



Original article

Comparative Evaluation of Polarization Colors of Collagen Fibers in Odontogenic Keratocyst and Ameloblastoma Using Picrosirius Red Stain (Histochemical Study)

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ABSTRACT

Aims: studying the quality and quantity of collagen fibers may help to delineate their influence on the aggressiveness and growth potential of the OKC and ameloblastoma. The aims of this study were to evaluate and compare the polarization colors, orientation, and organization of collagen fibers in the connective tissues of the odontogenic keratocyst (OKC), and ameloblastoma (AB) using picrosirius red (PSR) stain under a polarizing microscope with correlation to their biologic behavior.

Materials and method: polarization colors, orientation, and organization of collagen fibers in pre-diagnosed cases of OKC and AB, twenty each, and two control cases of dentigerous cyst (DC) were histochemically analyzed using PSR stain and a polarized light microscope.

Results: comparing the collagen fibers in the two lesions, there was no significant difference with respect to the polarization colors, orientation, and organization.

Conclusion: PSR staining and polarizing microscopy is a powerful tool to appraise the nature and arrangement of collagen fibers in odontogenic lesions. The connective tissue of OKC showed a predominance of green-yellow birefringence, and loosely packed fibers, this can be correlated to the aggressive nature of this lesion.

Keywords: *polarization colors, picrosirius red stain, polarizing microscope.*

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INTRODUCTION

Odontogenic tumors and odontogenic cysts are common lesions of the oral cavity, the epithelium, and mesenchymal tissues of the tooth-forming organ may give rise to a variety of odontogenic tumors, also odontogenic cysts are known to be derived from the epithelial rests lying in jawbones.¹

The odontogenic keratocyst (OKC) was described in 1956 by Philipsen as a developmental odontogenic cyst with distinctive histopathologic features². Ameloblastoma is a benign epithelial odontogenic tumor, it was introduced by Cusack in 1827.³

The role of connective tissue of odontogenic lesions regarding their growth potential, aggressiveness, and recurrence has been unfairly overlooked for a long time. Recent studies have underscored the fact that, the epithelium and connective tissue are nearly equally involved in

determining and predicting the clinical course of an odontogenic lesion, this holds true as the reciprocal interaction between odontogenic epithelium and mesenchymal tissue occurs at the early stages of odontogenesis, this is also applied to pathologic processes which lead to the development of various odontogenic tumors and cysts.⁴⁻⁶ Collagens, as a major constituent of connective tissue capsule in the wall of these lesions, can be accurately evaluated using picrosirius red stain and polarizing microscope.⁷

The PSR stain (F3BA) is an anionic dye-containing six sulfonate groups that chemically reacts with basic amino acids-rich collagens, this acid-base interaction allows each SR to bind to a collagen molecule alongside its axis; subsequently, the natural phenomenon of birefringence is enhanced when exposed to polarized light.⁸ Collagen

fibers demonstrate green, yellow, and red colors according to the amount of the light absorbed, in which collagens stand out against a black background, the color difference is largely dependent on packing, physical aggregation, and thickness of the fibers.^{8,9} This study was conducted in an attempt to evaluate and compare the polarization colors of collagen fiber bundles, and their pattern of orientation and organization in the connective tissues of OKCs and ameloblastoma with correlation to their biologic behavior.

MATERIALS AND METHODS

Sample selection

After requesting and obtaining the ethical approval from the Scientific Research Ethics Committee, University of Benghazi, Faculty of Dentistry to conduct this study, pre-diagnosed cases of OKC and AB; in addition to control cases of DC, were retrieved from the archives of the Department of Oral Medicine, Oral Pathology, Diagnosis and Radiology, Faculty of Dentistry, University of Benghazi, between 1996 and 2019. Cases with well-preserved histopathologic diagnostic criteria were included; whereas, inflamed fibrous stroma in which the characteristic diagnostic features were destroyed, and cases reported with a connective tissue disease were excluded.

A total of forty-two cases were selected for this study, twenty cases of formalin-fixed paraffin-embedded (FFPE) blocks each of twenty OKC (n=20), and twenty AB (n=20), and two cases of DC as controls.

Tissue processing and staining

From all selected forty-two FFPE blocks two sections of 4 μ thickness were taken, of which one was stained with hematoxylin and eosin (H & E) following the standard protocol, and then observed under a conventional light microscope to reassess diagnosis, and to be subjected to the selection criteria. The other section was stained with picrosirius red, the staining kit was purchased from Bio-Optica Milano S.p.A under the code number 04-121873 and LOT 0420, the kit contained four bottles, A: sirius red picrate solution, B: buffer solution (two in number), and C: Mayer's Hemalum.

Staining procedure: sections were brought to distilled water, and then put on 10 drops of **A** and left to act 50 minutes. Sections were rinsed briefly in distilled water, and put on 10 drops of reagent **B** which was left to act 2 minutes and repeated 2 times. Sections were briefly rinsed in distilled water and slides were drained. 10 drops of reagent **C** were used and left to act 3 minutes. Sections were blued in running tap water for 3 minutes, then dehydrated through ascending alcohols, and cleared in xylene. Sections were then viewed under a polarized light microscope for the analysis of collagen fibers.

Evaluation of collagen fibers in OKC and AB cases

The analysis of all selected cases stained with PSR was performed under a polarizing microscope using 10x and 40x magnifications in at least three separate fields.

Polarization colors of collagen fibers

The connective tissue of the two lesions showed polarization colors varying from green-yellow, yellow-orange and orange-red. The most predominant polarization color was determined in all cases, and this was performed during three separate periods of times to eliminate intra-observer bias, and the average reading for each case was recorded.

Orientation of collagen fibers

Collagen fibers orientation to the epithelial component was also observed, each case was classified according to the fibers orientation pattern as not parallel or parallel.

Organization of collagen fibers

The organization of collagen fibers was also evaluated in areas near to the epithelial component, and each case was classified as loose bundles of collagen fibers (loosely arranged and interwoven in all directions), or dense collagen fibers (well-defined organization with orderly organized collagen fibers forming collagen lamellae). All information obtained were documented in a chart for each case.

Statistical analysis

All obtained information was analyzed using SPSS format version 20 (Statistical Package for Social Sciences, Chicago, Illinois, USA) for statistical analysis. The polarization colors, orientation and organization of collagen fiber bundles in the two lesions were tested using Fisher's exact test and Chi-square test. Computations of P-value (<0.05) considered as statistically significant.

RESULTS

Polarization colors of collagen fiber bundles in the connective tissue of OKC and ameloblastoma

On evaluating PSR stained sections of control cases of DC they showed a predominance of orange-red and yellow-orange birefringence. OKC and AB cases revealed the following distribution of polarization colors of collagen fibers: green-yellow birefringence was the most predominant in OKC cases, and it occupied twelve (60%), followed by yellow-orange which was recorded in seven cases (35%), orange-red was documented in only one case (5%) (Figure 1,2).

On the contrary, most of the collagen fibers in AB cases exhibited a predominance of yellow-orange birefringence in eleven (55%), the green-yellow was documented in five cases (25%), and the orange-red color was noticed in four cases (20%) (Figure 3,4), (Table 1). (p-value= 0.086).

Figure 1 photomicrograph of PSR stained section of OKC showing a predominance of green-yellow birefringence of collagen fibers. 10x

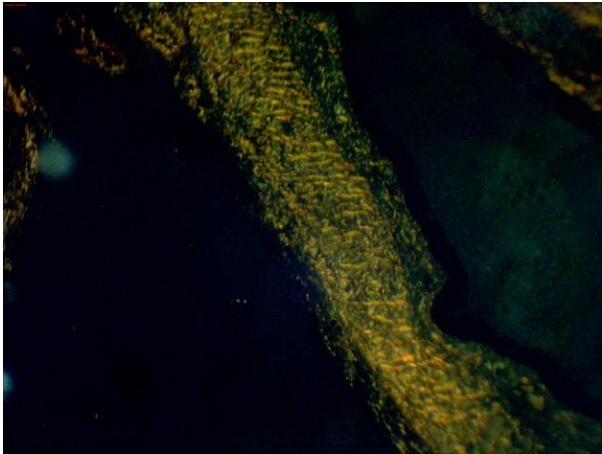


Figure2 photomicrograph of PSR stained section of OKC showing a predominance of green-yellow birefringence of collagen fibers.40x

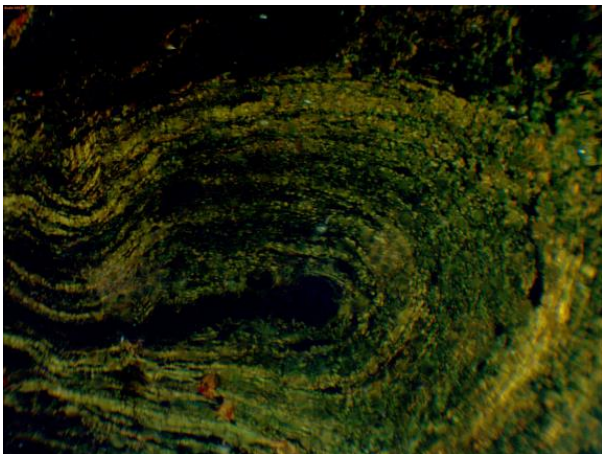


Figure 3 photomicrograph of PSR stained section of ameloblastoma showing a predominance of yellow-orange birefringence of collagen fibers. 10x

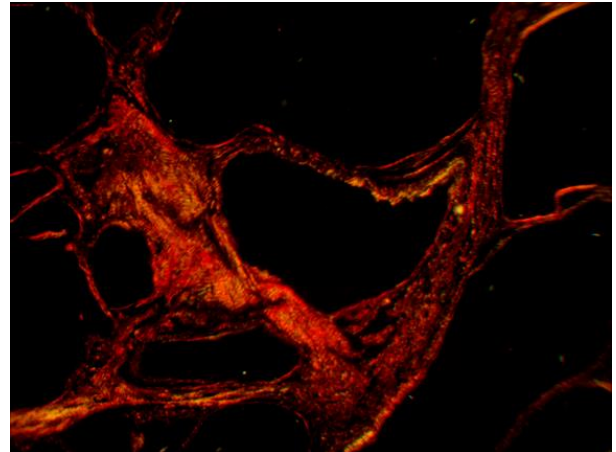
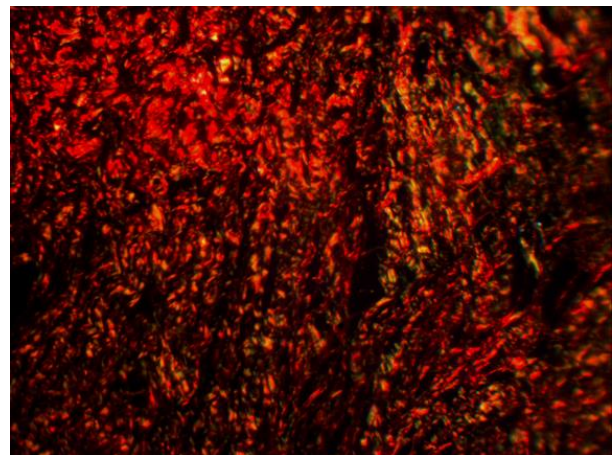


Figure 4 photomicrograph of PSR stained section of ameloblastoma showing a predominance of yellow-orange birefringence of collagen fibers. 40x



Collagen	OKC		AB	
	N	%	N	%
GY	12	60	5	25
YO	7	35	11	55
OR	1	5	4	20
Total	20	100%	20	100%

Table 1:
comparison of polarization colors of collagen fiber bundles in the stroma of OKC and ameloblastoma

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(*p-value= .086) (OKC: odontogenic keratocyst, AB: ameloblastoma, GY: green-yellow, YO: yellow-orange, OR: orange-red).

The orientation of collagen fiber bundles to the epithelial component in OKC and ameloblastoma:

The distribution of collagen fibers orientation pattern was the same in both OKC and AB cases, fibers were predominantly not parallel in eleven cases (55%), while parallel fibers were seen in nine cases (45%), (Figure 5, Table 2) (p-value=1.00).

Figure 5 photomicrograph of collagen fibers running parallel to the epithelial component in an OKC, 10x

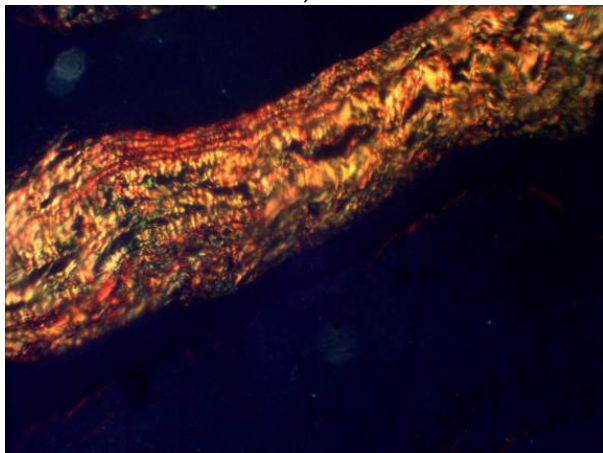


Table 2: comparison of orientation pattern of collagen fiber bundles in the of OKC and ameloblastoma

Collagen	OKC		AB	
	N	%	N	%
Not parallel	11	55	11	55
Parallel	9	45	9	45
Total	20	100%	20	100%

(*p-value=1.00)(OKC: odontogenic keratocyst, AB: ameloblastoma)

Organization pattern of collagen fiber bundles in the connective tissue of OKC and ameloblastoma:

Concerning the organization of collagen fibers it was demonstrated that, densely packed collagen fiber bundles were predominant in both OKC and AB accounting for eleven (55%) and twelve (60%) respectively; however, loosely packed fibers were documented in nine cases (45%) of OKC and eight cases (40%) of AB, (Figure 6, Table 3). (p-value=0.074)

Figure 6 photomicrograph of PSR stained section of ameloblastoma showing densely packed collagen fibers, 10x

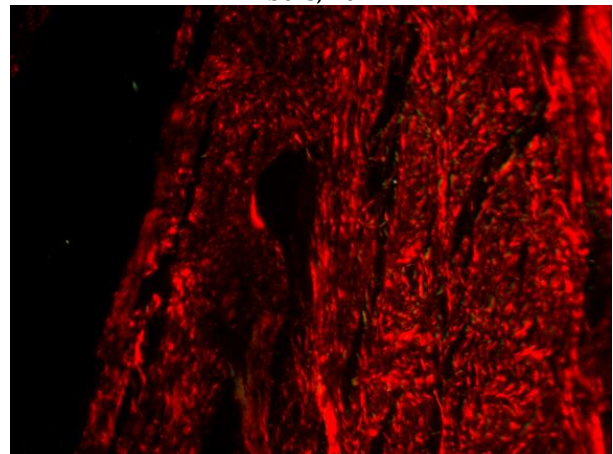


Table 3: comparison of organization pattern of collagen fiber bundles in the stroma of OKC and ameloblastoma

Collagen	OKC		AB	
	N	%	N	%
Dense	11	55	12	60
Loose	9	45	8	40
Total	20	100%	20	100%

(*p-value=.749) (OKC: odontogenic keratocyst, AB: Ameloblastoma).

DISCUSSION

This study was conducted in an attempt to assess, compare and investigate the difference in the polarization colors of collagen fiber bundles, and their pattern of orientation and organization in the connective tissue of OKCs and ameloblastoma with reference to their biologic behavior. This was a histochemical study, containing a total of forty two FFPE blocks of pre-diagnosed cases of OKC and ameloblastoma, twenty cases each, and two cases of DC were taken as controls, the sample selection was made upon inclusion and exclusion criteria.

Sections of 4μ were stained with PSR, and then viewed under a polarized light microscope using 10x and 40x magnifications in at least three separate fields; first, to record the most predominant polarization color the examination was conducted during three separate periods of times to eliminate intra-observer bias, and the average reading was taken for each case; second, patterns of orientation and organization of collagen bundles in each case were documented.

It was proposed that PSR staining and polarized light microscopy can effectively help to define the nature and arrangement of collagen fibers in odontogenic lesions, as mature thick well-packed

fibers demonstrate orange-red to red birefringence while immature thin loose fiber show a birefringence of green-yellow color.⁵ It has been documented that, the study of collagen fibers nature and the overall impact of connective tissue on the behavior of the OKC and ameloblastoma can help to explain the mechanism of expansion and reasons behind their aggressive growth and high recurrence rate.⁶

In the present study, it was observed that twelve cases (60%) of OKCs exhibited a predominance of green-yellow birefringence, this was in accordance with other several studies.^{5,10-13} Green-yellow polarization is related to loosely packed collagen fibers, this is in agreement with Sharf *et al.*¹⁴ who advocated that, physical aggregation of collagen molecules determine the polarization color; therefore, it was found that loosely packed collagens represented as green-yellow, this is also in conjunction with Junqueira *et al.*, and Montes *et al.*¹² who documented that, loose thin fibrils of type III collagen are found to physically form a loose meshwork in the ground substance which in turn exhibit a weak birefringence of green-yellow.¹⁵

It was also stated that, green-yellow color implies that stromal fibers consist of pro-collagen, intermediate, or pathologic collagens with disorganized pattern, such fibers are not structurally stable; hence, denature easily by the action of proteolytic enzymes of the stromal tissue. It has been shown that abnormal or immature fibers are found to be associated with aggressive lesions.^{5,12,16}

On the other hand, ABs demonstrated green-yellow birefringence in five cases (25%) which is less than OKC, this does not apply with reference to their biologic behavior as AB is an odontogenic tumor whereas OKC has been re-classified as an odontogenic cyst.¹⁷ Our finding contradicts the findings of Peddapeli K *et al.*¹⁶ in which AB showed slightly more of green-yellow color than the OKC.

Moreover, AB displayed more yellow-orange and orange-red birefringence than OKC cases, eleven (55%) and four (20%) compared to seven (35%) and one (5%) respectively. Our finding is in agreement with other studies.^{10,14,18} It was proposed that strong birefringence of orange and red denotes tightly packed collagens, and this is compatible with Junqueira *et al.*¹⁴ who claimed that, type I collagen are thick and closely packed; thus, fibers react strongly with PSR stain and produce long wavelength of orange and red color when viewed under PL.^{12,15}

On the contrary, Peddapeli K *et al.*¹⁶ reported that, OKCs showed more yellow-orange and orange-red when compared to ABs. Our findings can be justified as we have not included moderately and severely inflamed cases of OKC in an attempt to study this developmental lesion without the

recognized alteration on collagen fibers by secondary inflammation as evaluated by Kajikar MS *et al.*⁵, it was shown that infected OKCs revealing a severely inflamed stroma demonstrated orange to red birefringence; unlike non-infected OKCs which illustrated a predominance of green-yellow.

Also, it was reported that inflammation can induce a change in collagen fibers organization in inflamed OKCs by increasing the production of densely packed fibers; therefore, influence a change in the color of polarization.¹⁹ It has been documented that strongly birefringent collagen fibers have been correlated to odontogenic lesions with innocent behavior and less aggressive growth.^{12,13}

In the present study, the observations made on collagen fibers orientation showed equal cases of OKCs and ABs in which parallel fibers were reported in nine (45%) and not parallel in eleven (55%). Our findings were conflicting with the findings of other studies in which parallel fibers were predominant in OKC cases.¹⁶⁻²⁰ Parallel orientation has been considered to contribute to the separation between lining epithelium of OKCs from their supporting capsule, this was also linked to the different growth pattern of OKC from other odontogenic cysts, which in addition to daughter cysts explained the unceasing growth and high recurrence of the OKC.^{12,16}

Our study revealed that, loose collagen fibers distribution in OKCs was insignificantly more than ABs, nine (45%) compared to eight cases (40%) respectively. This is in agreement with Peddapeli K *et al.*¹⁶, it has been demonstrated that loosely packed collagen fibers are prone to degradation by collagenases; as a result, facilitating the growth and expansion of a lesion. Moreover, studies on the role of connective tissue of OKC advocated that, the function of the fibrous capsule of this lesion extends beyond structural support; it is rather involved in its pathogenesis and clinical behavior.^{5,16}

Conversely, dense collagen fibers in ABs were seen in twelve (60%) compared to eleven (55%) in OKCs, this can be attributed to the presence of mature densely packed fibers of long-standing lesions, this is consistent with the findings of Aggarwal P *et al.*²¹ However, our findings showed a contradiction with other studies.¹⁸⁻²⁰ It was shown that, cytokines and growth factors regulate the process of collagen and ECM components synthesis and degradation; in addition to defining the architecture of collagens within a tissue by influencing the activity of fibroblasts causing the production of thick mature fibers.¹⁵ Hence, collagen matures as the content of its proteoglycans and water decreases; subsequently, there is an increase in the fiber diameter with evidence of densely packed fibers organization.¹⁶

According to the observations made on the differences in the polarization colors, orientation and organization of collagen bundles in the connective tissue of OKC and AB they were found to be statistically insignificant (p -value=.086,1.00,.749 respectively). This was consistent with Raj Y. *et al.*⁵ and Ali AN *et al.*²⁰ However, our findings were conflicting with Peddapeli K *et al.*¹⁶ in which a significant difference was documented only between the means of yellow-orange; in addition, a difference was found in the orientation and organization of collagen fibers in OKC and AB.

This study showed some limitations, these limitations are:

- Selection bias, the study sample was not randomly chosen.
- Small sample size; hence, no firm conclusions can be drawn from this sample as a significant difference would be difficult to identify.
- Limited prior research on the same topic.
- Time constraints due to the current pandemic situation.
- Funding issues.
- Limited access to the literature.

Despite constraints, this research has shown a potential significance:

- Cases that were only applying to the diagnostic criteria of OKC and ameloblastoma were included; moreover, altered connective tissues by inflammation were excluded as inflammation does not play a role in the pathogenesis of these lesions.
- This research is filling the gap in the literature as being one of the few comparing the OKC and ameloblastoma in terms of collagen fibers characteristics.
- Despite the modest size of the sample, this sample can be considered relatively larger than the samples of other studies investigating the differences of collagen fibers among odontogenic lesions.
- Examination of all cases was performed in at least three different fields for three times separately to eliminate intra-observer bias and to insure accuracy of determining the most predominant color in each case.

CONCLUSION

- PSR staining and polarizing microscopy is a powerful tool to appraise the nature and arrangement of collagen fibers in odontogenic lesions.
- The connective tissue of OKC showed a predominance green-yellow birefringence, and loosely packed fibers, this can be correlated to the aggressive nature of this lesion.

- Studying and assessing collagen fibers of odontogenic lesions, especially lesions of diagnostic significance, may help to predict the clinical course of these lesions.

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Original article

Oral Cancer Awareness Among Dental Patients in Benghazi, Libya : A cross- sectional Study

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ABSTRACT

Background: Oral cancer is considered as one of the major public health problems in the coming decades. It is one of the most life-threatening conditions, and it is ranked as the 15th most common cancer in the world. Lack of awareness about the etiology, signs and symptoms of oral cancer might be the main causes of its delayed detection. Therefore, evaluation of patient's awareness towards oral cancer can play an important role in early detection and will lead to better prognosis. The aim of this study is to assess the level of dental patients' awareness towards oral cancer at Faculty of Dentistry, University of Benghazi.

Materials and Methods: A cross sectional study was conducted on dental patients during the academic year 2018-2019 at Faculty of Dentistry, University of Benghazi. Patients were surveyed for oral cancer awareness by using a pre-tested, self-administered questionnaire. The data was entered and tabulated into a computer using the Statistical Package for Social Science (SPSS Version 20 for Windows, SPSS Inc. Chicago, IL). Data analysis included descriptive statistics.

Results: The response rate was 87.4%. The study population comprised of 39.3% females and 60.7% males. Low educated patients represented 23.2% of participants. The majority of them (77.1%) have heard about the term of oral cancer with the mass media constitute 38.3% of their information sources. Less than half of patients had the idea that growth of abnormal tissue and continuous pain in jaw are considered as early symptoms of the cancer. Only 23.9% of participants believed that smoking is considered as a risk factor of oral cancer.

Conclusion and Recommendation: There is a lack in the awareness of early signs, symptoms, and etiology of oral cancer. Therefore, an intensive public education program to recognize the early warning signs, symptoms and etiologies of the cancer is recommended. In additions, individuals should be encouraged for self-oral examination and regularly visit the dental clinics. This would play an important role in early recognition of the cancer and better prognosis would be expected.

Keywords: dental patients, awareness, oral cancer, signs, symptoms and etiology.

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INTRODUCTION:

Oral cancer is considered as one of the major public health problems in the coming decades. It is one of the most life-threatening conditions, and it is ranked as the 15th most common cancer in the world.¹ A growing body of evidence shows an increase in its incidence in most countries, especially in the developing ones.² In eastern Libya, oral and pharyngeal cancer accounted for 4% and 2.2% of all cancers in males and females, respectively in 2003. Interestingly, in 2004 the number of new cases of oral and pharyngeal cancer had slightly increased in males and doubled in females.³ Gehani (2015) study represents that oral squamous cell carcinoma accounts for 55.2% of all oral cavity malignancies.

Epidemiologic evidence supports an association between alcohol consumption and various forms of tobacco consumption (smoking, snuff dipping, and chewing) as major factors contributing to oral cancer development.⁴ In addition to viral etiologies with human papilloma virus infection and Epstein barr virus, especially within the oropharynx, in younger people with no history of tobacco or alcohol abuse.⁵ However, other non-viral oral cancer lesions can develop anywhere but they are more commonly present in the tongue, buccal mucosa, floor of the mouth, and palate.⁴ Low intake of fruits and vegetables and genetic susceptibility have been reported as other contributing factors influencing the pathogenesis of oral cancer.⁶

In the early stages, oral cancer has no significant sign or symptom therefore, it is usually detected at advanced stage (III or IV). Following tumour mass development, tumour cells continue to proliferate, increase in size, invade the surrounding tissue and metastasize to distal organs aggravating the medical condition of the patients leading to death. Despite the recent advances in cancer therapy, this type of cancer has very poor survival rates worldwide; average of five-year survival rate of 50% has been reported.⁷

It has been argued that the mortality rate depends mainly on cancer stage which could be determined by clinical diagnosis. Evidence research has estimated that early diagnosis could increase the probability of cure and survival rates.⁸ Previous reports have illustrated that delay in diagnosis especially on the part of the patient, the time between the initial detection of symptoms and the first visit to a dentist,

may account for the detection of oral cancer lesions at advanced stages.⁹ One of the main causes of patient delay might be due to the lack of awareness and information about oral cancer signs and symptoms.¹⁰ Therefore, early diagnosis of the malignancy is possible and it may increase the survival rates. Similarly, oral cancers could be prevented and controlled if individuals know which risk factors are participating to it.

Previous questionnaire based studies in different developed countries including India², Iran¹¹ and Malaysia¹⁰ have revealed an alarming lack of awareness and knowledge of this type of cancer. The method utilized in all of these studies was self-administrated adapted questionnaires and in order to make sure that such testing measures were acceptable, reliable and valid, they were piloted in certain percentages of the targeted patients. In addition, the contents of such questionnaire were agreed among different experts in the searching area.

However, published data on awareness among Libyan population particularly who lived in Benghazi, are scarce. Therefore, the purpose of this study is to assess the level of dental patients' awareness towards the risk factors, as well as the signs and symptoms of oral cancer. The results obtained from the present study will be applied to assist the implementation of health education program, to increase public knowledge about oral cancer and attitudes towards early diagnosis in order to control and prevent the incidence of the cancer in addition to reduce its morbidity and mortality rate.

MATERIALS AND METHODS:

This cross-sectional study was conducted at the Faculty of Dentistry, University of Benghazi, Libya, during the academic year 2018-2019. Participants were adult dental patients (≥ 20 years) who agreed to take part in the study and signed the consent. Patients who attended the dental clinic on emergency basis and those with communication disabilities were excluded from the study. The interviews were conducted in the clinic waiting rooms where the participation was voluntary and anonymous. In order to make each participant feel as comfortable as possible, they were interviewed privately after a brief explanation of the objectives of the study and also responding to their questions and concerns. Each one took about 15 minutes to answer all the questions. However, Illiterate participants who were unable to read or fill in the questionnaire were interviewed for about 20

minutes and the researcher read each question and wrote the answers. Interviewer-administered questionnaire was adapted from previously validated items that have been applied in similar studies^{2, 12, 13} and it was translated from English into Arabic language to assess the patients' awareness regarding oral cancer. A pilot study was conducted by administering the questionnaire to a random sample of 20 participants. Modifications were subsequently made based on their feedback to ensure the validity of the questionnaire. Patients for appointments were approached for completing the survey and hence the survey targeted a convenience sample not driven by a defined sample size.

The questionnaire consisted of 26 closed ended questions covered four major sections. The first section concerned demographic data (age, gender, education level, occupation and smoking). The second section measured general awareness of oral cancer, by asking the patients whether they had heard of oral cancer and its means of knowledge, its contagiousity and treatability, and is it preventable or not. While the third section covered the respondents' knowledge of the cancer risk factors (smoking, smokeless tobacco, alcohol, family history, spicy food consumption, dietary deficiencies or imbalance, poor oral hygiene, HPV infection, and exposure to the sun). The fourth section in contrast examine the signs and symptoms of the cancer (abnormal tissue, ulcer, white or red spot, falling of teeth, reduced mouth opening, continuous pain, or numbness in jaw).

Data collection extended over a period of five months from November 2018 to March 2019. Response categories for each of the knowledge's questions were 'yes', 'no' and 'I don't know' and these answers were coded as 1, 2 and 3, respectively. Uncompleted questionnaires were excluded from the study. The data was entered and tabulated into a computer using the Statistical Package for Social Science (SPSS Version 20 for Windows, SPSS Inc. Chicago, IL). Data analysis included descriptive statistics based on the percentage of correctly answered questions. The total knowledge scores were represented as mean + standard deviation. Mann Whitney U test was used for comparing data as appropriate. The level of significance was set at P value equal to or less than 0.5.

RESULTS:

Five hundred questionnaires were distributed among Benghazi dental patients during five months. A total of 489 questionnaires had been returned back to

the researchers giving a response rate of 87.4%. The studying population comprised of 39.3% of females and 60.7% of males. Low educated and non-working patients represented 23.2% and 57% of participants, respectively (Table 1).

Table 1: Distribution of respondents by socio demographic profile

Variables		Per centages (%)
Age	20	19.5
	21-30	44.1
	31-40	16.3
	41-50	12.9
	50	7.2
Gender	male	60.7
	female	39.3
Level of education	low	23.2
	middle	45.9
	high	30.9
Working status	Non-working	57
	working	43
Smoking habit	smokers	28.7
	Non-smokers	71.3

The majority of the participants (77.1%) have heard about the term of oral cancer with the mass media constituted 38.3% of their sources of information (Figure 1 and 2). About 33.3 % of the patients were aware of the treatments available for

the cancer (Figure 3). While less than half of the patients had the idea that growth of abnormal tissue and continuous pain in jaw are considered as early signs and symptoms of the cancer development (21.7 % and 23.1%, respectively) (Figure 4). However, more

than half of the patients (71.3 %) were smoker and about 23.9% and 25.9% of the participates believed that smoking and alcohol consumption are considered as risk factors of the cancer (Figure 5).

Figure1: Percentage of participants who heard about oral cancer

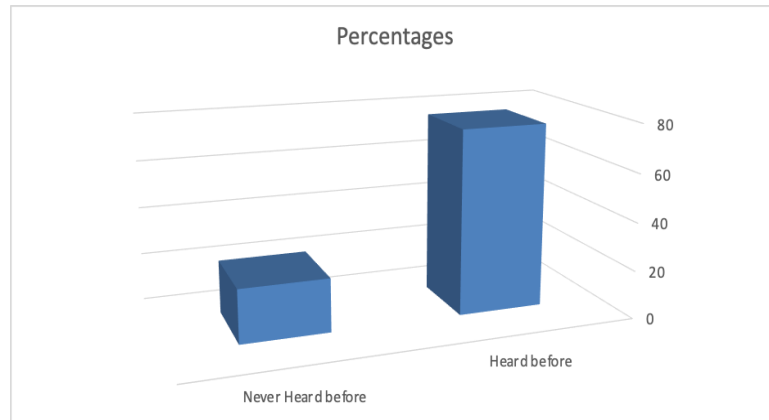


Figure 2: Distribution of participants according to the source of information from where they heard about oral cancer

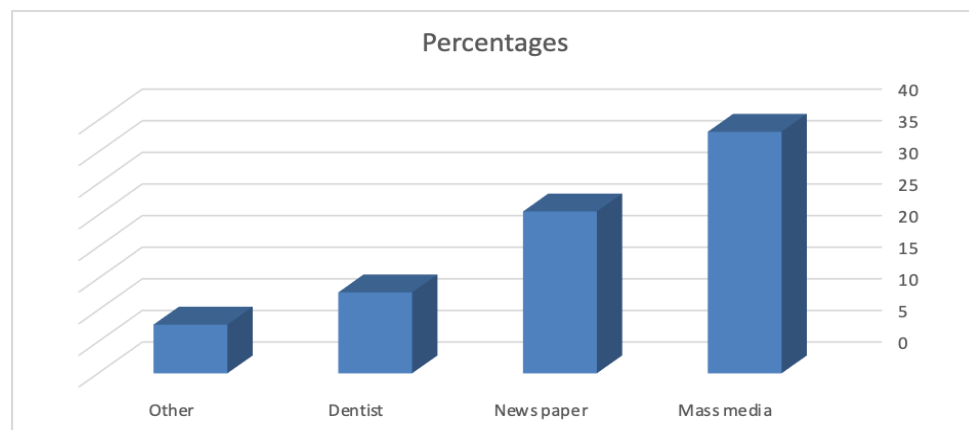


Figure 3: Percentage of participants against general awareness of oral cancer

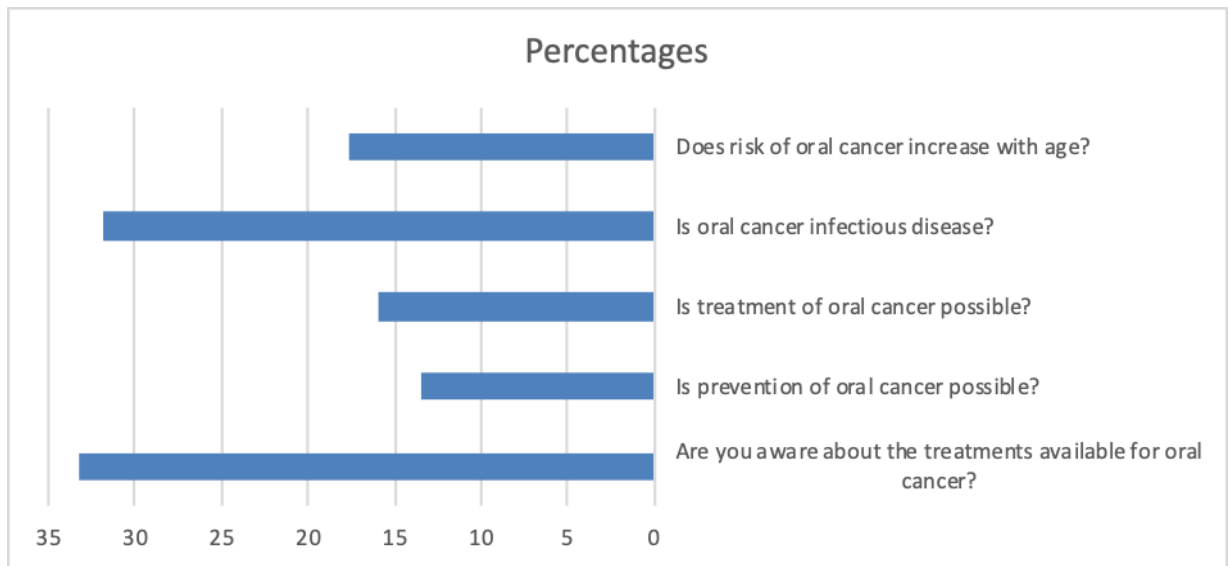


Figure 4: Percentage of participants across different clinical signs and symptoms of the oral cancer.

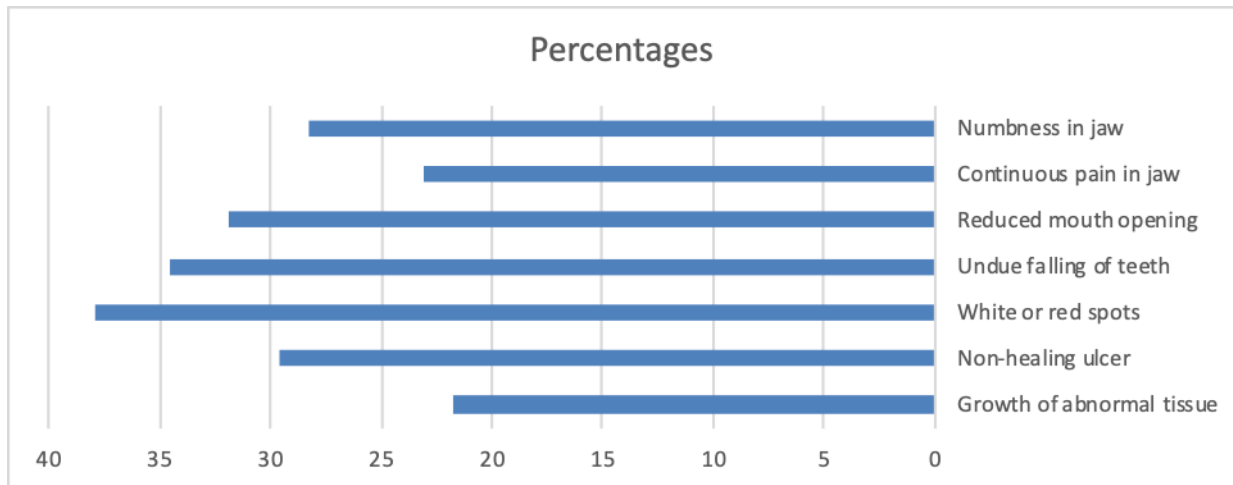
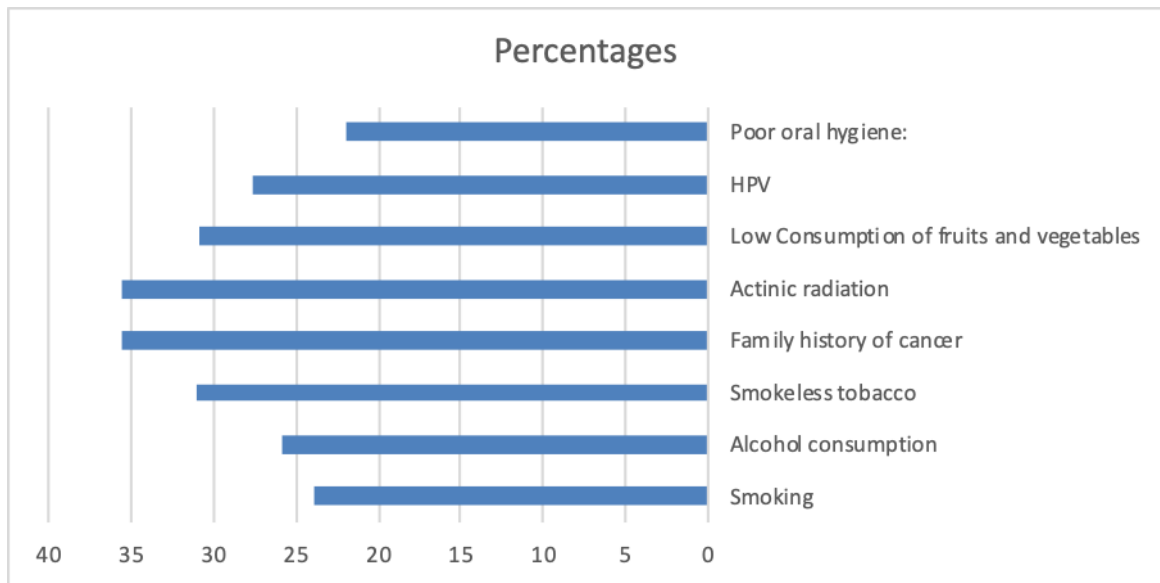


Figure 5: Knowledge of participants (%) about oral cancer risk factors

Regarding to general awareness, signs, symptoms and risk factors of oral cancer, Males (13.74 ± 2.94) had significantly higher awareness than females (12.68 ± 2.73) (Table 2). Furthermore, a significant difference was seen among various educated groups where the awareness was more in highly educated

ones (Table 3). Similarly, working participants represented a significant high statistical difference (Table 4). However, more than 70% of the participants were smokeless (Table 1) and interestingly the highest knowledge was observed among this group (Table 5).

Table 2: Mean value of general awareness, knowledge of signs & symptoms and risk factors of oral cancer according to gender

Variables	Male		Female		P *
	Mean	SD	Mean	SD	
General awareness	8.72	2.272	9.10	1.763	0.06
Knowledge of signs and symptoms	13.74	2.941	12.68	2.738	<0.001*
Knowledge of risk factors	16.01	4.785	15.94	4.740	0.777

Table 3: Mean value of general awareness, knowledge of signs & symptoms and risk factors of oral cancer among different levels of education

Variables	High		Middle		Low		P *
	Mean	SD	Mean	SD	Mean	SD	
General awareness	9.16	2.122	9.15	1.919	8.49	1.912	0.013*
Knowledge of signs and symptoms	13.90	2.663	12.58	2.767	13.27	3.010	0.001*
Knowledge of risk factors	15.89	4.517	15.93	4.734	16.10	4.984	0.945

Table 4. Mean value of general awareness, knowledge of signs & symptoms and risk factors of oral cancer according to the working status

Variables	Working		Not working		P *
	Mean	SD	Mean	SD	
General awareness	9.25	2.017	8.55	1.874	0.002*
Knowledge of signs and symptoms	13.53	2.958	12.78	2.753	0.013*
Knowledge of risk factors	16.11	4.622	15.86	4.855	0.639

Table 5: Mean value of general awareness, knowledge of Symptoms and risk factors of oral cancer among smoker & non-smoker groups

Variable	Smokers		Non-smokers		P *
	Mean	SD	Mean	SD	
General awareness	8.57	1.971	9.10	1.973	0.012*
Knowledge of symptoms	13.33	2.864	13.01	2.862	0.211
Knowledge of	12.72	2.747	16.59	4.729	0.005*

risk factors					
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DISCUSSION:

Carcinogens, including tobacco use, alcohol drinking and viral infection, are the major risk factors of oral cancer development.¹⁴ As the latter is one of the main concerning issues that increase the mortality rate of individuals¹⁵, it is believed that the only way to prevent and early detect it is via raising the bar of professional involvement and public awareness. This concept would be performed through implementation of certain educated program and using various education materials; for example, posters, TV and social networks.

In our study, the majority of the respondents 77.1% have been aware or heard about oral cancer previously (Figure 1), which is higher than a study made in 2015 in Riyadh city that showed only 53.6% of their participants had heard of the cancer¹⁶, while similar studies conducted in USA, India and Sri Lanka illustrated that 84%, 91.2% and 95% of participants were aware of it; Tomar and Logan¹⁷, Agrawal², and Ariyawardana and Vithanaarachchi¹⁸, respectively. This high prevalence rates in these countries might be due to the usage of various educational materials that enable them to be aware about this disease. One of the possible argument to the present result is that the data was collected from a single, health care system in the country and may not be universally generalizable to other population-based settings beyond the current environment which in turns raises the potential for selection bias within the targeted population of the current study.

A considerable portion of the sample (38.3%) in this study received their information via mass media (Figure 2) which reflects the importance of mass media in educating people about oral cancer and raising the awareness of it. These results confirm findings published in other studies reporting that mass media is a common and an effective source of information regarding oral cancer.¹⁶ Moreover, A Malaysian study assessed the impact of promoting oral cancer awareness on the public using a mass media campaign; the results showed a significant increase in the public awareness regarding general knowledge and etiol-ogical factors of the disease.¹⁹

Identification of early signs and symptoms of oral cancer is an important paramount for early detection of the disease and its treatment. In the present study, unfortunately, the knowledge pertaining the clinical presentation of oral cancer was remarkably unsatisfactory, with only less than half of the

participants were aware of different clinical signs and symptoms of the cancer (growth of abnormal tissue:21.7%; non healing ulcer : 29.6% and continuous pain in jaw ;23.1%) (Figure 4).These findings are consistent with most of the published studies, which reported lack of this awareness.^{12, 19} Therefore, raising in awareness and educating the public on the early signs of the cancer is mandatory for early diagnosis and treatment, which in turn leads to good prognosis.

It is known that the main risk factors for oral cancer are alcohol, smokeless tobacco consumption and smoking.²⁰ Public knowledge of the risk factors is one of the most important parameters for successful prevention of oral cancer in the community. In this study, the rate of participants who were aware of these risk factors were low (smoking: 23.9%; alcohol consumption;25.9%: smokeless tobacco: 31.1%) (Figure 5). These percentages are indeed lower than those reported in Sudan¹³, Australia²¹, Iran¹¹, UK²² and Riyadh, Saudi Arabia¹⁶. Decline rates in our study can be attributed to the improper aware of patients concerning effect of these factors on their health. Therefore, these results would indicate to an expected increase in oral cancer cases. That is why raising awareness and educating the public is important for prevention of this disease.

Several socioeconomic factors may affect the public oral cancer knowledge and awareness. This study reported that high-educated participants were observed to have better knowledge of the cancer (Table 3). Our result was in same line with studies in Sri Lanka¹⁸, Riyadh¹⁶ and Jordan²³. This finding might be due to that their exposures to mass media such as internet and social networks are wide as well.

CONCLUSION AND RECOMMENDATION:

The present study concluded that most of dental patients lack of the awareness regarding oral cancer and they have inadequate knowledge of the cancer risk factors, signs and symptoms. For this reason, an intensive public education program for the detection of early warning signs, symptoms and etiologies of oral cancer is recommended. Furthermore, population should be encouraged to examine their oral mucosa individually and regularly visit the dental clinics. These interventions would be play an important role in early detection of oral cancer which in turns leads to better prognosis.

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Original article

The Effect of Training Programs on the Performance of Healthcare Workers at Benghazi Medical Center (BMC)

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ABSTRACT

Background: Healthcare workers (HCWs) are considered as one of the cornerstones and play an important role inside the healthcare sector. However, health care workers (HCWs) in developing countries frequently work in challenging environments, mainly dealing with sciences and technology development. Thus, attention to involve the HCWs into training programs to meet their needs and to achieve a better level of work performance and to provide better health services in their institutions.

Methods: A descriptive cross-sectional study, aimed to explore the effects of training programs on healthcare workers performance at Benghazi Medical Center (BMC) in Benghazi city, Libya. 51 questionnaires were collected from March 2019 and end in June 2019 by using the impact of training on workers performance. Data were fed to statistical software package version 20.0 SPSS. Approval of the Ethical Committee of faculty of public health was obtained.

Results: High percentage (72.5%) of the study respondents had undergone training programs with healthcare institutions. The results also show that the majority of these respondents (59.5%) participated in training on the purpose of performance consideration and 75% associate training to their enhanced performance at work.

Conclusion: It may be indicated that training to a big extent can lead to an improved worker's performance but still it is not the only factor that can enhance worker's performance rather it is a combination of factors. Thus, further researches can be done on different factors in the healthcare sector, specifically concerning workers' performance factors such as training needs assessment, training programs design, development, are significant to study for exploring their effects on the workers' performance in the healthcare sector.

Key words: *Healthcare workers, Training effects, Performance.*

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INTRODUCTION

Improved capabilities, knowledge and skills of the talented workforce proved to be a major source of competitive advantage in a global market.¹ A good training program ensures that an employee discovers his talents and organizational interest. When this happens, an employee aligns his goals to the company objectives and this helps in meeting the organizational goals through high performance on the job.² More impetus has been given to the requirements to maximize the

performance of employees, mainly in the public sector, which is usually identified to be characterized by low productivity and poor performance.³ Thus, training is needed to raise employee's performance. If employees are trained, they will be aware of their job specifications, skills necessary to do the job and be capable to use new technology.⁴ Employees play a crucial role in every organizational set-up. Achieving organizational goals cannot be done without human resource

(employees).⁵ Attention on the people and the meeting of their needs is important to achieve corporate objectives, training and development is a very important component of human resource administration and management.⁶ Organizations make it necessary to provide long term and systematic training and development programs for their employees. This is because every aspect and activity of an organization involves people.⁷ Employee Development refers to the capacity and capability building on an employee, and thus as of whole organization, to meet the standard performance level.⁸

There are different ways of defining the term training. Workers to do sufficiently on confirmed task or job may define it as an organized development of the knowledge, skills and behavior need.⁹ Training is also seen as a planned process to modify attitude, knowledge or skill behaviour through learning experience to achieve effective performance in an activity or range of activities.¹⁰ These are the reactive and proactive approaches of training: The reactive approach tries to identify and find out a solution to the current needs and problems of the organization. On the other hand, a proactive approach is a forward-looking approach in which training is conducted in anticipation of the future needs of the organization.¹¹

Several advantages can be achieved through training, including the enhancement of job satisfaction among employees, in addition to commitment and collective empowerment.¹² Training is essential in the work environment. Without it, workers don't have a stronghold on their responsibilities or duties.¹³ Training programs help in making the acquaintance of employees with more advance technology and attaining robust competencies and skills to handle the functions and basics of newly introduced technical equipment.¹⁴ The purpose of training is basically to bridge the gap between job requirements and the present competence of an employee. Training is aimed at improving the behaviour and performance of a person and also it is a never-ending or continuous process.¹⁵

Employees who are trained regularly are well motivated, well-mannered and have enhanced confidence and self-esteem.¹⁶ Training provides a positive result to employees of the organization in the sense that they have more activist attitude towards their work as they can be more efficient, work healthier with fewer errors and require less supervision.¹⁷ This study aimed to find out the effects of training on healthcare workers performance at Benghazi Medical Center (BMC) in Benghazi city, Libya.

MATERIALS AND METHODS

Study design:

The study employed a cross sectional survey research design, aimed to find out the effects of training on healthcare workers performance at a governmental hospital affiliated to the Ministry of

Health namely: Benghazi Medical Center (BMC) in Benghazi city, Libya.

Data Collection:

A questionnaire was used as a method of data collection at BMC.¹⁸ Data was collected from March 2019 and end in June 2019 by using the impact of training on employee's performance questionnaire. The questionnaire included questions about the socio-demographic data and about training programs he/ she received in the past and their impact on his/ her performance.

Sampling and Population:

The population includes the HCWs who have received training, who are working in Benghazi medical Centre for more than a year. Thus, population consists of (51) healthcare workers (HCWs). The HCWs were informed regard their participation rights. They were assured about their free will to share or to refuse to share without any concerns and confirming that all data are treated with confidentiality.

Data Analysis:

Data for this study was analyzed quantitatively by Statistical Package for Social Sciences (SPSS version 20.0) was used to analyze quantitative data. Descriptive statistics were done. Tables, graphs and charts were used in presentation of data to consent visual simplicity of presented data.

RESULTS

Based on the demographics characteristics of the respondents (Table 1), majority of the respondents are females (84%), showing that more females are working in the healthcare sector and more interested participating in the training programs in comparison to males (16%). In age category; 29 respondents are between the ages (26 to 35 years) which represent the majority (60%), 16 respondents between the ages of (36 to 45 years) represent (31.4%) and 4 respondents between the age of (18 to 25 years) which is approximately (8%) of respondents.

The respondents from the healthcare sector hold a variety of educational levels falling between intermediate level and degree level. About 22% had the educational level of the intermediate diploma, (64.7%) Bachelor level, (11.8%) had higher diploma and (2%) had a master degree. It refers that there was limited skill among the HCWs and hence training can be a substantial requirement in this sector to follow for the development with new technology and improve worker's skills, therefore, increasing their performance at work.

Table 1 Demographic Characteristics of the respondents (n=51)

Variables	F requency	ercent %	ean ± SD.	edian
Gender				
Male	8			
Female	4	6		
	3	4		
Age (Years)				
18 – 25	4			
26-35	2	.8		
36-45	9			
46-55	1	6.9	5.14	2.0
>55 Year	6		±	
	2	1.4	.29	
	-	.9		
Social Status				
Single	2			
Married	1	1.2		
Divorced	2			
Widowed	8	4.9		
	2	.9		
	-			
Academic Level				
Intermediate	1			
Diploma High Diploma	1	1.6		
Bachelor	6			

Postgraduate	3	1.8		
	3			
	1	4.7		
Work Experience (Years)				
≤ 5	6	1.8		
6 to ≤10	2		.25 ±	
11 to ≤15	7	2.9	5.96	.0
≥ 16	1			
	7	1.6		
		3.7		

The results in Figure 1 also show that respondents from nursing department formed the majority with 12 participants, representing (23%), 9 trainees from the control infection department, representing (18%), 6 from dermatology department, representing (12%), and Medical laboratory, vaccination department, representing (8%), while only 2 HCWs at the emergency

department, surgery department and Internal medicine department, representing 4%. The rest of the other departments representing 2% (n=1). These results illustrate that the organizational structures of healthcare sector comprising of many departments which suggests that various levels of training may be needed to develop worker performance

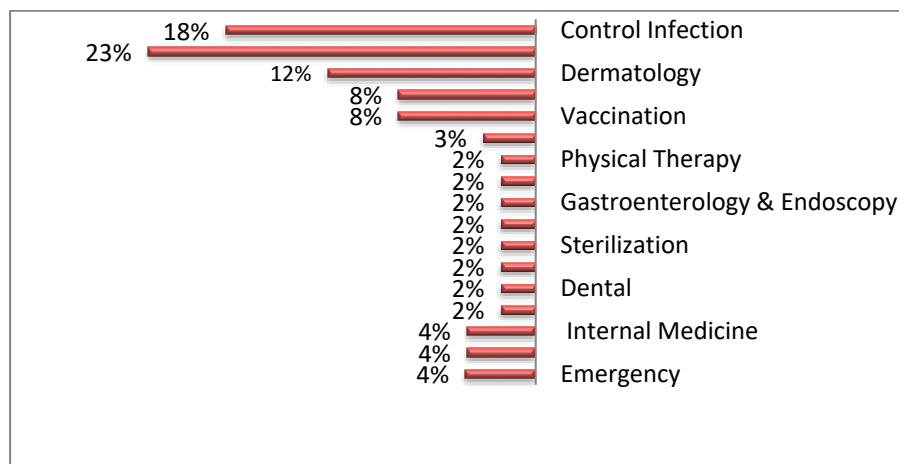


Figure 1: Distribution of the respondents according to Work Departments (n=51)

Table2 demonstrates that 37 respondents indicating 72.5% have experienced training with healthcare institutions.

The outcomes also demonstrate that the majority of respondents (59.5%) were selected to receive training on the purpose of performance consideration. While 56.8% of respondents informed that they participate in training on joining the company. Around 22% of HCWs stated their training selection according to their supervisors' recommendation in addition to compulsory for them.

The timetable of HCWs training as stated in the table above displays that the more than half of respondents (59.5%) were under no particular training timetable. It refers that they had no particular training timetable whereby they would need to link in training anytime as scheduled by the institution.

The most common approaches of facilitation recognized by the respondents as dominant during the training are lectures 30 represent (81.1%), while discussions represent (35.1%), presentations and demonstrations represent (27%). Seminars represent the remaining (10.8%). These outcomes can indicate that institutions strongly emphasis on lectures and discussions methods for their training programs.

Regarding effect of training methods on skill reveals that (73%) supposed that the training methods used affected their skills, and (35.1%) were satisfied with the quality of the training programs. (59.5%) from responded confirm on the relevance of training to respondents work. while the necessity for further training were also statistically highly percent was (58.6) it is for Improve performance.

**Table 2 Distribution of the
respondents according to HCWs
Performance (n=51)**

*More than one answer

HCWs Performance	Frequency	
Respondent's participation in training:		
No	14	7.5
Yes	37	2.5
Total	51	100
If Yes, * Selection for training: On joining the company	21	6.8
Supervisors recommendation	8	1.6
Compulsory for all HCWs	8	1.6
Upon employee request	4	0.8
Performance appraisal	22	9.5
Don't know	0	.0
Total	37	
Training schedule:		
Every 6 months	3	.1
Once a year	5	3.5
Every 2 years	7	8.9
No specific schedule	22	9.5
Total	37	100.0
* Methods of		

Based on the outcomes of Figure 2, HCWs trainees who involved in the present study (n=37) faced various challenges during their training programs. 30% of trainees complained from inappropriate timing. 13% reporting the training programs were on an irregular basis while 8%

indicated that there was a lack of efficiency in delivering knowledge. 3% reported there was the unavailability of capabilities. However, 43% of the trainees referred there were not any challenges regarding training program.

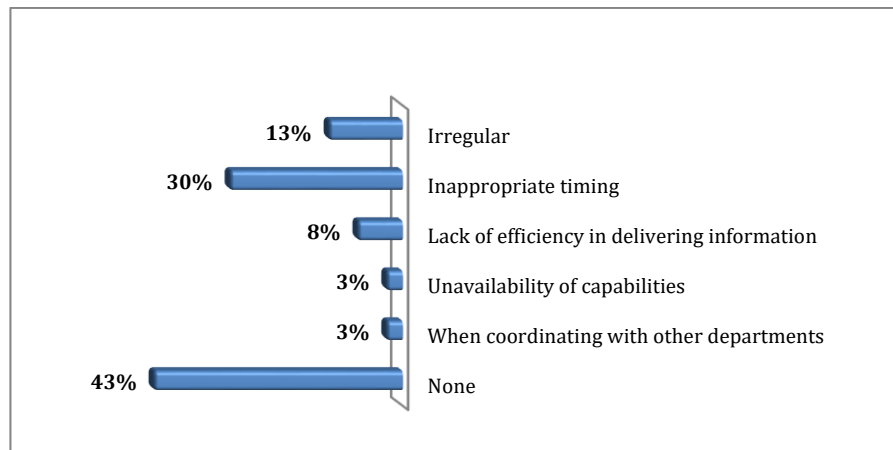


Figure 2: Challenges of Training Programs among respondent trainees (n=37)

As presented in Figure 3, the respondents HCWs reported their recommended methods to increase the level of future training programs. 39% suggested the importance of organizing a training schedule with their work time while 13% focused on practical training to improve HCWs skills and practices rather than focusing on increasing theoretical knowledge. 12% of the study respondents recommended increasing number of

training programs as well as coordination time table between different training courses Implementing in the same period. On the other hand, 4% of trainees preferred to completely discharge from their jobs during the training program and 2% asked for performing good behavior with trainees and motivate them.

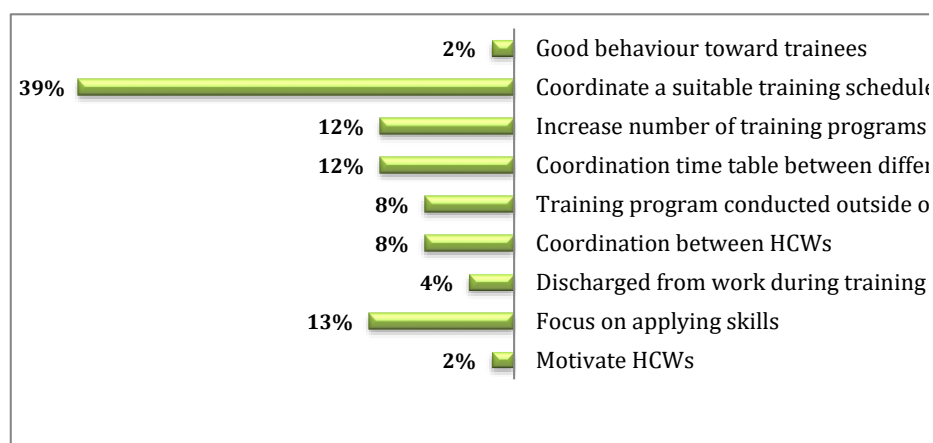


Figure 3: Recommended Methods to improve training programs

Table 3 Distribution of the respondents according to HCWs Performance (n=51) "Continue

Variables	Frequency	Percent %	Mean \pm SD.	Median
Gender				
Male	8	16	-	-
Female	43	84	-	-
Age (Years)				
18 – 25	4	7.8		
26-35	29	56.9		
36-45	16	31.4	35.14	32.0
46-55	2	3.9	\pm	
>55 Year	-	-	7.29	
Social Status				
Single	21	41.2		
Married	28	54.9		
Divorced	2	3.9	-	-
Widowed	-	-		
Academic Level				
Intermediate Diploma High	11	21.6		
Diploma Bachelor	6	11.8		
Postgraduate	33	64.7	-	-
	1	2		
Work Experience (Years)				
≤ 5	6	11.8		
6 to ≤ 10	27	52.9	9.25	
11 to ≤ 15	11	21.6	\pm	8.0
≥ 16	7	13.7	5.96	

**More than one answer*

DISCUSSION

This is a descriptive cross-sectional study on the Effect of Training Programs on the Performance of Healthcare Workers at Benghazi Medical Center (BMC). Based on the demographics characteristics of the respondents (Table 1), Only 2 respondents between (46 to 55 years) demonstrating approximately 4%. There are no respondents between the ages of (56 to 59 years). The data reveal that the healthcare sector employs mainly young workers. This similar to many evidence in the healthcare sector where the majority of the study population was females as well as the major work labor was in young age.¹⁹⁻²⁰⁻²¹ The data presented also in Table 1, show that More than half of the study respondents were married, representing approximately 55% whereas 41.2% were singles. Additionally, the mean of the study respondents was (9.25 \pm 5.96), ranging from 6 to up to 10 experience years and 11 to up to 15 experience years. On other hand, round 12% of respondents had experienced up to 5 years. They perhaps were graduated recently so they had limited knowledge of how to practice the job task. A study in Kenya assessed the level of practice among nurses after implementing training on (immunization pre-service training). It has been

established that the level of nurses' performance was very limited due to lack of experiences and practices as they were all recently graduated nurses, ²²⁻²³ have found similar outcomes after evaluating various healthcare facilities in developing countries among inexperienced staffs.

Respondent's participation in training (Table2), the remaining 14, representing a 27.5%, point out that they have not experienced any sort of training at work. This infers that the case institution does not provide training for all HCWs but probably for specific staffs. In contrast, the training may be directed for specific staffs from specific work levels. These finding agreed with any evidence that has high response rate among the study respondents toward undergoing training programs.¹⁹⁻²² The proportion of respondents who reported their requirements for training at work is approximately 11%. In contrast, a study was conducted to investigate the effects of such a training program (in immunization) on worker's performance of HCWs in the African area. The majority of training programs often recommended from supervisors and sometimes compulsory before conducting work. These training programs were implemented in term of improvement of the level of worker's performance, resulting in better health services about 19% of the respondents

reported their participation in training every two years, while 13.5% join in once a year, 8.1% every six months. Several studies argue that implementing specific training program based on the type of profession can significantly improve worker's performance.²⁴ Methods of facilitation at the training representing 35.1% is discussions, conversely, evidence in public health services in Kenya described that most of the HCWs depended on training programs in form of lectures (35% of the respondents) and seminar workshops (40% of the respondents) to be updated with the new knowledge regarding their jobs.²²

Regarding the effect of training methods on the skills (Table 3), the results existing that most of the respondents (73%) supposed that the training methods used affected their skills. But, 27% (n=10) believed otherwise. These findings agreed with some recent studies²⁴ this effect shows that the training methods conducted during training in the healthcare sector focus on certain skills so skill enhancement is emphasized. The findings also point out that most of the respondents (31 respondents) were satisfied with the quality of the training programs. This shows a clear release of training content to the trainees during the training program. In contrast, the 6 respondents indicated otherwise. More than half of the respondents (n=29) also stated the clear effectiveness of these training on their work.

A study conducted at first-level primary health centers in Bangladesh, more than 30% of the study respondents reported increases in work performance after participating training program. Using pre-and post-training tests to evaluate their knowledge. However, another evidence indicates the importance of conducting an assessment of the behavioural change of the HCWs after attending training programs. This assessment is critical in determining whether the training will have an impact on patient care and disease control indicators. At present, most evaluations of behavioural change have been conducted using qualitative research methods asking participants, for instance, whether they have applied newly acquired skills.¹⁹ It clear that post-training tests have limitations, as it can only assess whether trainees know about the guidelines or best practices, but cannot determine whether trainees have actually applied these strategies in their jobs.

Regarding the effect of training on the performance of the respondents HCWs, the outcomes clearly describe that the respondents signifying a high percentage of 75% associated training to their improved performance. In Nigeria, a study aimed to assess the level of knowledge, attitudes and practices among healthcare workers after participating training program, where 89% reported their higher improvement level.²¹ Investigated the training impacts on worker's performance, who reported partly improving in their knowledge and asking for further training on regular basis.

Study Limitations, Researchers views and Suggestions:

Small sample size because there were limited number of HCWs who have conducted training programs so there is a need to conduct further researches on the factors determining the implementation of training programs. In other hand, the researchers found that managers do not recognize regard the significance of training programs and its effect on employee performance because they believe that training raises the company cost. In an effort to correct this problem, management should decide that what these poor healthcare performers need is training. But if poor performance is caused by an actual lack of knowledge or skill, training will have little or no effect on the problem. Consequently, how do you recognize when training is the remedy? The researchers planned suggestions suitable for all companies to evaluate the employee performance in order to determine the accurate cause(s) of the performance problem and how best to correct it. These suggestions are:



- Evaluate the HCW's physical ability and health issues, to decide to what extent he/she can perform the job.
- Evaluate if the HCW's background knowledge and proficiency are compatible with tasks and duties.
- Evaluate how the HCWs recognize their jobs to understand their tasks and easy cooperate with their supervisors and aware the time work limits.
- Check if the HCW receives regular feedback on his/ her performance or not. So, if there is any incentive for good performance or punishment for bad performance.

CONCLUSION

Training programs can be considered as a motivational factor that can positively enhance the knowledge of the HCWs towards the job by which HCWs can become proficient in their jobs and they also become able to provide better services. Besides, training is seen as a useful means of handling with changes raised by technological innovation, organizational structuring and most notably it can play a key role to improve worker performance. However, still it is not the only factor that leads to good performance rather it is a combination of factors. A lot of future research can be done on different factors in the healthcare sector, but especially about workers' performance factors such as training needs assessment, training programs design, development is significant to study for exploring their effects on the resulting workers' performance in the healthcare sector. Thus, it is essential to examine any issues related to training and development in any healthcare sector. The role of managerial factors in workers' training and performance is also of unique importance and hence need to be also explored because a good manager either can increase or decrease the efficacy of training which in end affect workers' performance.

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Original article

**Prevalence of Impaired Dental Function Among Libyan Elderly Dental Patients:
Secondary Data Analysis**

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1. ABSTRACT

Background and Aim: This study was aimed to assess the prevalence of partial impairment of dental function among Libyan elderly population.

Methods: Secondary data analysis of previously collected data for a national survey of reasons of tooth extraction among Libyan adults. The data was extracted according to age of the participants (65 years of age or more). Having 20 or less functional teeth was considered as partial impairment of dental functions. Data was analyzed by SPSS version 25 to obtain frequencies and conduct bivariate analysis at $p \leq 0.05$.

Results: Of 165 elderly dental patients, the majority of them were males and from urban areas, 19% had 20 functioning teeth or less. The impaired dental function was higher among patients from rural areas and females ($p \leq 0.05$). Periodontal diseases and caries were the most common reasons for tooth loss in this group. Small number of participants had restorative treatment for tooth decay.

Conclusions: Partial impairment of dental function was observed in nearly the fifth of study group and appeared to be associated with social and gender differences. More efforts are required to understand this phenomenon and to offer dental care to those in need.

Keywords Tooth loss, Elderly, Libya, Secondary analysis.

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2. INTRODUCTION

The World Health Organization (WHO) predictions indicate that the global population is aging, and suggests that by the year 2050, the population aged 60 years or more will be twofold higher than the today's numbers.¹ Ageing, a foreseeable process, is usually measured by years and, there is an agreement that an individual aged 65 years or more is referred to as 'elderly'.² Ageing affects the integrity and function of human body, and

oral health is no exception. Several studies have found that severe tooth loss (edentulism) is associated with poor nutrition due to limited eating options, especially healthful fruits and vegetables and increased consumption of unhealthy processed foods rich in cholesterol and fats.³ Poor nutrition can lead to several systemic conditions and poor quality of life as an additional consequence of tooth loss. For example, individuals who lost their teeth can suffer from decreased self-esteem and overall well-being,

and socializing because they embarrassed to speak, smile, or eat in front of others.⁴

Periodontal disease and dental caries are the leading causes of tooth loss. However, causes such as trauma and prosthetic reasons have been reported.^{5,6} Risk factors for tooth loss include poor oral hygiene, tobacco use, dry mouth, gum disease (gingivitis) and some prescription medications. Tooth loss is preventable in most cases through proper oral hygiene, regular dental visits, avoiding tobacco and using products that help with dry mouth.⁷ Having 20 or 21 remaining teeth is regarded as functional dentition and has been set as global oral health goals.⁸ Many researchers have reported the importance of maintaining 20 or more teeth for oral health function, and this has been adopted by the WHO as a measure of functional dentition.^{9,10} In addition, there are health policies using this measure (having 20 or more teeth) as a health goal. For example, In Japan, having 20 or more natural teeth has been used as a goal of oral health policy since 1989, termed the "8020 (Eighty-Two) campaign".¹⁰

Previous studies in Libyan adults have demonstrated that extraction is quite common practice, with caries and periodontal diseases being the most common causes. However, as far as the authors concerned, no previous studies have attempted to investigate the impaired dental function in the adults and its related factors. Therefore, the present study aims to assess the prevalence of elderly dental patients who retain a minimum of 20 teeth after 65 years of age and its related factors.

3. MATERIAL AND METHODS

This study was based on a secondary analysis of data collected in a Multi-centre Cross-sectional study, conducted in six Libyan cities, covering the West, East and South of Libya. The data for the primary study was collected during the period between September 2016 and March 2017. The description of study design, data collection and sampling of the survey has been described elsewhere.¹¹ Permissions to access the primary raw data were obtained from the main author of the study.

The primary data included all dental patients aged 17 years or more who extracted their teeth in one of the dental clinics selected as study sites. A total of 2386 Libyan adults were recruited. Informed verbal consent was obtained from all participants. Data were collected by dentists in each research site who collected through clinical examination and interviews using especially designed form. The dental examination was done on dental chair unit used dental light, mouth mirror and dental probe. No other diagnostic aids such dental x-ray was used. The designed form contains information's on patient's demographic variables such as age; gender;

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education level; dental attendance pattern; occupation; place of birth; type of dental clinic; the type of tooth and reason for its extraction. The reasons for tooth extraction was categorized as following: caries, periodontal diseases, orthodontic treatment, prosthetic treatment, impaction, trauma, and other reasons.

Data of participants aged 65 years of age or more were extracted and analysed using SPSS software Version 25. Descriptive statistics were used to describe study sample characteristics and reasons of tooth extraction and prevalence of partial function impairment. Chi squared test was used to compare partial function impairment by participants' gender, and social class, and living area, at p value of 0.05.

4. RESULTS

A total of 178 elderly Libyan adults were included in the analysis. Table 1 shows the sociodemographic characteristics of the study subjects. The majority were males (70.2%), attended public dental clinics (73.6%). Only small proportion of the participants were from rural areas (9%) and attained university or higher educational level (16.9%).

Table 4: Socio-demographic characteristics of study sample (n=178)

<u>Socio-demographic characteristics</u>		Count	%
<i>Gender</i>	Male	125	70.2
	Female	53	29.8
<i>Clinic</i>	Public	131	73.6
	Private	47	26.4
<i>Education</i>	High school or less	146	82.0
	Higher than high school	30	16.9
<i>Residency</i>	Urban	162	91
	Rural	16	9

Figure 1 depicts the dentition status among the study subjects. Just less than the quarter had sound teeth (24.2%) whereas the majority had decayed (59%) or missing teeth (60.7%). The least common finding was the filled teeth which was observed in 15% of the participants. The most common reasons for tooth extraction among the study subjects were severe periodontitis (32.6%) and tooth decay (37.6%), followed by prosthetic reasons (23.6%). On the other hand, the least common reasons were trauma, impaction and failed endodontic treatment (1%) (Table 2).

Figure 1 shows the prevalence of Partial impaired function which was reported in 19 % of

study subjects. Table 3 presents comparison of PIF prevalence by study sample characteristics (gender, clinic types, education level and area of residency). Statistically significant differences were observed when PIF compared by gender and area of residence. Higher frequency of PIF was observed among females ($p=0.000$) and residents of rural areas ($p=0.001$).

Table 3: Comparison of partial impaired function (PIF)

by characteristics of study subjects			
Proportions of PIF in subgroups		Count (%)	P value
Gender	Male	15 (12.0)	0.000
	Female	19 (35.8)	
Clinic	Public	26 (19.8)	0.672
	Private	8 (17.0)	
Education	High school or less	28 (19.2)	0.917
	Higher than high school	6 (20.0)	
Residency	Urban	26(16)	0.001
	Rural	8 (50)	

Chi square test was used to compare subgroups at $p \leq 0.05$

Table 2: Reason of last tooth extraction among study sample (n=178)

Reasons for tooth extraction	Count	%
Restorable decayed tooth	6	3.3
Non-restorable decayed tooth	58	32.6
Severe periodontists	67	37.6
Trauma	1	0.6
Impaction	1	0.6
Prosthetic reasons	42	23.6
Pathology such as cystic lesion	2	1.1
failed restoration such as bridge or failed RCT	1	0.6

5.

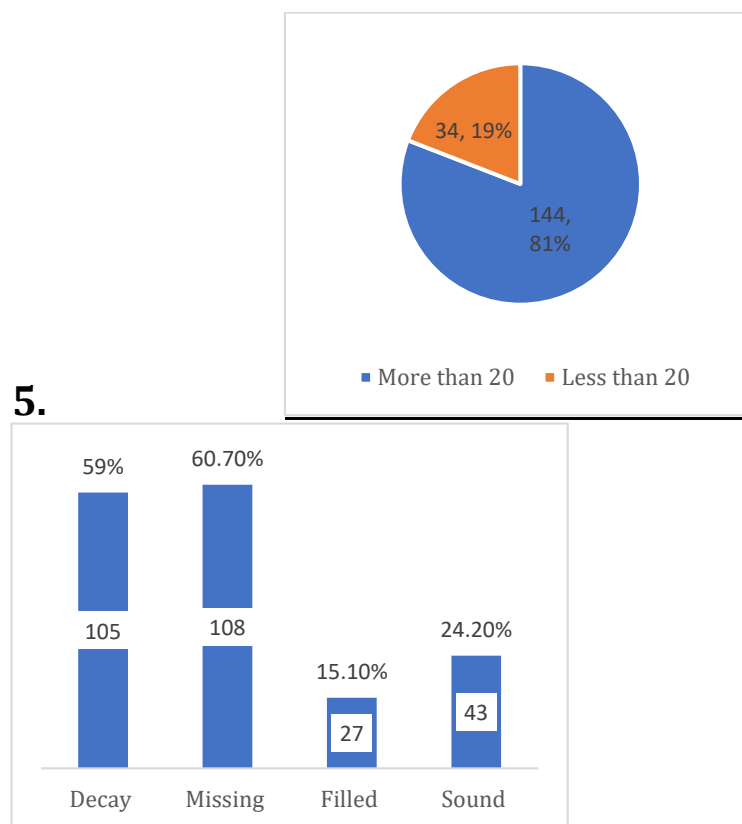


Figure 1: prevalence of Dentition status and Partial impaired function

6. DISCUSSION

To authors' best of knowledge, this is the first Libyan study to investigate partial impairment of dental function (having less than 20 functional teeth) among elderly. The study used secondary data analysis of previously collected data in a nation-wide survey of reasons of tooth extraction among Libyan adults. This approach saves time and efforts but also it has its own limitations such as no control over a chosen study population, variables of interest, and study design.¹² For example, the analysed data showed that the majority of participants were males, not educated and from urban areas which might be controlled if primary data was collected for the present study purpose.

In other words, representative proportions of these variable might be selected in a primary data was collected. We acknowledge this as a limitation of the present study and future research should consider these aspects. Nevertheless, the data used in this study appeared to fit well with the study aim and amenable to statistical analysis. Therefore, it can be claimed that the benefits of secondary data analysis outweigh the drawbacks in the present study. The prevalence of Partial impairment of functional dentation was reported in 19 % of study subjects. This means that considerable proportion of Libyan elderly keep their optimum functional dentition. Similar findings were observed in previous studies conducted in Brazil, Korea and Vietnam where the majority of adults had functional dentition.^{4,13,14} In line with the previous studies investigating the reasons of tooth loss among Libyan adults,^{11,15} The most common reasons for tooth extraction among the study subjects were severe periodontitis (32.6%) and tooth decay (37.6%), followed by prosthetic reasons (23.6%). This later appeared to be logic because of the increased demands of dental extraction in the elderly age group.¹⁶ This statement can be supported by present study finding that showed higher loss of function dentition among females.

Another interesting finding in this study was that patient from rural areas exhibited higher loss of

functional dentition, reflecting poorer dental services and utilization of care in these areas. The data shows that tooth loss and dental caries are very common among Libyan elderly with just less than the quarter had sound teeth (24.2%). On the other hand, a small proportion of the participants had filled (15%). These observations corroborate great deal previous findings among Libyan adults as well as children, and demonstrate highly unmet treatment needs,^{17,18} which can be attributed to low attention paid to oral health in a war-torn country. Previous research from other countries went through fiscal crisis, such as Greece, suggested that people tend to re-prioritize their demands and needs and what necessary or not.^{19,20} The financial crises in Libya has resulted in growth of the black market, weakened the Libyan dinar (LYD) and rapidly rising inflation.²¹ Consequently, the price of everything has increased up to 5-8 folds.²² Therefore, it is likely less educated males who forms the majority of the present study's participants prefer extraction over the expensive restorative treatment.

Further research using larger study sample and qualitative interviews is required to fully understand the reasons of loss of functional dentition, particular among females and rural areas. The present study although of small scale, it sheds light on the provision of dental care among Libyan elderly population and the need to provide appropriate care for this groups in order to maintain their oral and general health as well as ensure acceptable levels of quality of life.

CONCLUSIONS:

The present study suggests that nearly one fifth of Libyan elderly has lost their functional dentition and that there are regional and gender differences in the distribution of this condition. Further research is required to fully understand this phenomenon, and more efforts are needed to provide optimal dental care to this group.

Conflict of interests: none.

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Original article

Influence of Different Placement Techniques of Bulk-fill Resin Composite on Microleakage of Class II Cavity Preparation

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ABSTRACT

Aim: To evaluate the microleakage at the gingival seat of the class II cavity restored with bulk-fill resin composite using different placement techniques.

Materials and Methods: Two standardized class II cavities (MO and DO) were prepared in forty sound extracted human premolars. The cervical margin of the proximal box is located at 1 mm occlusal to the cemento-enamel junction (CEJ). The prepared teeth were divided into four groups of 10 teeth each (n=20 cavities) and restored with Tetric N-Bond total-etch adhesive, followed by resin composite, Tetric N-Ceram Bulk-fill placed in four different techniques: GpI; as bulk-fill in a single increment, GpII; horizontal layering, GpIII; oblique (wedge-shaped) layering, GpIV; vertical layering. All restored teeth were stored in distilled water for 24h at 37°C, thermocycled, and then soaked in 2% methylene blue dye for 24h. Teeth were then sectioned for microleakage evaluation using a stereomicroscope. Data were collected and statistically analyzed. Two specimens from each group were selected at random and examined under a scanning electron microscope (SEM) for marginal adaptation of restoration.

Results: No statistically significant differences in the microleakage score were observed between the four placement techniques ($P=0.610$). However, bulk placement had the highest mean and median score (3.0 ± 1.45 & 4). The horizontal, oblique, and vertical techniques had lower scores than bulk placement (2.30 ± 1.81 , 2.6 ± 1.66 , 2.45 ± 1.61) respectively.

Conclusions: Microleakage could not be eliminated by any of the tested placement techniques. Incremental placement techniques showed a lower score of microleakage compared with the bulk placement. The horizontal layering of bulk-fill composite showed the best results in terms of the marginal seal with tooth structure.

Keywords: Microleakage, Class II cavity, Bulk-fill composites, placement techniques.

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INTRODUCTION

Resin composites are widely used tooth-colored restorative materials in dentistry owing to their ability to replace the biological tissue in both appearance and function.¹ However, the suitability of these materials has been limited by its inherent polymerization shrinkage,² that compromises the integrity of the resin composite-tooth interface leading to gap formation between the cavity walls and the restoration.³ This implies a clinically undetectable passage of bacteria, fluids, molecules from the oral cavity to the tooth

structure known as microleakage causing secondary caries and failure of the restoration.^{4,5}

Microleakage is one of the most frequent happenstance problems in posterior composite restorations especially in class II cavity preparations at the gingival seat which create a great challenges to the dental surgeons. This is due to difficulties in restoration technique, curing process and continuous exposure to subclavicular fluid,⁶ particularly when the cervical margin of the preparation in the dentin.⁷ Microleakage could be the outcome of several clinical

factors that accompanied by increased polymerization shrinkage stress such as the high C-factor,⁸ light-curing scenarios,⁹ and variety of placement techniques of resin composites.¹⁰

To minimize the shrinkage stress, and consequently to decrease the microleakage with improvement in the marginal integrity and durability of resin composite restorations several approaches have been introduced such as; modification in material's formulations for properties optimization, the use of liner or base,⁴ and incremental layering technique for placement of resin composite.¹¹

Regarding modification in material formulations, a new category of resin-based composites (RBCs) called "Bulk-fill" composites have been introduced to dental practitioners.¹² These materials are claimed to offer a single increment placement ranging from 4-5 mm thickness instead of the conventional 2 mm increment.¹² This makes the material simple to use due to the decrease in the number of clinical steps, and quicker working time. In addition, literature reported that bulk-fill composites produce less shrinkage stress and cuspal flexure in standard class II cavities,¹³ and preparation of high C-factor design while maintaining a high degree of cure.¹⁰

The incremental layering technique has been accepted as the gold standard for the placement of resin composite restorations.¹⁴ This technique included packing the material incrementally into the cavity preparation, thus the contraction stress that occurred on one increment could be compensated by the next one.¹⁵ In addition, several studies reported that the direction of the composite increment placed in the cavity showed great influences on the shrinkage stresses and microleakage.^{4, 14, 15} The horizontal placement technique has been reported to increase the shrinkage stresses between the opposing cavity walls.⁴ The oblique (wedge-shaped) layering technique reduces the C-factor and limits the development of contraction forces between opposing walls and hence decreases the polymerization shrinkage stresses.^{16, 17} Vertical layering technique reduces the gap formed at the gingival margin, hence reduces postoperative sensitivity and secondary caries.¹⁸ Some studies found no influence of placement techniques of composite resin on microleakage.^{19, 20} Whereas other investigators found that the diagonal/oblique layering technique had the most leak-free margins when the proximal box ended on

enamel.²¹ Other investigators reported better results with the vertical layering technique compared to oblique layering.²² On the other hand not much information is available on the effect of using various incremental placement techniques of bulk-fill composites on microleakage. Therefore, this *in vitro* study was conducted aimed at evaluating the marginal microleakage at the gingival seat of class II cavity prepared at 1mm occlusal to the cemento-enamel junction (CEJ) restored with Bulk-fill composite resin packed in the cavity preparations using four different placement techniques.

MATERIALS AND METHODS:

The materials used in this study were one commercial bulk-fill resin composite; a Tetric N-Ceram bulk-fill, and one universal bonding system; Tetric N-Bond (Ivoclar Vivadent) with total etching technique as shown in Table 1.

Specimen preparation:

A total of 40 sound premolars teeth with neither carious lesion nor restoration, recently extracted for orthodontic reasons from several dental clinics were collected and used in this study. The extracted teeth were examined by an illuminated multi-powerhead magnifier to ensure that they were free of any defects. Then teeth were cleaned with an ultrasonic scaler and immersed in normal saline (0.9 % isotonic saline) till the time of use which was no longer than one month. The normal saline was changed every 3 days.²³

The teeth were mounted vertically to a level of 2 mm below the cement enamel junction in readymade plastic containers used for ice quips of 2.5 cm height and 3cm diameter filled with fast setting dental stone. Using a number #245 fissure bur under air-water cooling high-speed handpiece, two standardized class II cavities (MO and DO) were prepared on mesial and distal aspects of each tooth. The cavity dimensions were 4.0 mm bucco-lingual, 2.0 mm axially, and a cervical margin located at 1mm occlusal to the cemento-enamel junction (CEJ) with no enamel bevels. A new bur was used after every five cavities preparations.²³ A total of eighty Class II cavities with parallel walls, rounded internal line angles, and cervical margins established at 1 mm above the CEJ were prepared.

Table 1: Details of the materials used in the study

Product Name (Manufacturer)	Composition	Manufacturer instructions for use
Tetric N-Ceram Bulk Fill (Ivoclar Vivadent)	Dimethacrylate (19-21% wt). Inorganic filler (75-77% wt.), ~ 0.04-3 mm size. barium glass, prepolymer, yttriumtrifluoride, and mixed oxide. Additives catalysts, stabilizers, and pigments (<1.0% weight).	Applied in increment of 4 mm to the cavity walls. Light-cure for 20s. Additional polymerization from the buccal and palatal aspects after removal of the metal matrix.
Tetric N- Bond (Ivoclar Vivadent)	Phosphoric acid acrylate, HEMA, Bis-GMA, urethane dimethacrylate, ethanol, film-forming agent, catalysts, and stabilizers.	Apply a thick layer of Tetric N-Bond on the enamel and dentin gently using a microbrush. Remove the excess material by a gentle stream of air. Light-cure for 10s.

Restorative procedure:

Universal Tofflemire retainer (AISI 420 German stainless steel) with a metal matrix band of 0.05 mm (No 1001/30, Kerr Hawe SA, Bioggio, Switzerland) was applied for all cavities. The walls of each cavity were acid-etched with 37% phosphoric acid for 15s, rinsed with water for 15s, and dried with absorbent points. Tetric N-Bond, a light-curing, nano-filled single-component, dental adhesive (Ivoclar Vivadent, Liechtenstein) was applied to all prepared cavities with microbrush according to manufacturers' instruction, and then light-cured for 10s using a light-emitting diode (LED) curing unit (Elipar S10; 3M ESPE, St. Paul, MN, USA). The prepared teeth were then divided into four equal groups of 10 teeth each with a total of 20 cavities (n=20) for each placement technique. A Tetric N-Ceram Bulk-Fill composite (Ivoclar Vivadent) was packed according to placement technique as following: **GpI** (control): A bulk-fill (single increment) technique. **GpII**: Horizontal layering technique in three increments of less than 2 mm thickness each, starting from the gingival seat of the preparation towards the occlusal surface. **GpIII**: Oblique layering (wedge-shaped) technique in three increments in an oblique manner from wall to floor of the cavity. **GpIV**: Vertical layering technique in three layers packed vertically started from one wall (buccal) to the other wall (lingual) of the cavity.

Each increment was photo-activated with LED light-curing unit for 40s from the occlusal surface. Additional curing for 20s was performed on the proximal surface after removal of the matrix band and the retainer. The output of the light-curing unit was checked using a curing radiometer (Blue phase Meter II) to ensure a light intensity of at least 1000 mW/cm².

All the restorations were finished with a 30-fluted tungsten carbide finishing bur (Diatech Dental AC, Heerbrugg Switzerland) with water coolant to remove any excess material, particularly in the cervical region. This is

followed by polishing with rubber points in a low-speed hand-piece and Soflex polishing discs to promote a smooth surface. Restored teeth were then stored in distilled water at 37°C for 24 hours before testing to ensure a complete polymerization process.

Thermocycling:

The specimens were subjected to thermal cycling to simulate the oral environment. The number of cycles used was 5000 cycles equivalent to 6 months. Dwell times were 25 seconds in each water bath thermocycling machine (Robota automated thermal cycle; BILGE, Turkey) with a lag time of 10 seconds. The low-temperature point was 5°C, and the high-temperature point was 55°C.²⁴

Microleakage test:

The apex of each tooth was sealed by wax, and the remaining surfaces except for 1 mm around the restoration margins were covered with two layers of nail polish. The specimens were then immersed in a solution of 2% methylene blue dye (Supreme organization for drugs, Germany) for 24 hours at 37°C. Subsequently, the teeth were cleaned of the dye using brushes and rinsing with water, then were sectioned mesio-distally with a low-speed diamond disc under water spray at 1 mm above the gingival seat perpendicular to the long axis of the tooth.²⁵ The dye penetration along the cavity walls (including axial and gingival margins) was assessed under a stereomicroscope (Nikon Eclipse E600, Tokyo, Japan) at X35 magnification. The image of the restoration was captured and transferred to a computer equipped with an image analysis software program (Image J 1.43U, National Institute of Health, USA), where the microleakage was assessed. The depth of dye penetration was analyzed based on the graded scale used in previous studies.^{4, 19, 26} Score 0: No dye penetration, score 1: Dye penetration extending to 1/3rd of the cervical wall, score 2: Dye

penetration extending to 2/3rd of the cervical wall, score 3- Dye penetration into the whole of the cervical wall, and score 4: Dye penetration into the cervical wall and axial walls toward the pulp.

Marginal adaptation analysis using a scanning electron microscope (SEM):

For morphologic evaluation of the dentin/resin interfaces by SEM (JEOL, JSM-7610FPlus Field Emission SEM, Phillips, Holland) two teeth as representative specimens from each testing group were sectioned longitudinally perpendicular to the bonded surface using a low-speed diamond disc under copious water coolant. After surface polishing, teeth were immersed in 6 ml/liter hydrochloric acid (HCl) for 30 seconds to demineralize the minerals within the hybrid layer, then washed with water for one minute. The specimens were then immersed in 1% sodium hypochlorite (NaOCl) for 10 minutes to dissolve exposed collagen beneath the hybrid layer, and then thorough rinsing with water for 5 minutes. The specimens were dehydrated in ascending concentrations of alcohol, subjected to critical point drying, and then were gold-sputtered. The hybrid layer and the resin tags at dentin/resin interfaces were observed with SEM at a magnification of X1000.²⁷

Statistical analysis was carried out using a distribution test to evaluate the proportions of microleakage scores within each testing group and within the overall study specimens. Kruskal Wallis test was used to compare microleakage across different techniques of bulk-fill composite resin. Additionally, the Mann Whitney U test was used to compare microleakage score by tooth surface.

RESULTS:

Results of the microleakage test:

Results of the study demonstrated that microleakage occurs in the four different placement techniques. Figure 1 shows the distribution of microleakage among the study specimens (80 restorations). No microleakage (score 0) was observed in less than a quarter of study specimens; 19 restorations (23.8%). On the other hand, nearly half of the specimens; 36 restorations (45%) demonstrated score 4, where the dye infiltrated up to the whole length of the gingival wall and along the axial wall. 15 restorations (18.8%) showed a score 3 of microleakage. Scores 1 and 2 were minimum and representing as 2(2.5%), and 8(10%) respectively.

Results of the study showed that packing of the composite with bulk technique as a single increment (GpI) demonstrated the lowest level of no microleakage (score 0); 3 restorations (15%), and the highest level of

microleakage (score 4); 11 restorations (55%), followed by scores 3 (20%) and 2 (10%) (Figure 2). No microleakage was seen in 35% of the composite restorations placed with the horizontal layering technique (GpII). Score 4 was the most common pattern of microleakage in this group (40%) followed by scores 3 (20%), and score 2 (5%) respectively. Score 1 was not observed among this group (Figure 3). Oblique layering technique (GpIII) illustrated that 25% of the restoration showed no microleakage. Very similar to the former techniques, score 4 was the most prominent in this group and observed in 45% of the restorations, followed by score 3 (20%), and 2 (10%) respectively. Score 1 was not observed in this group (Figure 4).

Among the vertical layering technique (GpIV); 20% of the restorations exhibited no microleakage. However, similar to the former techniques, score 4 was the predominant type of microleakage; 40% of the restorations. On the other hand, unlike previous layering techniques, a score 1 was observed in 10% of restorations (Figure 5). To compare the proportions of no microleakage (score 0) in the four placement techniques; It can be observed that the horizontal layering technique demonstrated the least microleakage, whereas the bulk techniques showed the highest level of microleakage. Nevertheless, these differences were not statistically significant ($p=0.565$) (Figure 6).

Results of the Kruskal Wallis test (Table 2) revealed that there were no statistically significant differences in microleakage scores between the different placement techniques ($P=0.610$). However, the bulk technique had the highest mean and median for microleakage score (3.0 ± 1.451 & 4). The incremental placement techniques show better results than the bulk placement technique regarding the microleakage score. Although the horizontal, vertical, and oblique techniques have a similar median score, the horizontal technique has the lowest mean score (Table 2). Results of the Mann Whitney U test revealed that there were no statistically significant differences for the overall mesial and distal restorations.

Table 2: Comparison of microleakage scores by different placement techniques (n=80)						
Placement technique	Number of specimens	Mean score	SD	Median	Mode	P-value
Bulk (GpI)	20	3.00	1.451	4	4	0.610
Horizontal (GpII)	20	2.30	1.809	3	4	
Oblique (GpIII)	20	2.60	1.667	3	4	
Vertical (GpIV)	20	2.45	1.605	3	4	

Results of marginal adaptation analysis using a scanning electron microscope (SEM):

The SEM photomicrograph of the bonded resin/dentin interface of bulk placement specimens showed a thin hybrid layer with long and broken dentin resin tags arranged perpendicular to the interface with a continuous gap along with the interface (Figure 7-I). Figure 7-II illustrated the SEM image of the resin/dentin interface of the specimen that belonged to the oblique technique. The bonded interface showed a thin hybrid layer with many long with resin tags at the interface with a small continuous gap along the bonded interface. On the other hand, it was observed that when the composite was placed with the horizontal technique, a uniform, thick hybrid layer with numerous short resin tags penetrating inside the dentinal tubules was visible, provided a marginal continuity and good composite adaptation with no gap at the tooth restoration interface (Figure 7-III). The good bond was observed only with the horizontal placement technique. The SEM images of the vertical placement technique showed a non-uniform hybrid layer with short, and thin resin tags extend to a small distance of dentinal tubules with gap formation along with the interface (Figure 7-IV).

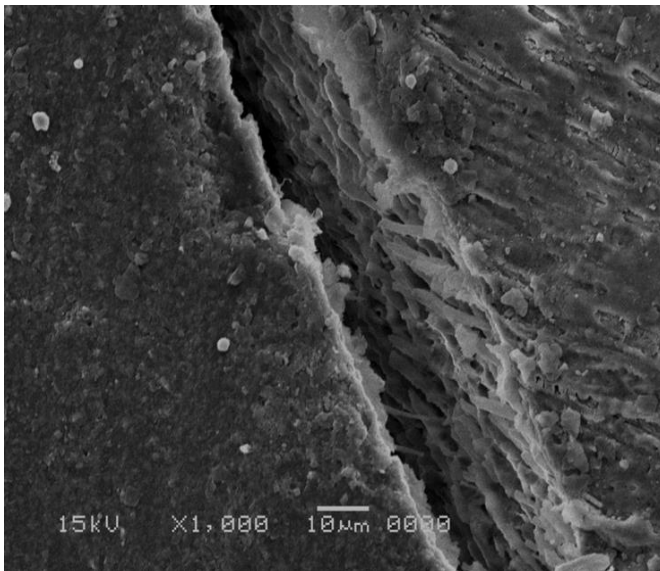


Figure 7-I: SEM photomicrograph of resin/dentin interface of bulk placement group of showing a thin hybrid layer, long and ruptured dentin resin tags arranged perpendicular to the interface with continuous gap along interface.

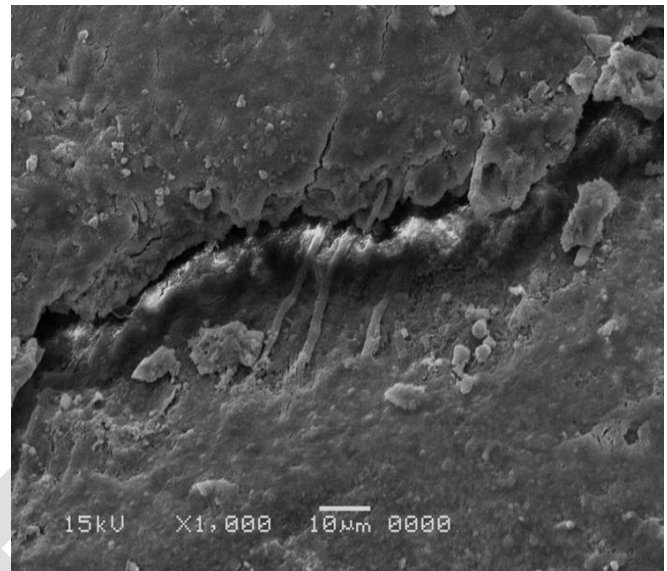


Figure 7-II: SEM photomicrograph of resin/dentin interface of oblique technique showing a thin hybrid layer with many long resin tags with small continuous gap along interface.



Figure 7-III: SEM photomicrograph of resin/dentin interface of horizontal placement technique showing hybrid layer with numerous short resin tags penetrating inside the dentinal tubules.

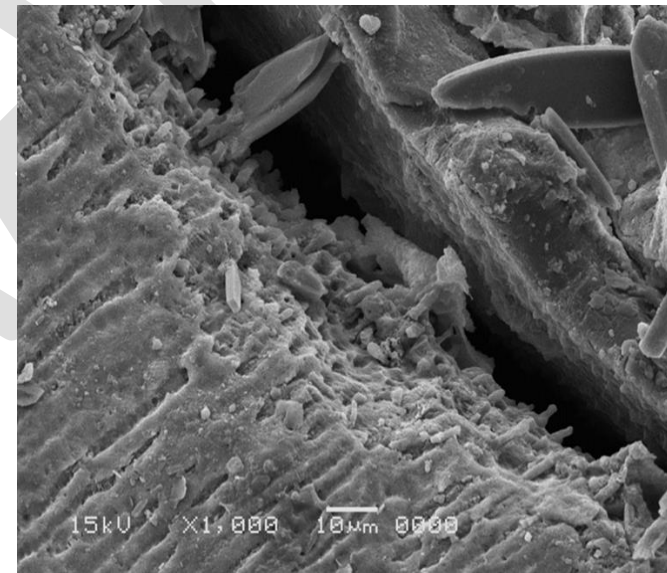


Figure 7-IV: SEM photomicrograph of resin/dentin interface of vertical placement, shows a non-uniform hybrid layer, with short, thin resin tags extend in to small distance of dentinal tubules with gap formation along the interface.

Figure 7I-7IV: SEM photomicrograph of resin/dentin interface of specimen's using four different placement techniques.

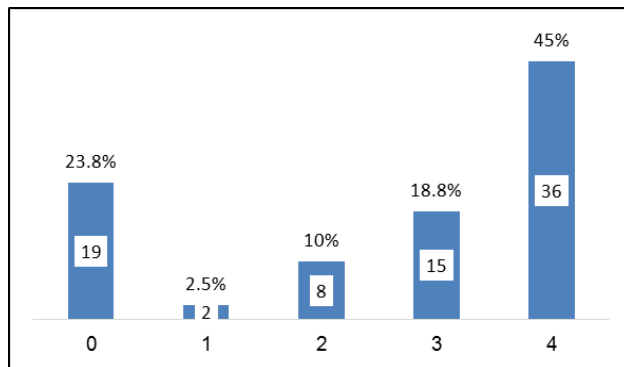


Figure 1: Microleakage scores (0-4) and numbers of composite restorations among the study

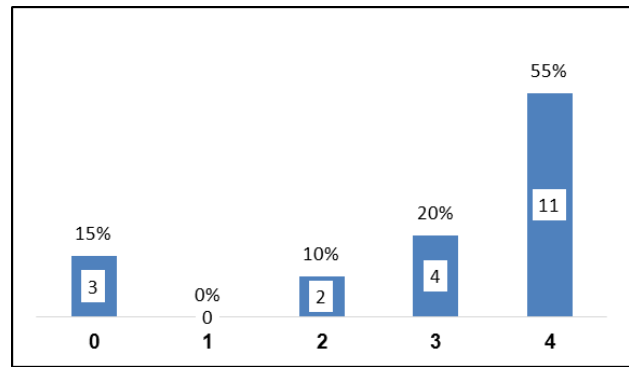


Figure 2: Microleakage scores (0-4) of composite restorations using a bulk technique

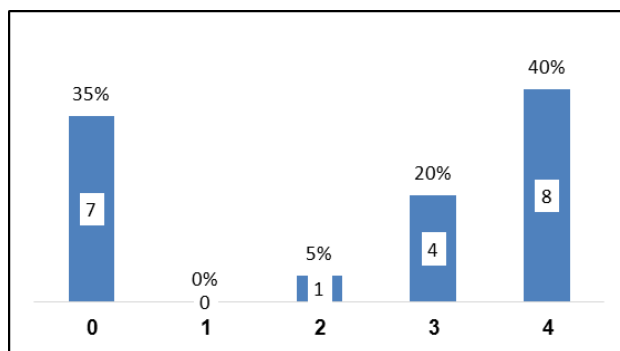


Figure 3: Microleakage scores (0-4) of composite restorations using horizontal layering

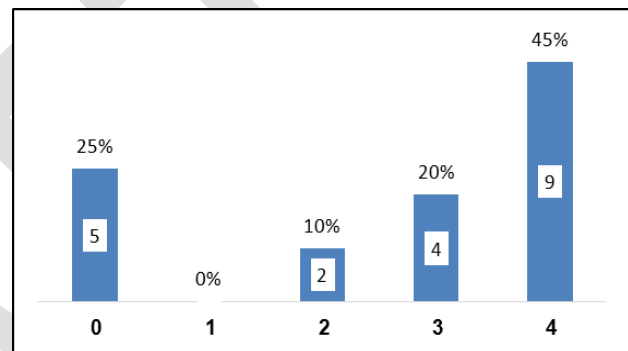


Figure 4: Microleakage score (0-4) of composite restorations using oblique layering

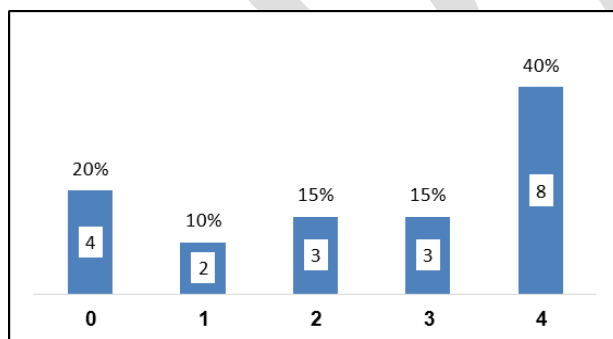


Figure 5: Microleakage scores (0-4) of composite restorations using vertical layering

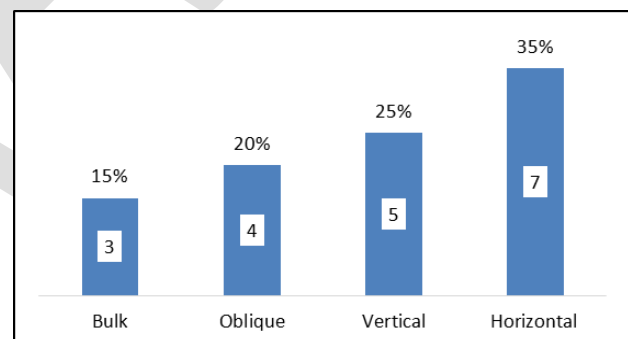


Figure 6: Distribution of no microleakage, score zero in bulk-fill composite restorations in different techniques (n=80) using Fisher exact test for

Figures1-6: Microleakage scores of composite restorations using different placement techniques.

DISCUSSION:

This in vitro study was performed to assess the microleakage of class II cavity restored with bulk-fill composite resin material packed in the cavity preparations using four placement techniques. The outcomes of the study showed that microleakage is quite common in different types of composite placement techniques. These findings could be attributed to the polymerization shrinkage which is considered an inherent property responsible for the main shortcoming of composite resin material.²⁸ In addition, polymerization shrinkage increases in a cavity with high a C-factor as in the case of class II cavities. These results could be also related to the restorative procedures and curing protocol.²⁹ Literature reported that the differences in the volumetric changes between the resin and the tooth structure during temperature changes induced microleakage and gap formation.³⁰

Moreover, the manufacturer claimed that Tetric N-Ceram bulk-fill composite has a high viscosity, with a nanofiller content of 79-81% wt. producing a volumetric shrinkage of 1.74% and shrinkage stress of 1.1 MPa.³¹ Others added that the viscosity of the bulk-fill restorative material influenced the proportion of gap-free marginal interface and the internal adaptation in dentin.³²

The obtainable results of the distribution of microleakage scores within each group denoted that, the highest percentage of specimens belonged to score 4, while the lowest one was recorded for score 2 among the entire testing groups. The absence of score 1 in some groups could be ascribed to the fracture of the undermined enamel at the unbeveled gingival margin during specimens preparation and sectioning. Or it could be due to the propagation of minute cracks in dental enamel during thermocycling owing to repeated hot and cold cycles which allow dye penetration to dentin.³³

Statistical analysis (Table 2) revealed that there were no statistically significant differences in microleakage scores between the different placement techniques ($P=0.610$), although incremental placement techniques showed less microleakage than bulk placement technique. The explanation for these results could be attributed to the fact that when composite was placed inside the cavity in a single increment, the material contacted four walls at a time leaving only two free unbounded surfaces. In such a case, the C-factor is high,³⁴ and therefore possibility of gap formation and adhesive bond failure.³⁵ Another possible explanation could be related to ineffective or inadequate curing at the deeper layer of the composite restoration.³⁶ Our findings were in line with previous studies that demonstrated that the placement of a large increment of bulk-fill resin composite into a

cavity increased the potential of creating high shrinkage stress and induced more strain.^{35, 37} This is because the buccal and lingual walls are pulled together which might cause adhesive failure and enhanced microleakage.^{35, 37}

Our results coincided with Özel and Syman, 2009,³⁸ Giachetti et al., 2006,¹⁴ who suggested that using layering or increment techniques in Class II cavities reduces the adverse effects of polymerization shrinkage and marginal gap formation comparing to the bulk technique. The authors reported that their results were attributed to the use of a small volume of material with the incremental technique which reduces the ratio of bonded to unbonded surfaces, thus decreases the C-factor and stresses on the composite restoration. Thus provides minimal contact with the opposing cavity walls during polymerization.^{14, 38} On the contrary other investigators found no significant difference between bulk and incremental techniques when evaluating microleakage of class II composite restorations.³⁹ Cecelia and Aranha in 2004 added that Tetric Ceram composite placed in bulk technique did not differ from the incremental technique, and had significantly less microleakage than Surefil composite either in bulk or in increment techniques.⁴⁰

The horizontal placement technique (Gp II) of the bulk-fill composite showed the least microleakage among all testing groups (Figures 3). According to Welime, 2014 the horizontal placement technique was ranked as the easiest to use clinically amongst the incremental placement techniques.⁴¹ Other researchers added that this technique is an acceptable method for resin composite insertion at enamel margins since standardized layers of equivalent volume allow superior control of the polymerization shrinkage levels.³⁵ In the same context, others added that the location of the gingival wall at enamel margins improves bonding since enamel is a better substrate for bonding especially with a selective total-etch adhesive system that creates microporosities, allowing penetration of the adhesive, thus forming a micromechanical bonding with the resin composite restoration.⁴²

Our findings were in agreement with Frankenberg et al., 2007 who found that the horizontal layering technique had the best marginal and bond qualities compared to the vertical and oblique layering techniques.¹⁷ Yumei et al, 2009 reported that the shrinkage of a single horizontal thin layer of composite generates remarkably less tensile force than the contraction of a bulk of composite that fills the whole cavity.⁴³

Perhaps the most important contribution of horizontal incremental technique would be an

adequate polymerization for bulk-fill composites and an adequate degree of conversion of the material in this thickness, as it was postulated by Campas et al., 2014 in their study.⁴⁴ In this regard, the result could be related to the benefits claimed by the manufacturer that higher translucency and light transmission properties of bulk-fill resin were enhanced, and modified by adding prepolymer shrinkage stress relievers, polymerization modulators chemically embedded in the center of polymerizable resin backbone, high-molecular-weight base monomer to optimize flexibility and network structure and highly light-reactive photoinitiator system, benzoyl germanium (Ivocerin) to enable rapid polymerization and greater curing depth.⁴⁴ On contrary to our result, several authors reported that using this technique for composite application leads to an increase in the C-factor, and thereupon increases the shrinkage stresses between the opposing cavity walls which lead to microleakage.^{45, 46}

The next best result of microleakage distribution was the oblique technique (GpIII) (Figure 4). Our results were in agreement with Lopes et al.,⁴⁷ and Loguerio et al.,⁴⁸ who documented that the combined simultaneous different layers using the oblique placement technique may result in much more shrinkage stress and microleakage. In addition, Sillas and Jose, found that the oblique technique exhibited significant microleakage despite the reduction in C-factor, when investigated the marginal adaptation of class II adhesive restorations.⁴⁹ The vertical placement technique in the current study produced the highest microleakage proportion among the increment placement techniques. It has been reported that; to eliminate microleakage and a gap formation at the gingival wall in a restoration packed with vertical technique, the curing process should be started from outside or behind the corresponding wall in which the increment is packed.⁵⁰

The results of the current study revealed that there were no statistically significant differences in the overall mesial and distal restorations. It is believed that the location of the preparation on either mesial or distal aspects of the tooth should not significantly affect the results because all cavities on both sides were prepared similarly and restored with the same type of composite resin. This result may reflect the standardization which was followed in each step during specimens' preparation and testing.

A scanning electron microscope (SEM) was chosen to be used for marginal adaptation analysis in the present study. The quality of the marginal seal at the gingival margins of restorations was evaluated according to Sabatini et al., 2010 who categorized the gap criteria into two categories;⁵¹ 1. No gap: This

means the margin appears with smooth and uninterrupted tooth-restoration continuity. 2. Presence of gap: means a distinct gap exists at the tooth restoration margin. Assessment of the SEM images of the selected specimens showed that only the horizontal layering technique of the bulk-fill composite demonstrated good quality hybrid layer with a marginal continuity and good adaptation with no gap at the tooth restoration interphase. This result could be possibly contributed to the adequate polymerization and the sufficient degree of conversion of the bulk-fill composite increments (Figure 7-III). Evaluation of the SEM images of the other placement techniques revealed that there is inadequate bonding to tooth structures probably due to contraction forces resulting from polymerization shrinkage that might give rise to gap formation at cavity wall and restorative material interface. layer

Marginal integrity of resin composite restoration might be affected by various factors including the cavity size, the angle at which enamel prisms and dentinal tubules are cut based on their location, the procedure in which dental hard tissues are conditioned, the layering protocol, and the polymerization technique used.⁵² Therefore in the present study it appears that differences in the placement techniques were responsible for differences in gap formations.⁵³

CONCLUSIONS:

Within the limitations of this in vitro study and based on the results, it can be concluded that: microleakage could not be eliminated by any of the tested placement techniques despite the significant advances in composite materials and dentin bonding systems. Incremental placement techniques showed a lower score of microleakage compared with the bulk placement technique. The horizontal layering of bulk-fill composite showed the best results in terms of the marginal seal with tooth structure.

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DRAFT



Original article

Primary Malignant Tumors of Oral and Maxillofacial Region in Libyan Population: A single institution experience of 171 cases

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ABSTRACT:

background: Globally, a significantly high rate of mortality was caused by oral cancer; however, epidemiological data of oral cancer is still scanty, particularly in Arab countries. This retrospective study was designed to provide a systematic analysis of the histological subtypes of oral and maxillofacial malignant neoplasms in Libyan population during two decades, and to compare the results with previous reports from Libya and other countries.

Method: data were gathered using patients medical record and biopsy files, and the histological slides were reevaluated. The tumors were classified according WHO criteria (2017).

Results: Carcinoma was found in 145 patients, forming (84.7%) of all the malignant oral and maxillofacial neoplasms, followed by lymphoma and sarcoma (6.4%), and neural crest cell tumours (2.3%). Males were more affected than females at a ratio of 1.3:1. Most of the patients (70.8%) were above the age of 40 with a mean age of 51.7 years old at the time of the diagnosis. Only 1% of the malignant neoplasms were odontogenic in origin. Squamous cell carcinoma (67.5%) was the most common epithelial neoplasm, followed by mucoepidermoid carcinoma (9.7%) and adenoid cystic carcinoma (4.1%). The tongue was the most common site for squamous cell carcinoma (41.1%), followed by buccal mucosa (16.8%).

Conclusion: compared to previous reports from eastern and some western countries; the prevalence of malignant neoplasms of oral and maxillofacial region in Libyan populations is considerably low.

Keywords:

Libyan population, malignant, oral and maxillofacial tumors, squamous cell carcinoma.

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INTRODUCTION:

Worldwide, more deaths were estimated to be caused by cancer compared to strokes and coronary heart diseases.¹ According to the GLOBOCAN (Global cancer incidence, mortality and prevalence) 2018, there are continuous global demographic and epidemiologic changes raising the expectations of

increasing cancer burden over the next decades, this estimate is as high as 20 million new cancer cases are expected to emerge by the year 2025.² The estimation presented by the GLOBOCAN 2018 was 18.1 million new cases, and 9.6 million deaths which is significantly high compared to 14.1 and 8.2 million respectively in

2012, and this impact will be more pronounced in countries of middle and low income.² Head and neck cancer represents the 6th-9th most common cancer, with squamous cell carcinoma (SCC) being the most common oral malignancy forming about 80-90% of all malignant neoplasms of oral cavity.³ The estimated annual mortality due to oral SCC is incredibly high (40-50%), this burden is expected to increase to almost two folds by 2030.⁴

To date, research about malignant neoplasms of orofacial region in Arab countries in general and Libya in particular is relatively lacking, and the epidemiological data is still scanty in this part of the world. One study was conducted during the period of (1991- 2007) reviewing all the cases of orofacial malignancy that have been diagnosed at faculty of dentistry in Benghazi-Libya, and revealed a lower incidence of oral cancer in Libya compared to other countries.⁵ The aim of this study is to investigate the relative frequency of primary malignant tumors in oral and maxillofacial region in the population of Benghazi over the last two decades, and to determine the most common malignant neoplasm during the study period. The results will be then compared to a previous study in Libya and other studies from some neighbouring Arab countries, in addition to parts of eastern and western countries.

METHODS:

The study was designed as a retrospective review of all the cases diagnosed with primary malignant neoplasms of orofacial region between the year 2000 and 2020 at the Department of Oral Medicine, Pathology, Diagnosis and Radiology- Faculty of Dentistry, Benghazi University. A total of 2036 patients with oral and maxillofacial malignant tumors were reviewed using their medical records and biopsy files; the gathered information included patient's age, gender and the specific site of the lesion. The histological slides were re-evaluated by the authors to confirm the diagnosis and to grade particular tumors according to the World Health Organization classification (WHO 2017).⁶ All the cases were analysed in relation to age, gender, site, and

histological subtype of the lesion. Exclusion criteria: Metastasized tumours, and those with controversial diagnosis were excluded.

For statistical analysis, all descriptive and quantitative data analysis and graphs were performed using the Statistical Package for the Social Sciences (SPSS) software, version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was used for the variables including: age, gender, and site of the lesion. Chi-square test was used to assess the significance, and the level of statistic difference was set at < 0.05.

RESULTS:

During the 20-years period, a total of 2036 specimens were histopathologically evaluated; of these, 171 (8%) specimens were diagnosed as malignant tumors. To provide comprehensive understanding; the malignant neoplasms were broadly classified according to their tissue of origin into three main categories: Carcinomas, sarcomas, lymphomas, and neural crest cell tumors. Carcinomas constituted the highest value of all the malignant lesions (84.7%), followed by lymphomas and sarcomas with an equal percentage (6.4%). Malignancies that have been histologically classified as neural crest cell tumors represent the smallest proportion of the study sample (2.3%). Gender based analysis revealed that males 57% were more affected than females 43%; with male to female ratio being 1.3:1.

Most of the patients diagnosed with primary malignant oral and maxillofacial neoplasms were of older age group (70.8% were above the age of 40), with mean age of the incidence being 51.7 years old. Only 6% of malignant tumors were found in patients below the age of 20, and about 2% were found in children aging less than eleven years. Patients with a histological diagnosis of carcinoma were older than those with sarcoma and lymphoma ($p < 0.05$). (Table 5) (Figure 1)

Squamous cell carcinoma (SCC) forms the bulk of the epithelial neoplasms (67.5%), followed by mucoepidermoid carcinoma (MEC) (9.7%), and adenoid cystic carcinoma (AdCC) (4.14%). About 99% of the tumors were non odontogenic.

Table (1) Malignant tumors according to age and sex 2000-2020

Diagnosis	All cases	%	Cases with known age	Age range (years)	Mean age	Age SD	No. of males	No. of females	M:F ratio
Carcinoma	145	84.7%	141	14-93	53.5	17.4	85	60	1.3:1
Sarcoma	11	6.4%	11	5-70	34.18	22.66	4	7	0.6:1
Lymphoma	11	6.4%	11	4-72	45.09	23.48	6	5	1.2:1
Neural crest	4	2.3%	4	30-85	58.33	27.53	2	2	1:1
Total	171	100					97	74	

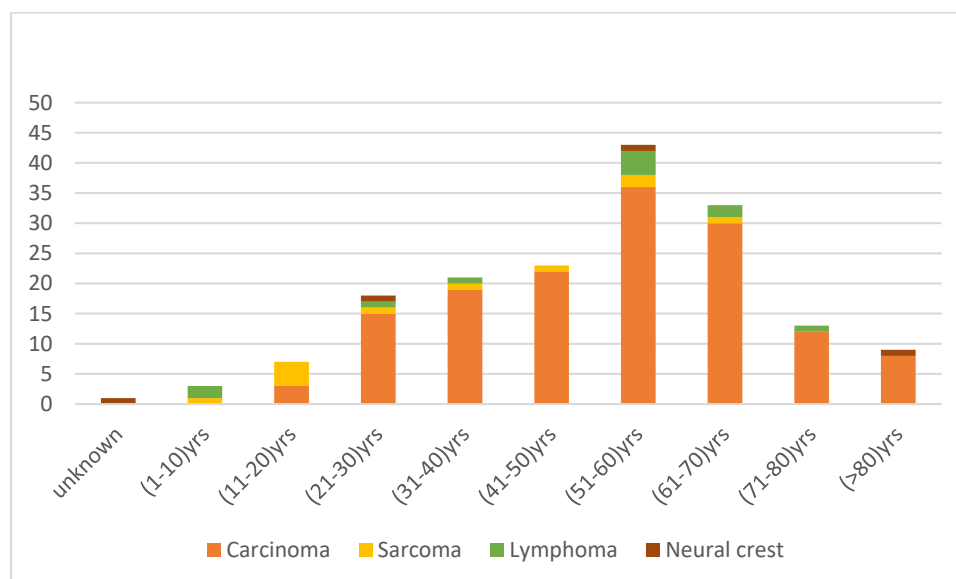


Figure (1) Age distribution of malignant oral and maxillofacial tumors

1. Carcinoma:

Carcinoma constituted about 7% (145 of 2036) of the total samples diagnosed during this period, and around 85% (145 of 171) of the malignant lesions. The patients ages ranged from 14-93 years (mean = 53.5 years), and most of them (74.9%) were aging 40 years or above. The peak age of incidence was during the sixth and the seventh decades of life. Males were slightly more affected than females at a ratio of (1.3:1).

Site related analysis revealed the following: Tongue (n = 41), (28.9%), cheek (n = 26) (18.3%) and palate (n= 18) (12.7%) were the most common sites.

Among the epithelial tumors, SCC (67.5%) was the most common neoplasm, forming about 57% of all the primary malignant tumors in the study sample, and around 5% of all the biopsied lesions during the study period. The mean age of the patients at presentation was 58 years and about 57% of them were males. (Figure 2)

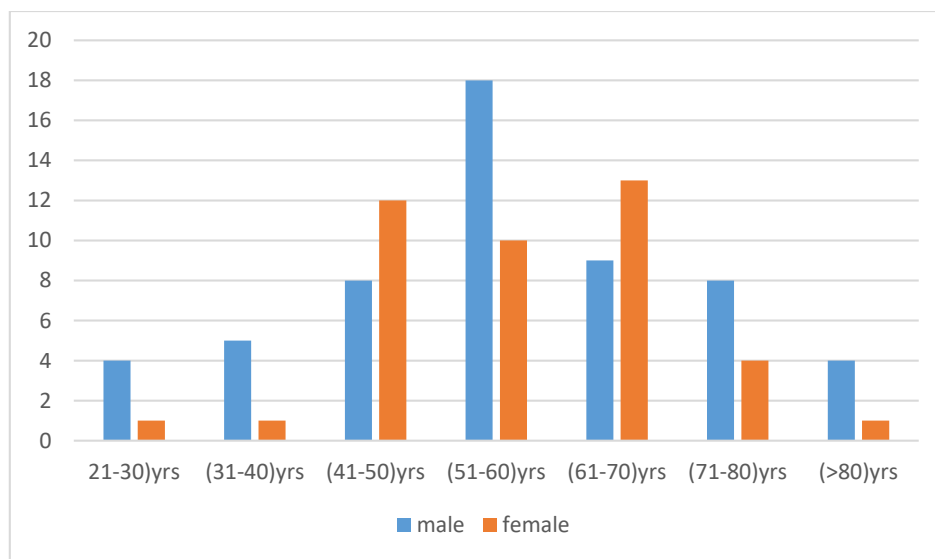


Figure (2) Age distribution of squamous cell carcinoma

The tongue was the most common site of SCC making about (41.1%), followed by the buccal mucosa (16.8%). Further analysis of the histological subtypes of SCC revealed that most of them were of a moderately differentiated type (43.6%). (Table6)

Salivary gland tumors constituted about 24% of the epithelial tumors, with the highest proportion being formed by MEC (40%), followed by AdCC (17%). The peak age of incidence was 31-40 years old. (Table 7)

Table (2) Site distribution of histological subtypes of squamous cell carcinoma

Anatomic Site	Well differentiated	Moderately differentiated	Poorly differentiated	Total (%)
Tongue	12	22	5	39 (41.1%)
Buccalmucosa	8	5	3	16 (16.8%)
Upper gingiva/ Alveolar ridge	3	4	4	11 (11.6%)
Lower gingiva/ Alveolar ridge	3	4	3	10 (10.5%)
Lower lip	3	4	1	8 (8.4%)
Palate	0	0	2	2 (2.1%)
Retromandibular area	1	2	0	3 (3.2%)
Floor of mouth	0	0	3	3 (3.2%)
Ventrum of tongue	0	1	0	1 (1.1%)
Upper lip	1	0	0	1 (1.1%)
Submandibular region	0	1	0	1 (1.1%)
Total	31	43	21	95

*The site of the lesion was unknown in three cases

Table (3) Age distribution of malignant salivary glands tumors:

Histological subtype/age	11-20	21-30	31-40	41-50	51-60	61-70	>80	Total (%)
MEC	0	4	7	0	1	2	0	14 (40%)
AdCC	0	0	2	1	0	2	1	6 (17.1%)
PLGA	0	2	1	0	2	0	0	5 (14.3%)
ACC	0	1	0	0	0	0	0	1 (2.9%)
CAexPA	1	2	1	0	0	1	0	5 (14.3%)
NOS	0	0	0	0	1	0	0	1 (2.9%)
Salivary duct Carcinoma	0	0	0	0	0	1	0	1 (2.9%)
PCAC	0	0	1	0	0	0	0	1 (2.9%)
Undifferentiated Adenocarcinoma	0	0	0	0	0	0	1	1 (2.9%)
Total	1	9	12	1	4	6	2	35 (100%)

PLGA: Polymorphous low grade adenocarcinoma, CAexPA: Carcinoma ex.pleomorphic adenoma, ACC: Acinic cell carcinoma, PCAC: Papillary cystic adenocarcinoma, NOS: Not otherwise specified, AC: Adenocarcinoma.

information regarding patient's age, gender, site of the lesion, as well as its histological sub-type.

Primary malignant tumors constituted 8.17% of all the biopsied lesions during the last two decades, which seems to be close to that reported in Benghazi by Subhashraj *et al* in 2009.⁵ Most of the patients were above the age of 40; and most of them were older than those diagnosed with sarcoma or lymphoma ($P < 0.05$). Males were more affected than females by a ratio of 1.3:1, which is in agreement with previous reports worldwide.⁷

Amongst all the collected records, SCC was the most prevalent malignant neoplasm forming about 57% of all the malignant tumors of oral and maxillofacial region, which is relatively higher than that recorded in 2009 (41%).⁵ In fact, this is an alarming indicator for the increased incidence of SCC among Libyan population, and further studies are required to investigate the reason beyond this trend. Similarly, 90% of the malignant oral lesions in Egypt were SCC; which is in agreement with the previous global reports on oral cancer.⁸ However, the percentage is much higher than that recorded in Libya, possibly due to the less frequently encountered risk factors; including smoking especially in women, consumption of spicy food as well as sun exposure,⁹ this is also the case in Iraq where 70 % of oral malignancy were SCC.¹⁰ In Jordan, the prevalence was also higher than that reported in our study (84%), and this could be explained by the wider use of Nargil or Shisha (water pipe) by both genders, beside cigarette smoking. In addition, about 17% of the patients in Jordan were alcohol drinkers¹¹ which is an unacceptable habit in Libya, both legally and socially. Similar percentage of SCC was reported in Yemen (84%), but with different associated risk factors; these were tobacco and Qat chewing which was recognized as a popular habit in Yemen,¹² and other eastern countries like Saudi Arabia.¹³

Males were more affected than female at a ratio of (1.3:1) which is consistent with previous reports.^{5,14} The reason beyond that could be the more likelihood of men to be exposed to carcinogens particularly smoking and alcohol drinking, which is considered more stigmatic in females, particularly in Arab populations. Even though, Yemen and Saudi Arabia showed greater percentage of oral SCC in women compared to Libya and other Arab countries. In fact, this was attributed to the popular habit of tobacco chewing (Shamma and Qat) which is socially accepted for females in these countries.¹³

Importantly, the reported prevalence of SCC in females in this study was reasonably higher (43%) than that recorded by Subhashraj and his colleagues in 2009 in Benghazi (37%),⁵ raising the question whether an underlying risk factor became more encountered in Libyan females over the last decade. This trend was also evident in the follow-up 2010

2. Lymphoma:

During the last two decades, about 0.54% (11 of 2036) of the biopsied lesions were diagnosed as lymphomas, and constituted 6.4% (11 of 171) of the total primary malignant neoplasms. Non-Hodgkin's lymphoma (54.6%) was more common than Hodgkin's lymphoma (45.5 %). Burkett's lymphoma was found in only two patients who were in their first decade of life, and accounted for 33% of all non-Hodgkin's lymphoma cases. About (36.4%) of the patients diagnosed with lymphomas presented with submandibular lymph node involvement.

3. Sarcoma:

Tumors of mesenchymal origin accounted for about 0.5% (11 of 2036) of the total biopsies done during the study period, and 6.4% (11 of 171) of the primary malignant neoplasms. The age of the patients ranged from 5 to 59 years, about 62.5% of the patients were below 20 years old, and most of them were in their second decade of life. Rhabdomyosarcoma and osteosarcoma were the most common, and each of them represented (36%) of the malignant mesenchymal lesions. The reminder was represented by three cases of different mesenchymal tumors; these are: chondrosarcoma, malignant fibrous histiocytoma, and granulocytic sarcoma. Each of them formed (9%) of all sarcoma cases.

4. Tumors of neural crest cells:

Neural crest cells tumors formed 2.3% of all malignant neoplasms of oral and maxillofacial region, and represented by four lesions; two of them were malignant melanomas affected two female patients aging 60 and 85, and two cases of olfactory neuroblastoma in two male patients aging 12 and 30.

DISCUSSION:

In light of the previously conducted study by Subhashraj *et al* in 2009 for the primary malignant orofacial tumors among the Libyan population over a period of 17 years from 1991 to 2007 at the Department of Oral and Maxillofacial Surgery-Benghazi University, which found that the incidence of oral malignancy was impressively low compared to other countries in Africa and some European countries, as this was considered the first report on primary malignant tumors of orofacial region in general and the incidence of oral cancer in particular, among the Libyan population.⁵ Therefore, we designed this retrospective review of all the cases diagnosed with primary malignant neoplasms of oral and maxillofacial region over the last two decades at the Department of Oral Medicine, Pathology, Diagnosis and Radiology, Faculty of Dentistry- Benghazi University to summarize and compare the prevalence of oral and maxillofacial malignant lesions in Benghazi, with detailed

Africa and the Middle East.^{25,26} The situation is different in some eastern countries like Yemen, Saudi Arabia, and India where buccal mucosa, vestibule, and gingiva were the most affected areas by SCC. In fact, this is attributed to cultural variation in use of tobacco, as these countries showed greater preference of tobacco and betel nut chewing.^{13,16,27} The second most common site in our study was the buccal mucosa (16.8%), while the retromolar area and the floor of the mouth are the least affected sites (3% and 1% respectively). These variations in certain anatomical sites among different countries could be explained by the different cultural behavior, geographical areas of particular countries, as well as the various genetic buildup of different ethnic groups.²⁸ Another factor which needs to be considered is that many patients seek treatment at advanced stages of the disease where it becomes difficult to identify the initial site of the lesion.

The most common histological subtype in our study was the moderately differentiated SCC, which might be associated with poor prognosis. However, other factors need to be considered; these include: size and site of the lesion, pattern of invasion, tumor margins, and lymph node involvement.¹²

Malignant odontogenic tumors are exceedingly rare lesions arising from the remnants of odontogenic tissues and odontogenic cysts in jaw bones.²⁹ In the current study, only two lesions were diagnosed as malignant odontogenic tumors, and both of them were malignant ameloblastoma. Malignant salivary gland tumors accounted for 21% of all the malignant lesions of oral and maxillofacial region, and 40% of them were mucoepidermoid carcinoma. This is almost consistent with most of the previous reports.^{5, 30, 31}

Lymphomas are a heterogeneous group of malignant tumors of the hematopoietic system characterized by the aberrant proliferation of mature lymphoid cells or their precursors.³² In this study, lymphomas accounted for about 6.4% of all malignant lesions, which is close to that reported in 2009,⁵ but relatively higher than that reported from Jordan¹¹ and Iran.³³ Non-Hodgkin's lymphoma was more common (54.6%) than Hodgkin's lymphoma (45.5%), which is in agreement with earlier reports from different countries.^{11,33,34} Burkitt's lymphoma is a very aggressive non-Hodgkin's lymphoma associated with Epstein -Barr virus.³⁵ About 33% of non-Hodgkin's lymphomas in our study were confirmed to be Burkitt's lymphoma, and were found in two patients aging less than 10 years. Of all cases of lymphomas, Burkitt's accounted for only 18%, which is significantly less than that reported from other African countries like Nigeria.³²

Sarcomas are aggressive malignant tumors of mesenchymal tissue, they are less common than carcinomas and more frequently seen in younger age group.^{11,36} In our study, sarcomas form around 0.5%

Benghazi cancer update report (BCR) where the incidence of oral cancer was almost doubled in females.¹⁵ Apart from smoking, Libyan women might be exposed to other risk factors; these include: poor oral hygiene which is well known to be greatly related to oral cancer in the absence of smoking and/or alcohol drinking,¹⁶ chronic irritation from teeth or faulty restorations which is considered either as an initiating or a modulating factor in carcinogenesis.¹⁷ Although there is no documented data regarding the dietary habits among the Libyan population which is considered another risk factor for oral cancer; Libyan food is usually rich in fat and carbohydrates, with less vegetables and fruits. Green vegetables and fruits are considered as a rich source of beta-carotene and vitamin E which are proven to be strongly related to the prevention of carcinogenesis, and treatment of oral precancerous lesions.¹⁸ Another dietary risk factor that can be discussed to explain the increased prevalence of oral cancer among females is malnutrition and iron deficiency anemia. Anemia is a substantial indicator of cancer risk, particularly iron deficiency anemia which represents 50% of all the causes of anemia worldwide.¹⁹ However, in the absence of well documented data regarding the prevalence of iron deficiency anemia among Libyan women with oral cancer, it would be untimely to conceive its role as an etiological factor. Thus, research should be encouraged to conclude whether such relationship exists.

The role of human papilloma virus (HPV) in cancer pathogenesis has been an area of considerable research over the last years, and its relationship with oropharyngeal and SCC has been recently established although the exact underlying pathogenesis is still obscure.²⁰ Therefore, it would be important to consider HPV as a possible associated risk factor particularly in younger age group. If that could be proven, then vaccination would be an effective prophylactic measure. Furthermore, identification of viral particles in such patients indicates a more favorable prognosis compared to those with HPV negative lesions.^{20,21}

Most of the patients diagnosed with SCC were from older age group with 53.5 years old being the mean age at diagnosis, which is consistent with previous reports on oral cancer worldwide.^{22,23,24} However, recent studies demonstrated a significant change in this trend, where an increased incidence in oral cancer has been reported in younger age groups (< 35 years old) with relatively unclear associated risk factors.²⁵ Thus, HPV and changing dietary habits should be considered as possible etiological factors for oral cancer in young adults.

Site based analysis revealed that the tongue was the most common site of SCC (41.1%) which is consistent with previous reports from Libya,⁵ western countries,^{22,23} and some parts of North

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of all biopsied lesions and 6.4% of all malignant lesions. Most of the patients were below the age of 20, with almost no sex predilection. The most common sarcomas were rhabdomyosarcoma, and osteosarcoma with an equal percentage of (36%), which is in conformity with previous reports from Iran and Nigeria and.^{36,37} The other mesenchymal tumors found in this study were: chondrosarcoma, fibrous histiocytoma, and granulocytic sarcoma. Each of them formed about 9% of all the mesenchymal tumors.

Tumors of neural crest cells represented the smallest component in our study sample (2.3%); as they presented as two cases of melanoma, and two olfactory neuroblastoma cases. Oral malignant melanoma is a very rare malignant neoplasm of melanocytes forming only 0.5% of all oral malignancies and < 1% of all other melanomas.³⁸ Olfactory neuroblastoma is an uncommon malignant neoplasm arising in the olfactory epithelium at the roof of the nasal cavity, and usually associated with distance metastasis and poor prognosis.³⁹

In this study, despite the effort we made to include all the malignant oral and maxillofacial lesions in Libyan populations of Benghazi, even those biopsied at private sector; some cases could be missed. This includes patients who were diagnosed in other countries or in private hospitals, or those whom biopsies were not sent for histopathological examination, in addition to undiagnosed patients with malignancy. However, the data in this study is less likely to be affected by the previously mentioned conditions since majority of the specialized health care service is provided for free by the Ministry of Health and most of Libyan people are educated with a literacy rate about 80%.⁵

In conclusion, compared to neighboring Arab and western countries, Libya has got a lower incidence of oral and maxillofacial malignancy, which is in confirmatory with the previous report in Benghazi.⁵ This encourages more research to investigate the inherent etiological factors for this variation, particularly behavioral factors which were evident among eastern countries. A part from smoking, the role of HPV and diet should be further investigated, especially in women, and young nonsmokers. As a considerable proportion of SCC cases in this study were of well differentiated grade, research should be encouraged to investigate the presence of HPV particles in such lesions. If this was the case, then vaccination against HPV would be a helpful prophylactic measure. Considering its association with risk factors, oral cancer is a potentially preventable disease. Therefore, public education and motivation could effectively contribute in the reduction of the incidence of oral cancer.

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