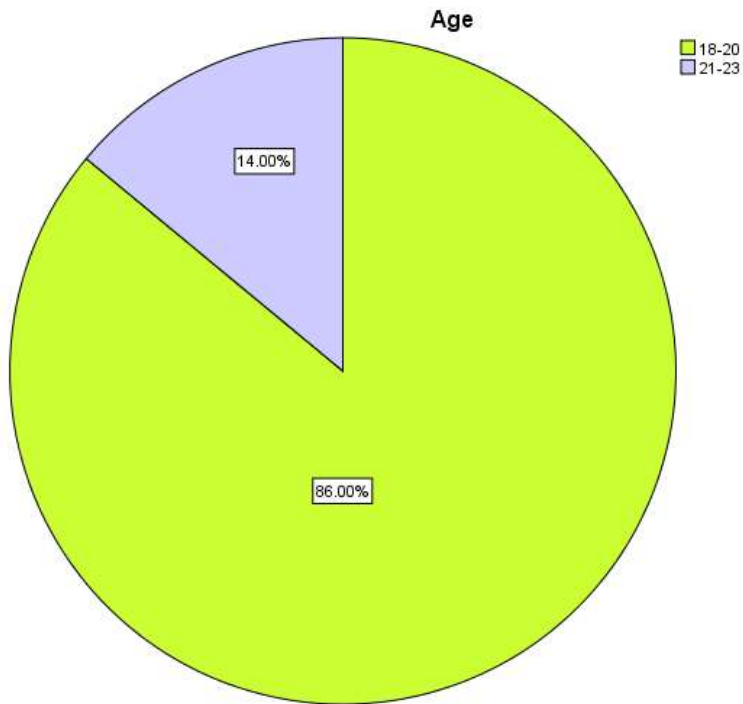
Age

Figure 1 Pie chart represents the age of the population. Periwinkle denotes age group of 18-20 and light yellow denotes 21-23. Majority were from the age group of 18-20(86%) followed by the age group of 21-23(14%).
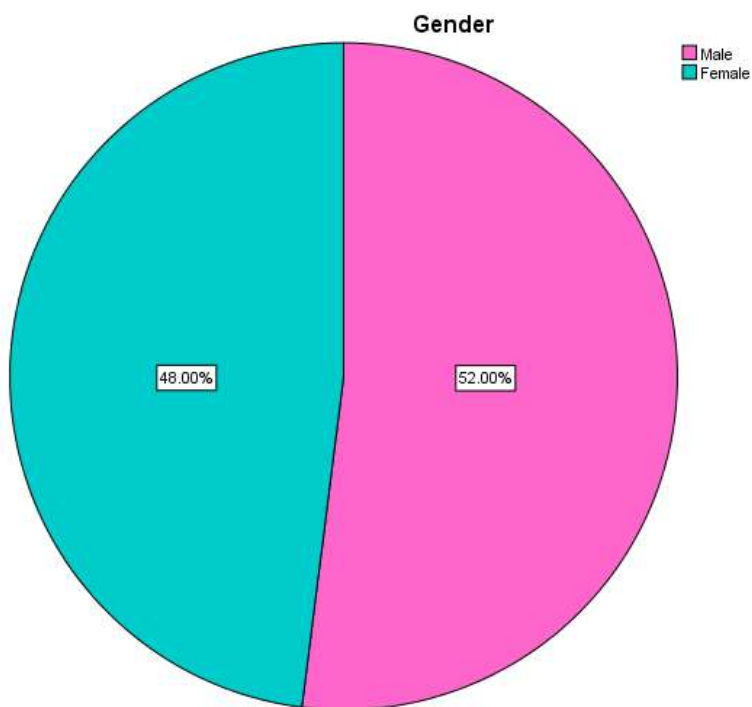
Gender

Figure 2 Pie chart represents the gender of the participant. Pink denotes males and dark cyan denotes females. Majority were male(52%) followed by female(48%).
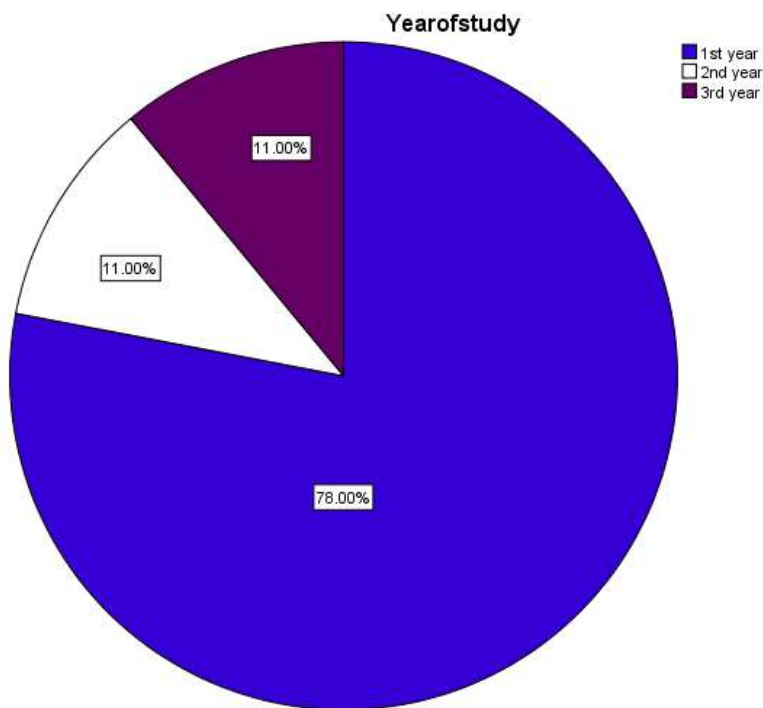
Figure 3 Pie chart represents the year of study. Dark blue denotes first year, white denotes second year and violet denotes third year. Majority were from first year (78%) followed by second and third year students (11% each)
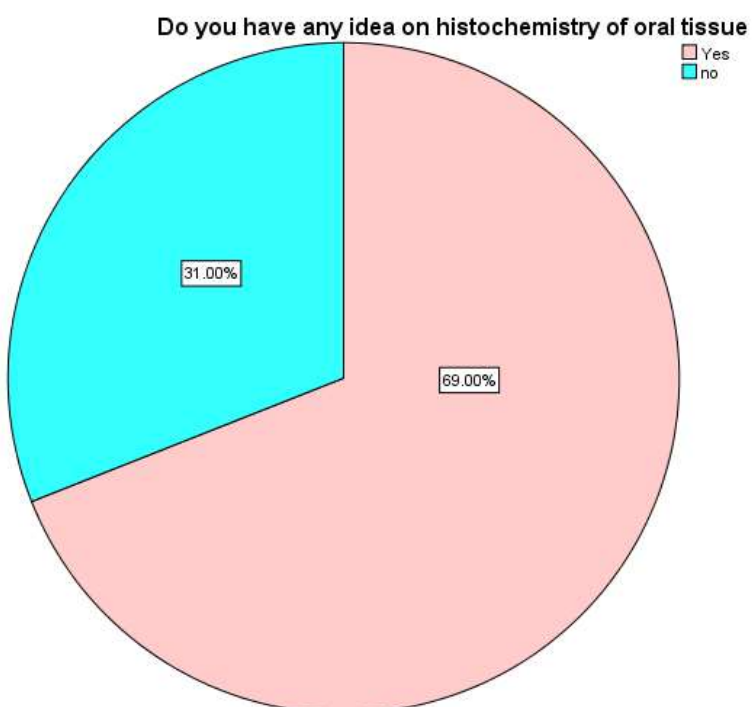
Figure 4 Pie chart shows the response for ideas on histochemistry of oral tissue. Peach color denotes Yes and cyan blue represents No. Majority (69%) were aware whereas 31% have no idea about histochemistry of oral tissues.
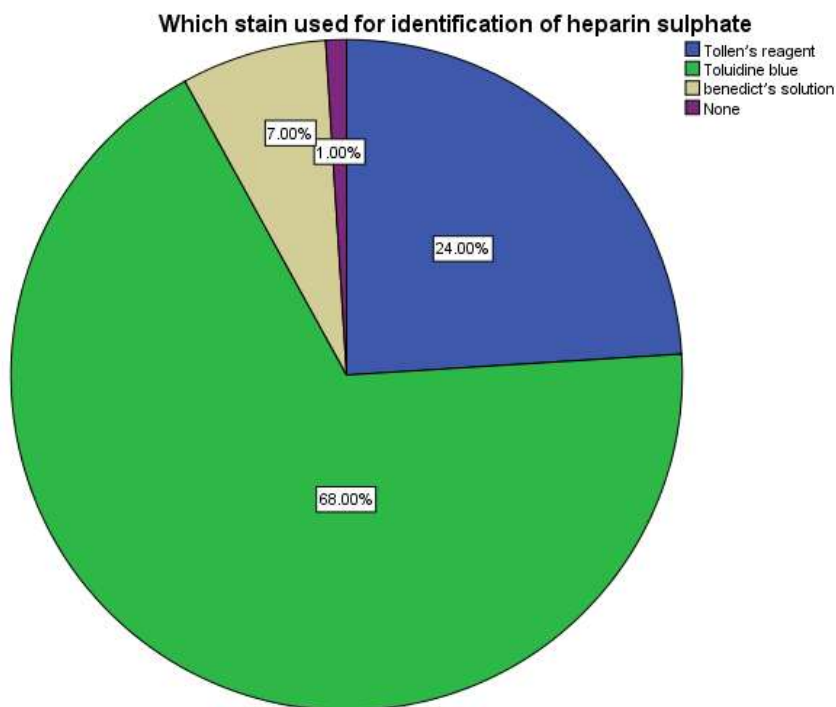

Figure 5 Pie chart shows the percentage of response for stain used for identification of heparan sulphate. Here green denotes toluidine blue, blue denotes tollen's reagent, beige denotes Benedictus solution and violet denotes none of these. Majority(69%) of the population were aware of toluidine blue stains used for identification of heparan sulphate whereas 24%(tollen's reagent),7%(benedict solution) and and rest 1%were unaware about stains used for identification of heparan sulphate.
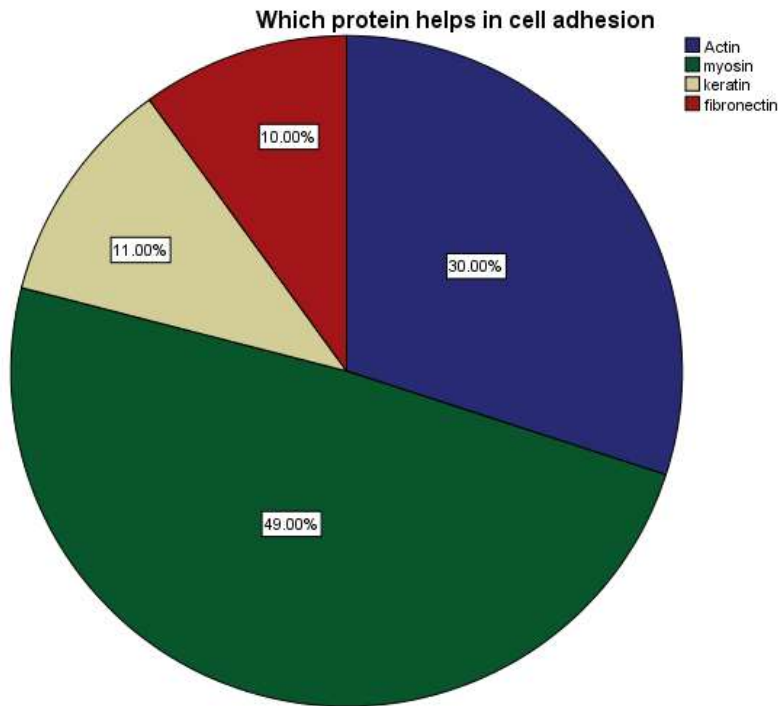
Figure 6 Pie chart shows the percentage of response for protein which helps in cell adhesion. Dark green denotes myosin, dark blue denotes actin, beige denotes keratin and red denotes fibronectin. Only 10%(fibronectin) of the population were aware about the protein which helps in adhesion whereas 49%(myosin), 30%(actin) and 11%(keratin) were unaware about the protein which helps in adhesion.

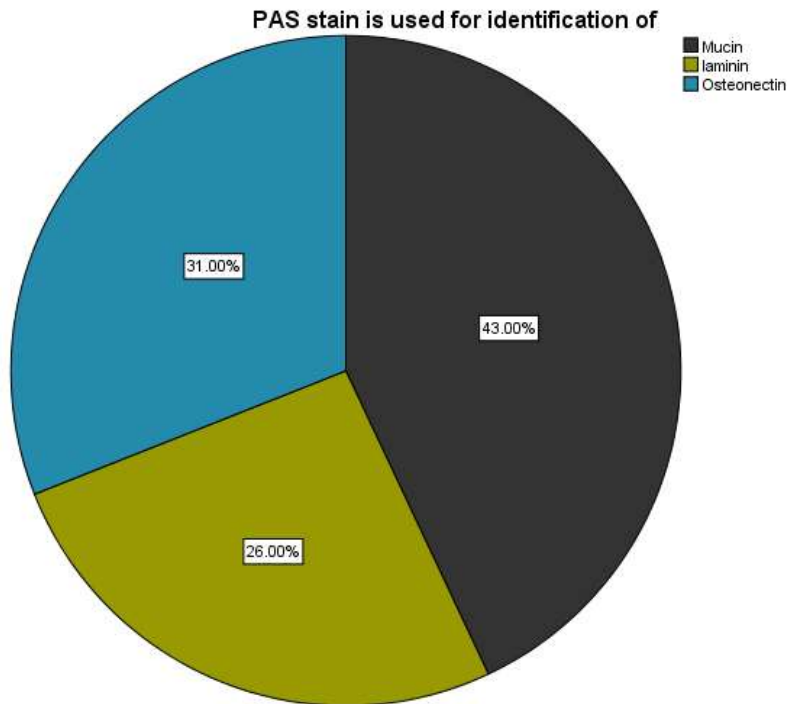**PAS stain is used for identification of**

Figure 7 Pie chart shows the percentage of response about use of PAS stains. Brown denotes mucin, mustard denotes laminin and glacial blue denotes osteonectin. Majority (43%) of the population were aware of PAS stains used for identification of mucin whereas 26%(laminin) and 31%(fibronectin) were unaware about them .

Which stain is used to differentiate between muscle and collagenfibre
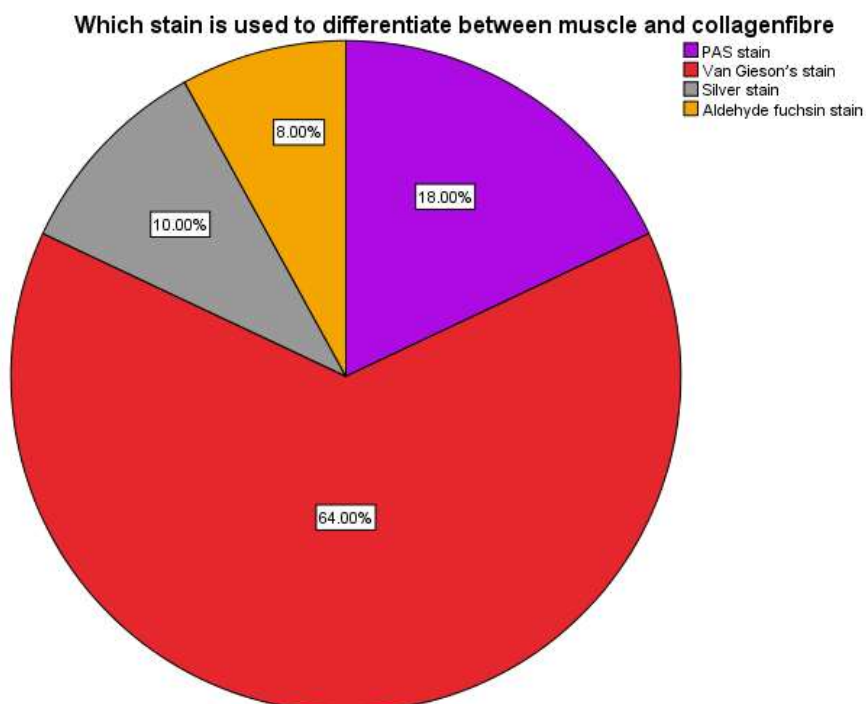
Figure 8 Pie chart shows the percentage of response for stain used in differentiation between muscles and collagen fibres. In which purple denotes PAS stain , Rose Madder denotes van gieson's stain,grey denotes silver stain and dark yellow denotes aldehyde fuchsin stain. Majority(64%) of the population were aware of van gieson's stain used in differentiation between muscles and collagen fibres whereas 18%(PAS stain), 10%(silver stain) and 8%(aldehyde fuchsin stain) were unaware about stains used in differentiation between muscles and collagen fibres.
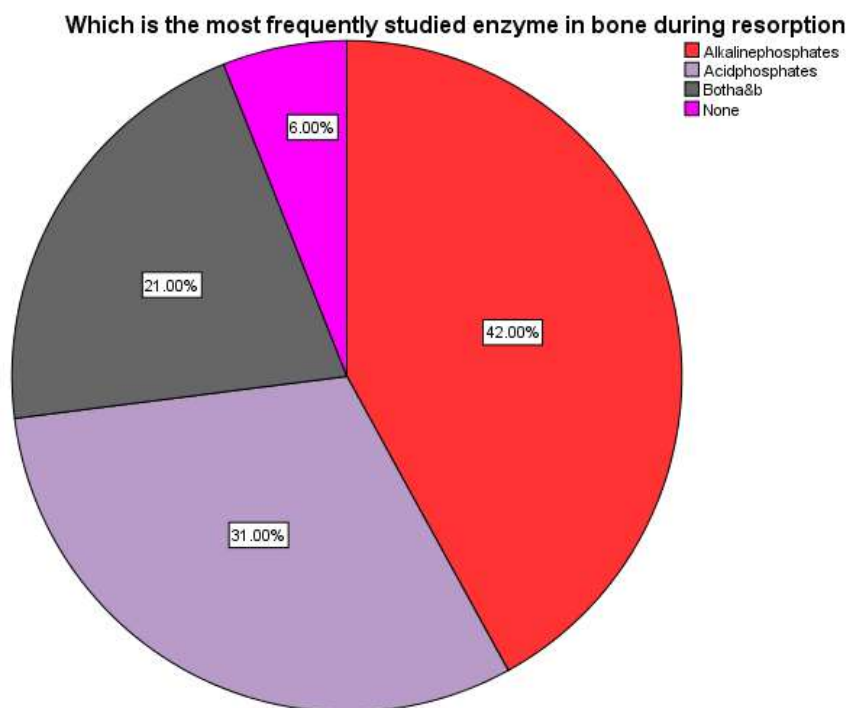

Figure 9 Pie chart shows the percentage of response for frequently studied enzymes during bone resorption. In which orange red denotes alkaline phosphatase, light purple denotes acid phosphates, dark gray denotes both a and b and dark pink denotes none. Majority of 42%(alkaline phosphatase) were aware whereas 21%(both a and b), acid phosphate(31%) and 6%(none)of the population were unaware about enzymes involved in bone resorption.

What is the fixative used for histochemical staining of nucleic acid

Rossman'sfluid
carnoy'smixture
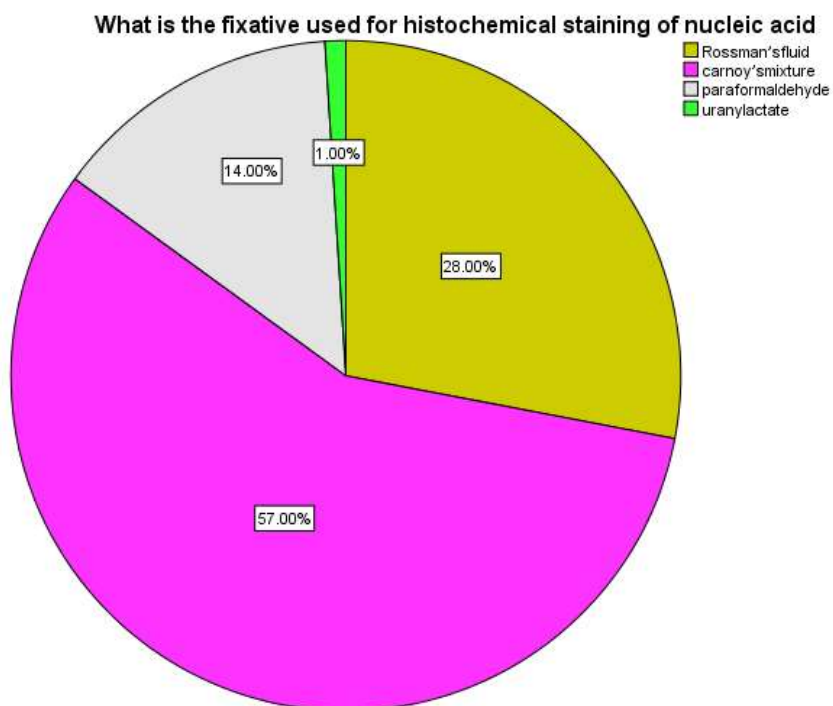paraformaldehyde
uranylactate

14.00%

1.00%

28.00%

57.00%

Figure 10 Pie chart shows the percentage of response for fixatives used for nucleic acid staining. Green yellow denotes rossman's fluid, pink denotes carnoy's mixture, silver denotes paraformaldehyde and light green denotes urinary lactate. Majority(57%) of the population were aware whereas 28%(rossman's fluid), 14%(paraformaldehyde) and 1%(urany lactate) were unaware about fixatives used for nucleic acid staining.

Which of the following used for study of lipids

Alcianblue
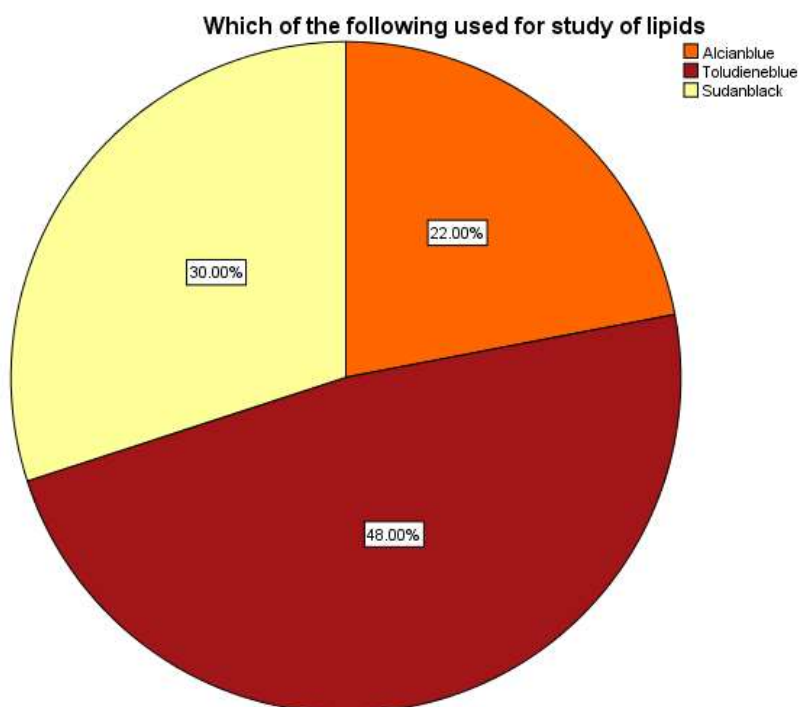Toludieneblue
Sudanblack

30.00%

22.00%

48.00%

Figure 11 Pie chart shows the percentage of response for stain used in study of lipids. In which orange denotes alician blue, dark red toluidine blue and yellow denotes Sudan black. Only 30%(sudan black)

of the population were aware whereas 48%(toluidine blue) and 22%(urany lactate) of the population were unaware about stains used in study of lipids.
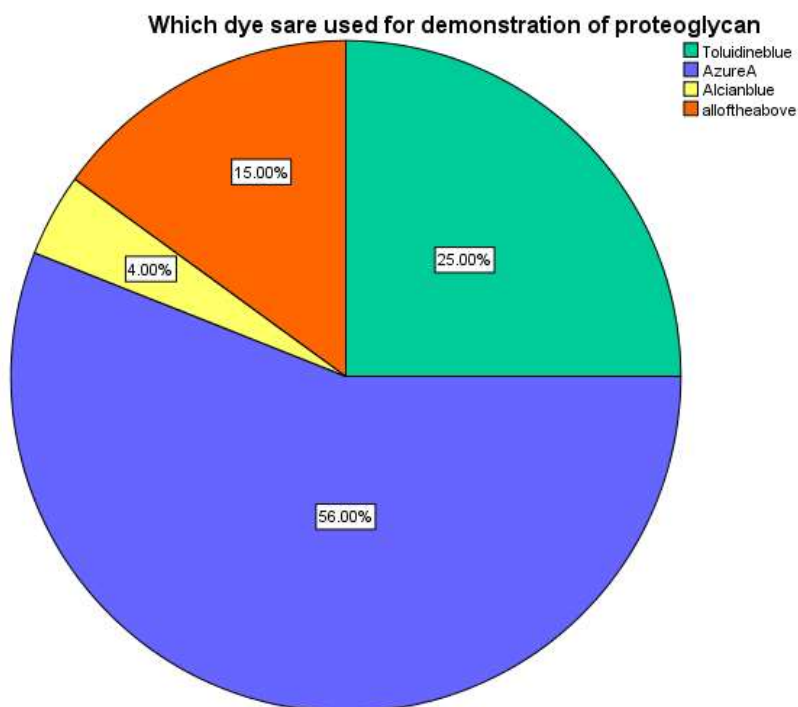

Figure 12 Pie chart shows the percentage of response for dye used for demonstration of proteoglycan. In which aqua blue denotes toluidine blue, light blue denotes azure a, yellow denotes alician blue and orange denotes all of the above. Only 15% of the population were aware whereas 56%(azure A), 25%(toluidine blue) and 4%(urany lactate) were unaware that toluidine blue is used for the demonstration of proteoglycan.
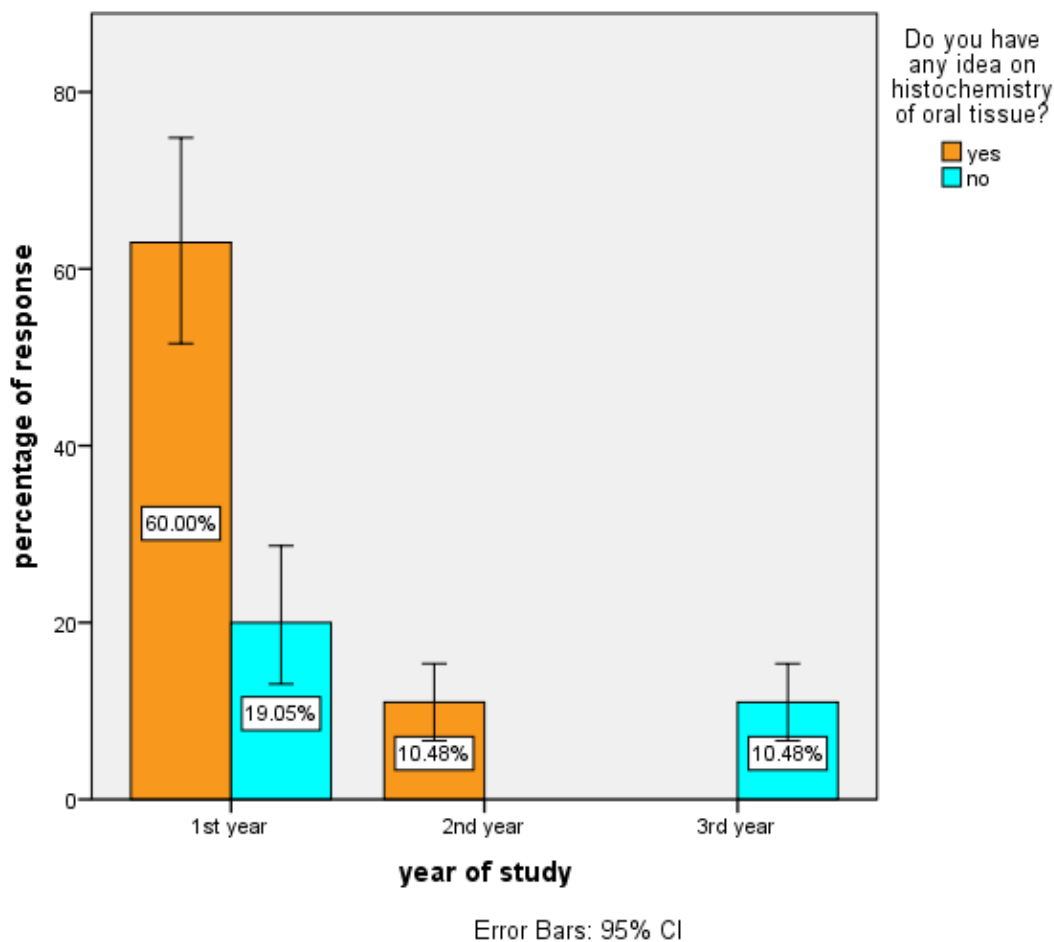
Figure 13 : The above bar graph represents the association between year of study and idea about histochemistry. X axis represents the year of study and Y axis represents the percentage of responses. Cyan represents NO and saffron represents YES and the majority of first year respondents(60%) were more aware about histochemistry of oral tissue than second year respondents(10.48%). Pearson chi square test shows p value is 0.00 which is statistically significant.
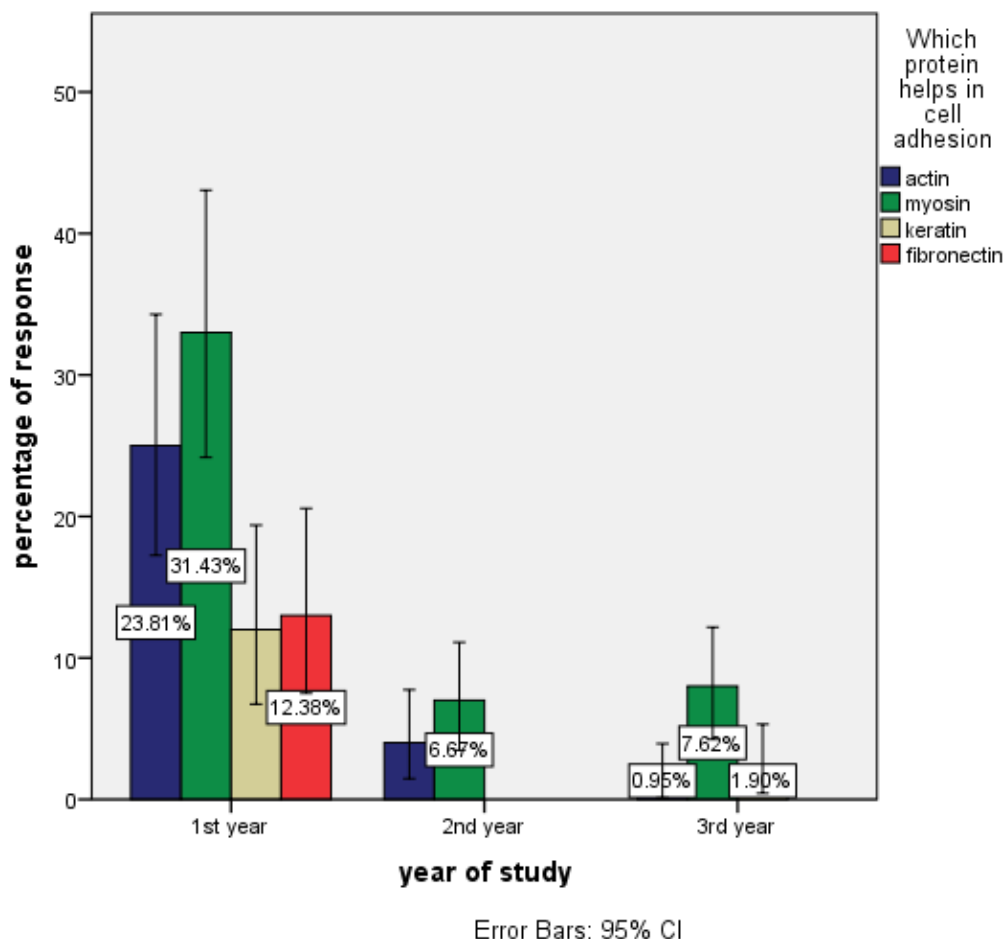
Figure 14 : The above bar graph represents the association between years of study and students who were aware that the protein helps in cell adhesion . X axis represents the year of study and Y axis represents the percentage of responses. Here dark green represents myosin , dark blue represents actin, beige represents keratin and red represents fibronectin. Majority (12.38%)of first year students were aware that fibronectin is the protein which helps in cell adhesion when compared to third year students(1%). Pearson chi square test shows p value is 0.00 which is statistically significant.
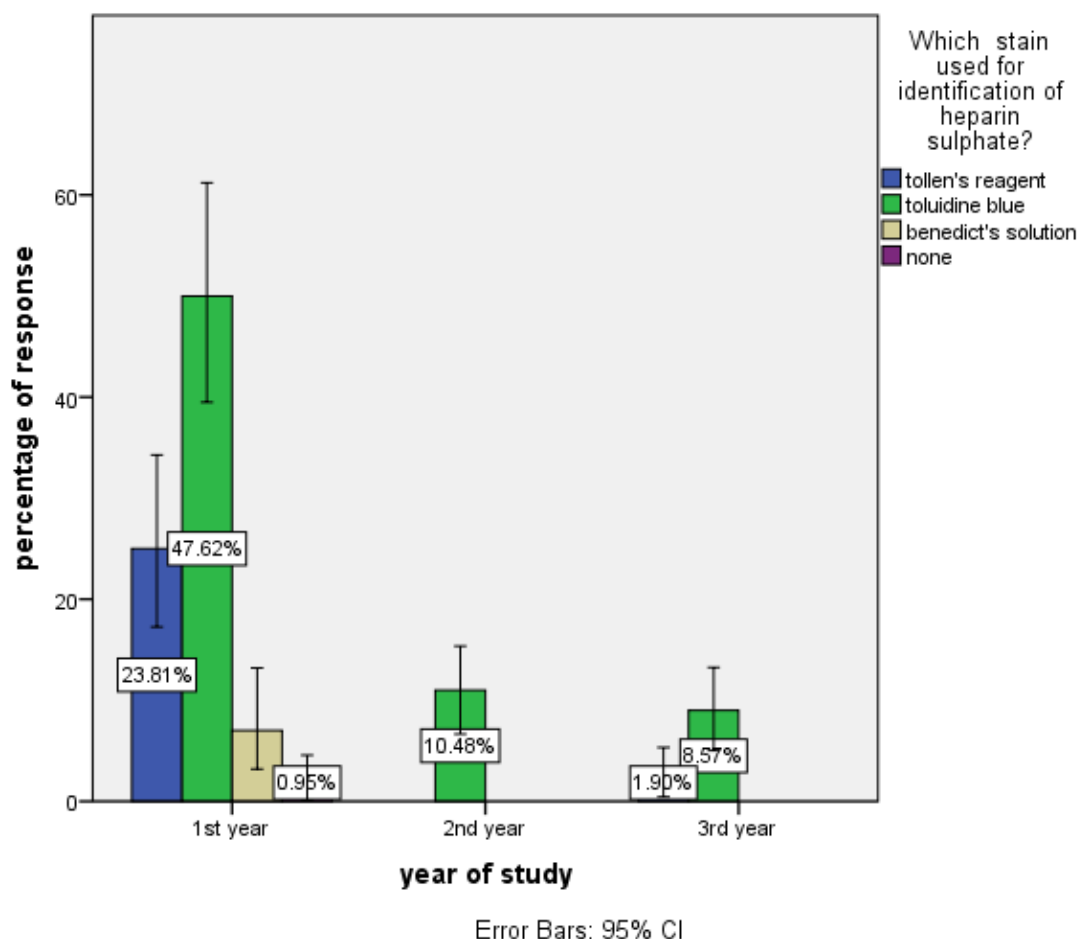
Figure 15 : The above bar graph represents the association between the year of study and knowledge about stain used for identification of a heparan sulphate. X axis represents the year of study and Y axis represents the percentage of responses. Here violet represents none ,blue represents tollen's reagent, beige represents benedict's solution and green represents toluidine blue. Majority (47.62%) of first year students were more aware that toluidine blue stain helps in identification of heparan sulphate than second years (10.48%). Pearson chi square test shows p value is 0.00 which is statistically significant.

DISCUSSION

In our present study, the majority of first year dental students were aware about the histochemistry of oral tissue whereas only a few of second year dental students were aware of histochemistry of oral tissue due to their lack of practice on a particular topic. Majority (64%) of the population were aware of van gieson's stain used in differentiation between muscles and collagen fibres. Only 10% of the population were aware that fibronectin is the protein that helps in adhesion.

Histochemistry seeks to elucidate the characteristics of metabolism in tissue cells and interstitial media. It involves changes in cell properties in the process of development and the connection between the work, metabolism, and renewal of mature cells and tissues(25). A basic principle of histochemical methodology is the bonding of a definite chemical component of cells with a stain or

the formation of a stain in the process of the reaction (26). In our present study, 69% were aware about histochemistry whereas 31% were unaware about histochemistry of oral tissues.

PAS stain is a staining method which is used for identification of polysaccharides such as glycogen and mucus substances such as glycoproteins. PAS stain is used for identification of mucin(27). In our present study, 43% of students were aware of PAS stains. Acid mucin is a type of mucin stain which contains sialic acid. Acid mucin can identify most of the tissue like tissue present in the salivary gland (28–30). Van Gieson's stain is a mixture of picric acid and acid fuchsin.Van Gieson's stain is used to differentiate between muscle and college fibre and in this present study, 64% of the participants were aware of the same (31)12,31).

Heparan Sulphate is a linear polysaccharide found in all animal tissue. Toluidine blue is a basic thiazine metachromatic dye with high affinity for acidic tissue components (14). Heparan sulphate can be identified by toluidine blue stain and only 68% of students were aware about that (28). Cell adhesion is the process by which cells form contacts with each other or with their substratum through specialized protein complexes. Cell adhesion is helped by a protein called fibronectin and only 10% were aware about the cell adhesion molecule in this study(28,29). Carnoy's solution is a fixative composed of 60% ethanol, 30% chloroform and 10% glacial acetic acid, 1 gram of ferric chloride. Majority(57%) of students responded that carnoy's mixture is the fixative used for identification staining of nucleic acid (32,33). This study was done on a small scale population, in future, such studies should be done on a larger scale population for better accuracy. The theoretical lectures should be combined with the practical sessions in the lab to make them understand the histochemistry of each stain upon the cells and matrix which gives better understanding of the subject in depth.

CONCLUSION:

The study establishes that first year undergraduate students have adequate knowledge on Histochemistry of Oral tissues. Attending practical classes simultaneously with theory classes has an effect of reinforcement of the subject thereby resulting in increased knowledge in the topic studied.

CONFLICT OF INTEREST:

All the authors declare that there was no conflict of interest in present study.

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