Abstract: Objective: Biopsy is the gold standard for the diagnosis of oral lesions. Correct management and care of biopsy at all steps (before, during and after obtaining a biopsy) is highly important to provide proper tissue samples for microscopic assessment by pathologists. This study aimed to assess and compare the knowledge of dental students (DSs) and general dentists (GDs) on post-oral biopsy instructions. Material and Methods: A questionnaire including two parts was used: 1) Demographic data and self-evaluation of biopsy knowledge by the participants and 2) 11 items about the correct oral biopsy storage and transport to a histopathology laboratory. The data collected from the questionnaires were analyzed by STATA. Results: 48 GDs and 131 DSs participated in this study. The knowledge score of the DSs (5.43±2.01) was significantly lower than GDs (8.33±1.78) (p<0.05). Moreover, there was no significant relationship between GDs' knowledge and their working experience, age, gender and the university they graduated from. However, there was a significant relationship between DSs' school year and their knowledge. Conclusion: The findings showed that the knowledge of DSs was lower than GDs. Since, these students will care for the oral and dental health of the community in the future, upgrading their training (by improving the quantity and quality of theoretical and practical training) is necessary to both understand the different aspects of biopsy, and to be familiar enough with proper oral biopsy storage and transport processes. Keywords: Knowledge; biopsy; students, dental; dentists; Mouth Neoplasms; Surveys and Questionnaires.
(GD) sobre las instrucciones posteriores a la biopsia oral. **Material y Métodos:** Se utilizó un cuestionario que constaba de dos partes:

1) Datos demográficos y autoevaluación del conocimiento de la biopsia por parte de los participantes y

2) 11 ítems sobre el correcto almacenamiento y transporte de la biopsia oral a un laboratorio de histopatología. **Statistical analysis:** Analizó los datos recopilados de los cuestionarios.

**Resultados:** 48 GD y 131 SD participaron en este estudio. La puntuación de conocimiento de los DS (5,43 ± 2,01) fue significativamente menor que la de los GD (8,33 ± 1,78) (p <0,05). Además, no hubo una relación significativa entre los conocimientos de los GD y su experiencia laboral, edad, género y la universidad de la que se graduaron.

**Palabra Clave:** Conocimiento; biopsia; Estudiantes de Odontología; odontólogos; Neoplasias de la Boca; Encuestas y Cuestionarios.
**Table 1.** Knowledge score based on DSs school year, work experience of GDs and university of graduation of GDs.

| Variable                              | N (%) | Knowledge score |
|---------------------------------------|-------|-----------------|
| School year of Dental students        |       |                 |
| 1<sup>st</sup>                         | 22 (16.8) | 4.23 ± 1.51     |
| 2<sup>nd</sup>                         | 23 (17.6) | 4.65 ± 1.69     |
| 3<sup>rd</sup>                         | 23 (17.6) | 5.43 ± 2.19     |
| 4<sup>th</sup>                         | 24 (18.3) | 5.92 ± 2.28     |
| 5<sup>th</sup>                         | 21 (16.0) | 5.86 ± 1.90     |
| 6<sup>th</sup>                         | 18 (13.7) | 6.72 ± 1.36     |
| University of graduation of General dentists |       |                 |
| Tehran                                | 21 (43.7) | 8.48 ± 1.78     |
| Hamadan                               | 20 (41.7) | 7.95 ± 1.90     |
| Tabriz                                 | 4 (8.3)  | 10.50 ± 0.71    |
| Kerman                                 | 2 (4.2)  | 8.00 ± 0.00     |
| Shiraz                                 | 1 (2.1)  | 10.00 ± 0.00    |
| Work experience of General dentists   |       |                 |
| < 10 years                             | 20 (41.7) | 8.11 ± 1.81     |
| 10-20 years                            | 23 (47.9) | 8.13 ± 1.68     |
| >20 years                              | 5 (10.4)  | 9.40 ± 1.95     |

**Table 2.** Self-evaluation of biopsy knowledge by DSs and GDs and also sources of such knowledge.

| Self-evaluation questions                          | Answers | Dental students | General dentists |
|---------------------------------------------------|---------|----------------|-----------------|
| Do you have any knowledge on proper biopsy storage and transport? | Yes       | 51 (38.9) | 37 (77.1) |
|                                                  | No       | 80 (61.1) | 11 (22.9) |
| If your answer to the above is “Yes” do you evaluate your knowledge to be adequate? | Yes       | 29 (56.9) | 20 (54) |
|                                                  | No       | 22 (43.1) | 17 (46) |
|                                                  | Academic Education | 33 (64.7) | 22 (59.5) |
|                                                  | Reading Scientific Literature | 8 (15.7) | 7 (18.9) |
|                                                  | Academic Education + Conferences | 2 (3.9) | 4 (10.8) |
|                                                  | Academic Education + Reading Scientific Literature | 0 (0.0) | 3 (8.1) |
| What is the source(s) of your knowledge?          | Academic Education + Reading Scientific Literature + Conferences | 1 (2.0) | 1 (2.7) |
|                                                  | Conferences | 2 (3.9) | 0 (0.0) |
|                                                  | Peers (other students/colleagues) | 4 (7.8) | 0 (0.0) |
|                                                  | Reading Scientific Literature + Conferences | 1 (2.0) | 0 (0.0) |

**Table 3.** Reasons for not sending biopsy samples to histopathological laboratory.

| Reasons                                                                 | Dental students | General dentists |
|------------------------------------------------------------------------|-----------------|-----------------|
| Biopsy is not a dentist duty                                           | 12 (27.9)       | 14 (43.7)       |
| Biopsy is not applicable for a patient                                 | 2 (6.2)         | 0 (0.0)         |
| There is no need for histopathological assessment when a lesion is clinically diagnosed | 4 (9.3)         | 0 (0.0)         |
| Lack of patient’s consent for biopsy                                   | 0 (0.0)         | 2 (6.2)         |
| Lack of proper academic education/training                              | 24 (55.9)       | 11 (34.4)       |
| Biopsy is not a dentist duty + Lack of enough education/training       | 2 (4.6)         | 0 (0.0)         |
| Others                                                                 | 1 (2.3)         | 3 (9.4)         |
Table 4. Frequency distribution of GDs, and DSs, answers to the questionnaire items.

| Item | Correct Questionnaire item                                                                 | Dental students answer | General dentists answer |
|------|-------------------------------------------------------------------------------------------|------------------------|-------------------------|
| 1    | Histopathological assessment of each abnormal oral tissue sample taken as biopsy is necessary. | Correct: 82 (62.6%)    | Incorrect/no answer: 49 (37.4%) |
| 2    | The entire lesion/all the tissue piece excised during a biopsy should be sent to histopathological assessment | Correct: 84 (64.1%)    | Incorrect/no answer: 47 (35.9%) |
| 3    | The entire lesion/all the tissue pieces excised during a biopsy should be sent to one histopathological laboratory | Correct: 63 (48.1%)    | Incorrect/no answer: 68 (51.9%) |
| 4    | The collected biopsy sample should be immediately placed in fixative.                      | Correct: 76 (58%)      | Incorrect/no answer: 55 (42%) |
| 5    | The best fixation method is to immerse the sample in a container containing suitable fixative. | Correct: 65 (49.6%)    | Incorrect/no answer: 66 (50.4%) |
| 6    | 10-15% buffered formalin is the most suitable fixative for routine histopathological assessment of tissue samples using a light microscope. | Correct: 66 (50.4%)    | Incorrect/no answer: 65 (49.6%) |
| 7    | The sample should be immersed in sufficient volume of fixative (10-20 times the volume of sample). | Correct: 44 (33.6%)    | Incorrect/no answer: 87 (66.4%) |
| 8    | A plastic container with a wide opening is recommended for sample transfer to laboratory.   | Correct: 41 (31.3%)    | Incorrect/no answer: 90 (68.7%) |
| 9    | The sample container should have a screw-cap.                                              | Correct: 58 (44.3%)    | Incorrect/no answer: 73 (55.7%) |
| 10   | Before sending biopsy to laboratory name of patient, clinician and site of biopsy should be labeled on body of container. | Correct: 78 (59.5%)    | Incorrect/no answer: 53 (40.5%) |
| 11   | Recording and sending comprehensive information (such as clinical patient history and clinical/radiographic findings) play important role on correct histopathological interpretation and diagnosis. | Correct: 54 (41.2%)    | Incorrect/no answer: 77 (58.8%) |

RESULTS.

A total of 131 out of 180 DSs and 48 of 87 GDs participated in this study voluntarily.

Descriptive results

Of 131 DSs, with a mean age of 23.33±2.98 years old, 85 (64.89%) were male and 46 (35.11%) were female. Also, the highest and the lowest number of DSs were in the fourth (n=24) and sixth (n=18) school years, respectively (Table 1). Self-evaluation of biopsy knowledge by DSs as well as sources of such knowledge is presented in Table 2.

Moreover, 67.2% (n=88) of DSs had positive and 32.8% (n=43) had negative attitudes about sending a biopsy to a laboratory in the future. Table 3 presents the stated reasons for not sending biopsy to the laboratory. DSs knowledge mean score was 5.43±2.01 (ranging from 0 to 10). Table 1 presented the knowledge score of DSs based on their school year. The frequency distribution of DSs responses to each question is also presented in Table 4.

Of 48 GDs, 28 (58.83 %) were male and 20 (41.67 %) were female and their mean age was 39.62±5.75 years old. Their mean years of work experience was 11.93±4.68 years (ranging from 4 to 25 years), and the majority (21, 43.7%) graduated from Tehran University of Medical Sciences (Table 1). Self-evaluation of biopsy knowledge by GDs and sources of such knowledge are presented in Table 2. The findings showed that 66.7% of GDs (n=32) had never sent a biopsy to a laboratory; the reasons are presented in Table 3. GDs knowledge score mean was 8.33±1.78 (ranging from 3 to 11). Knowledge scores of GDs, based on their work experience and the
Acquired their knowledge during their dental education. Furthermore, not only in the curriculum, but even afterwards, what was taught about biopsy in academic educational settings compared to other sources seemed to be of greater importance and to play a more prominent role. As such, it is important to pay attention to both the quantity and the quality of theoretical and practical training to obviate the educational weaknesses in this area. Taking an oral biopsy sample is a simple surgical procedure, and all GDs should be able to do it. The majority of GDs reported that they never sent a biopsy sample to the laboratory. However, some stated that taking a biopsy is not the duty of a dentist or that they did not receive any training in this area.

The majority of DSs, who stated that they would not do biopsy in the future, believed that lack of training was the main reason. Murgod et al. reported that the main reasons for dentists to not perform a biopsy were patients' lack of consent to the procedure, the dentists' lack of adequate experience/ expertise in this area and not having the required instruments to do the biopsy. On the other hand, Bataineh et al. put the greatest emphasis on the absence of adequate training in the dental curriculum.

In the present study, no significant correlation between knowledge of GDs and their work experience, age, gender and the university they graduated from was found. Similarly, Bataineh et al. found no significant correlation between the work experience of GDs and their knowledge about biopsy. However, Lopez-Jornet et al. showed that GDs with a longer work experience were more willing to perform biopsy.

The knowledge score of DSs had a significant correlation with their school year and age. The increasing age of the DSs reflected their more advanced academic development. The knowledge score of the 6th year DSs was higher than that of DSs at lower school years, which could be due to a more comprehensive theoretical knowledge acquisition, increased clinical experience and performing a higher number of biopsy cases compared with DSs still at lower school years. Improper oral biopsy storage and transport could be due to the lack of knowledge in this area.

Therefore, it is vital to perform assessments and to offer the necessary training based on the results. Also, it is necessary to determine the knowledge level of DSs and GDs in order to design the correct curriculum in dental schools to reach this goal.

Analytical results

Kruskal-Wallis test showed that there is a significant correlation between the knowledge score of DSs and their school year ($p<0.05$). However, the knowledge score of the males ($5.62±1.96$) and the females ($5.74±2.08$) DSs were not significantly different (Mann Whitney test, $p=0.28$). There was no significant correlation between the knowledge score of GDs and the university they graduated from ($p=0.16$) and the years of work experience ($p=0.247$). No significant difference was found between knowledge scores of males ($8.46±1.81$) and females ($8.15±1.75$) ($p=0.78$).

On the other hand, Spearman's correlation test showed a significant correlation between age and knowledge score of DSs, so that their knowledge score was significantly increased by an increase in their age (correlation coefficient=0.235, $p=0.006$). This correlation was not significant for GDs (correlation coefficient=-0.034, $p=0.818$).

Comparison of the knowledge score of DSs and GDs showed a significant difference in terms of the correct oral biopsy storage and transport. The knowledge score of GDs in this area was significantly higher than that of DSs (Student t-test, $p=0.0001$).

DISCUSSION.

Taking a biopsy sample for histopathological study is the most effective strategy to correct diagnosis of oral lesions. In addition to the errors related to taking a biopsy, delay fixation, use of a fixative solution without considering type of diagnostic technique, improper storage and transport of oral biopsy can also result in an incorrect interpretation by pathologists.

In this study a higher percentage of GDs believed that they had knowledge on proper oral biopsy storage and transport compared to DSs. Unfortunately, the difference between the two groups was considerable. This difference, especially in relation to the DS who are still busy with their studies at the university and have the opportunity to acquire more knowledge in this area, was discouraging. However, this finding could be due to the inclusion of DS in lower school years in the study, who had not yet received theoretical and practical training in this area.

The majority of GDs and DSs reported that they acquired their knowledge during their dental education. Furthermore, not only in the curriculum, but even afterwards, what was taught about biopsy in academic educational settings compared to other sources seemed to be of greater importance and to play a more prominent role. As such, it is important to pay attention to both the quantity and the quality of theoretical and practical training to obviate the educational weaknesses in this area. Taking an oral biopsy sample is a simple surgical procedure, and all GDs should be able to do it. The majority of GDs reported that they never sent a biopsy sample to the laboratory. However, some stated that taking a biopsy is not the duty of a dentist or that they did not receive any training in this area.

The majority of DSs, who stated that they would not do biopsy in the future, believed that lack of training was the main reason. Murgod et al. reported that the main reasons for dentists to not perform a biopsy were patients' lack of consent to the procedure, the dentists' lack of adequate experience/ expertise in this area and not having the required instruments to do the biopsy. On the other hand, Bataineh et al. put the greatest emphasis on the absence of adequate training in the dental curriculum.

In the present study, no significant correlation between knowledge of GDs and their work experience, age, gender and the university they graduated from was found. Similarly, Bataineh et al. found no significant correlation between the work experience of GDs and their knowledge about biopsy. However, Lopez-Jornet et al. showed that GDs with a longer work experience were more willing to perform biopsy.

The knowledge score of DSs had a significant correlation with their school year and age. The increasing age of the DSs reflected their more advanced academic development. The knowledge score of the 6th year DSs was higher than that of DSs at lower school years, which could be due to a more comprehensive theoretical knowledge acquisition, increased clinical experience and performing a higher number of biopsy cases compared with DSs still at lower school years. Improper oral biopsy storage and transport could be due to the lack of knowledge in this area.

Therefore, it is vital to perform assessments and to offer the necessary training based on the results. Also, it is necessary to determine the knowledge level of DSs and GDs in order to design the correct curriculum in dental schools to reach this goal.
In this study, the items (correct answers of the questions asked in the questionnaire) were as follows:

1: Histopathological analysis of each abnormal oral tissue sample taken as biopsy is necessary to achieve a final diagnosis.\textsuperscript{3,5}

2 and 3: The entire lesion/all the tissue pieces excised should be sent to one laboratory.\textsuperscript{12} Sending only a part of a sample to a laboratory may result in missing of histopathological changes and inaccessibility of pathologist to important microscopically diagnostic features in other parts of the sample (which were not sent to the laboratory). This can lead to controversial diagnoses by different laboratories which can adversely affect the proper management of the disease in the patient.

4: The collected biopsy sample should be immediately placed in fixative.\textsuperscript{4}

Delay between tissue collection and its immersion in fixative can result in activation of tissue enzymes, autolysis and protein degradation.\textsuperscript{13} These processes can cause changes in tissue and make it histopathologically undetectable.\textsuperscript{14}

5: For optimal fixation, the best method is to immerse the sample in a container containing suitable fixative.\textsuperscript{15}

Wrapping the sample in a gauze, cotton or tissue paper can cause its dehydration and drying and the tissue may remain un-fixed.\textsuperscript{13} Moreover, it would be difficult to separate the dry tissue from the gauze\textsuperscript{14} and this may damage it. Freezing the tissue sample, instead of fixing it, is also incorrect since it can cause cellular changes and epithelial perforations due to the formation of ice crystals.\textsuperscript{3,16}

6: At present, 10%-15% buffered formalin is the most suitable fixative solution for routine histopathologic assessment of tissue samples under a light microscope.\textsuperscript{5,16} Biopsy samples immersed in solutions such as water, saline or alcohol often show abnormal cellular and structural changes, which can cause problems in reaching a correct diagnosis.\textsuperscript{17} Samples are not properly fixed in these solutions\textsuperscript{3,14} and tissue autolysis continues.\textsuperscript{16}

7: The sample should be immersed in sufficient volume of fixative to ensure its adequate fixation.\textsuperscript{1} The sample should also be submerged in the fixative.\textsuperscript{15} This volume varies from 10 to 20 times the volume of sample in the literature.\textsuperscript{1,14} If the sample is not completely immersed in formalin, it may become dehydrated; dehydration confers a dry appearance to the sample and causes cracking and over-staining of tissue sections.

8: A plastic container\textsuperscript{12} with a wide opening\textsuperscript{1} is recommended to transfer the sample to the laboratory. Using a glass container is risky since it may break and hurt people. Moreover, small glass particles may penetrate into the tissue sample and damage the microtome used for tissue sectioning.\textsuperscript{12,18}

Formalin also increases the tissue consistency and results in its hardening.\textsuperscript{1} The opening of the container used for sample transfer should be wide 1 in order to allow an easy and safe removal of the tissue from the container.

9: The tissue sample container should have a screw-cap.\textsuperscript{12} Formalin is classified as a carcinogen.\textsuperscript{19,20} Contact of formalin with skin and breathing its vapor are dangerous for the person who carries the container.\textsuperscript{20} It seems that press-to-seal containers (such as some injection bottles) have a higher risk of leakage and evaporation of fixative compared with screw-cap containers.

10: All biopsy containers should be labeled.\textsuperscript{14} These labels should be stuck to the body of the container, not to the caps.\textsuperscript{21} In the laboratory, after opening the caps of similar containers, there is a possibility of caps misplacing and as a result label misplacing, thus, serious errors may occur. The label should contain the name of patient, the name of clinician and site of biopsy.\textsuperscript{1,14}

Having a label is so important that the laboratory can refuse to accept the containers without proper labeling and send them back to the clinician for correct labeling.\textsuperscript{12,18}

11: Histopathology assessment includes microscopic and macroscopic study of tissue as well as its relation to clinical history and any imaging findings.\textsuperscript{4} Thus, it is necessary to provide the pathologist with comprehensive patient history, information about the lesion and name and address of the clinician in charge of the patient.\textsuperscript{22}

Such information should be recorded in the histopathological assessment request form 4 and sent to the laboratory along with the biopsy sample.\textsuperscript{4} This form is the first line of communication between the clinician and the laboratory.\textsuperscript{18} By doing so, the pathologist gets an idea of what the dentist observed and what they think about the lesion.\textsuperscript{23}

**CONCLUSION.**

In this study the knowledge of GDs was significantly higher than that of DSs. However, the knowledge of both groups highlighted the need for further training.
programs both before and after graduation.

Considering our small sample size, our results may not be generalizable to the entire population of DSs and GDs in Iran. However, our findings highlighted the need for knowledge improvement in this area.

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