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Oral Cytological Changes Among Yemeni Qat Users

Abdulghani S. Shaalan*[1], Hussain G. Ahmed [2], Mohammed S. Abdelaziz [1]

*1- College of Medical Laboratory Sciences, Sudan University of Science and Technology.
2- College of Medical Laboratory Sciences, University of Khartoum

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ABSTRACT

This study aimed to detect cytological typical changes in buccal mucosa among qat users. A total of 599 individuals were investigated, of whom 499 were qat chewers (cases) and 100 were non-qat users (control). The cytological smears were obtained from buccal mucosa using wooden tongue depressor, and participants underwent the Papanicolaou stain for cytological changes and AgNOR staining for evaluation of the mean number of AgNOR dots per nucleus. A significant correlation between qat use and oral cytological changes was found among cases compared to controls. Cytological atypia were verified among 25(5%) of the cases compared to 1(1%) of controls, (P= 0.02). Hence, keratinization was identified in 143(28.7%) of the cases compared to 11(11%) of controls, (P= 0.000). Acute inflammatory cells was identified in 55(11%) of the cases compared to 4(4%) of controls, (P= 0.03). Viral infection (koilocytes) detected by cytology in 53(10.6%) of the cases compared to 4(4%) of controls, (p= 0.02). and confirmed by ELISA in 45 of cases and in 2 of control. Fungal infection was observed in 16(3.2%) of the cases compared to 1(1%) of controls, (p= 0.05). Also a significant relation between oral epithelial atypia and duration, intensity of qat chewing. Statistical analyses revealed a greater mean number of AgNORs per nucleus in (cases) qat chewers (3.24) compared to (1.36) in non qat users (controls) (P= 0.00). And the mean of AgNOR count increase with duration of qat chewing and this was found to be statistically significant (P= 0.002). This study concluded that the qat chewers a risk factor caused cytological changes in oral mucosa and this cytological changes particular oral epithelial atypia associated with duration and intensity of qat chewing. In addition to the mean AgNORs count is a useful indicator for cellular proliferation activity in oral epithelium and for prediction of the risk of exposure to certain carcinogenic elements that may induce oral cancer.

المستخلص

هدفت هذه الدراسة لكشف التغيرات الخلوية في الغشاء المخاطي للفم عند مستخدمين القات. وجرى تقييم 599 فرد, منهم 499 حالة (ماضغون للقات) و100 عينة ضابطة (غيرماضغون للقات). تم تحضيرمسحات من الغشاء المخاطي للفم باستخدام أعواد خافض أللسان وخضعت العينات لصبغة بابانيكولا لتقييم التغيرات الخلوية وصبغة AgNOR لتقييم متوسط عدد النقاط لكل نواة. وجدأن هنالك علاقة ذات دلالة إحصائية بين استخدام القات والتغيرات الخلوية للفم بين الحالات باالمقارنة مع الضوابط. وتم التحقق من خلايا لانمطية بين 25(5%)

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من الحالات بالمقارنة مع11(11%) من الضوابط (P= 0.02). وبالتالي تم تحديد التقرن في 143(28.7%) من الحالات بالمقارنة مع 11(11%) من الضوابط (P= 0.000). تم تحديد الخلايا الالتهابية الحادة في 55(11%) من الحالات بالمقارنة مع4(4%) من الضوابط (P= 0.03). ولوحظ عدوى فيروسية في 53 (10.6%) من الحالات بالمقارنة مع 4(4%) من الضوابط (P= 0.03). تم إجراء كشف تاكيدي لفيروس (HPV) باستخدام ELISA حيث شوهد (45) من الحالات بالمقارنة مع (2) من الضوابط. ولوحظت العدوى الفطرية في 16(3%) من الحالات بالمقارنة مع 1(1%) من الضوابط (p= 0.05). وكشفت ألتحليلات ألاحصائية أن متوسط عدد نقاط AgNOR لكل نواة عند مستخدمي القات (3.24%) باالمقارنه مع (1.36%) في العينات الضابطة بدلالة احصائية (P= 0.03). كذالك وجدت علاقة ذات دلالة إحصائية بين ظهور ألخلأيا ألانمطية لظهارة الفم وفترة، وكثافه مضغ القات. كما لوحظ أرتفاع في متوسط عدد نقاط AgNOR مع العينات التي وجد بها خلايا لانمطية, تقرن, عدوى فيروسية. خلصت هذه الدراسة الى أن استخدام القات هو عامل مؤثر للتغيرات الخلوية للفم مع وجود علاقة ذات دلالة إحصائية لارتباط الخلايا ألانمطية مع مدة وكثافة مضغ القات. بالإضافة إلى أن متوسط عدد نقاط AgNORs للنواة هو مؤشر مفيد لنشاط الانتشار الخلوي في ظهارة ألفم وللتنبؤ بخطر ألتعرض لبعض العناصر المسببة للسرطان التي ربما تسبب سرطان الفم.

***KEYWORDS*:** Qat, Atypia, Oral cytology.

INTRODUCTION

Oral cancer is one of the ten common cancers in the world. It accounts for 5% of all malignant tumors of which 60% are diagnosed at advanced stage. Most oral cancers are squamous cell carcinomas, and the vast majority of oral squamous cell carcinomas (OSCC) are preceded by precursor lesions that can be present as leukoplakia, erythroplakia, or erythroleukoplakia [1]. Earlier detection and follow-up of oral precancerous lesions are important to predict their potency to turn into malignancy [2]. Qat, is a herbal product consisting of leaves and shoots, has been used in Yemen for centuries and its usage is widely spread [3]. Qat is usually chewed at special social gathering, but it is also used frequently during work [4]. It was estimated that 61.1% of the adults in Yemen were current qat users [5]. High frequencies of oral cancers were reported from countries that have high rates of qat usage [6]. The high relative frequency of oral SCC may be related to the habits of chewing tobacco and qat[7] There are three main alkaloids present in qat leaves: cathinone, cathine, and ephedrine[8]. The potentiality of qat to induce cancerous changes has been investigated in vitro using human cell line [9]. Qat extract, cathinone, and cathine induced cell death by apoptosis, in addition to structural chromosomal changes, such as those seen in cells in the development of cancer [10] In addition; the use of qat has also been shown to produce a variety of oral mucosal changes, such as acanthosis, hyperkeratosis, and epithelial dysplasia [6]. The use of exfoliative cytology (EC) for diagnosis or screening of oral mucosal lesions has received a considerable attention [11, 12] and remain the simple and useful method in oral cancer screening programs in remote areas lacking advanced diagnostic facilities. Qat is the most danger killer in Yemen in all aspects as economical, social, and health. Qat spreading in large area in

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Yemen, the farmers used large agriculture toxicity which affect human health that lead to many diseases such as cancer. Therefore, the aim of this study was to assess direct effect of qat on baccul mucosa, with possible association with precancerous and cancerous changes.

MATERIALS and METHODS

Five hundred and ninety nine individuals were randomly selected for this study. Five hundred were cases and the 100 were control. The target groups were qat chewers (cases) and non qat chewers (control) volunteers living in the city of Amran, Yemen. All cases and control were apparently healthy. All the study subjects were males; the age was ranged from 20 to 45 years old with a mean age of 28 years. And the case study current qat user for at least 3 years with a frequency chewing of qat once or twice per day. Oral cancer patients, persons with bad oral hygiene, tobacco users, and alcohol consumers were excluded from the study. Each participant was asked to sign a written ethical consent before taking the specimen.

Sample collection

Smears were obtained from the buccal mucosa were using wooden tongue depressor, the contents were prepared on clean glass slides. All smears fixed immediately for 15 minutes in 95% ethyl alcohol. Blood samples were collected then serum was separated from cells by centrifugation for ELISA.

Sample Processing

Smears were stained using the Papanicolaou and grocott staining method for positive smear with fungi described by Bancroft [13], Silver nitrate method for NOR protein sites described by Paiva [14]. Enzyme linked immuno-sorbent assay (ELISA) were applied to IgM anti-HPV antibodies in the serum of human chewing qat. (anti HPVkits. ACON laboratory, inc. USA)

Cytological Assessment of the Results

The presence of two or more of the following features indicated the presence of epithelial atypia: nuclear enlargement associated with increased nuclear cytoplasmic ratio, hyper chromatism, chromatin clumping with moderately prominent nucleolation and irregular nuclear borders, bi or multi nucleation, increased keratinization and scantiness of the cytoplasm, and variations in size and/or shape of the cells and nuclei [15].

Data Analysis

Data was analyzed using statistic package for social sciences (SPSS) computer program. Mean was calculated, Chi- square and independent test was used (P value <0.05).

RESULTS

Five hundred and ninety nine individuals participated in this study, of whom 499 were cases and 100 were controls. The ages were ranged from 20 to 45 years old with a mean age of (28 ± 1.17) years. The study group consisted were males and the great majority of the participants were found at age range less than 25 years representing (41.1%) followed by age ranged, 26-30 years and 31-35 years, constituting (28.2%) and 79(32.2%), respectively, seen in Table (1).

Table 1: Distribution of cases and controls by age.

Age range	Number	Percent
< 25 years	246	41.1
26-30	196	32.7

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31-35	79	13.2
36-40	39	5.6
41+	39	5.6
Total	599	100

The distribution of the cases and the controls by cytological findings is shown in Table (2), cytological atypia (Dyskaryosis) was detected among (5%) of the cases and among (1%) of the control. Consequently, the relation between qat use and cytological atypia was statistically significant (P= 0.02). Hyperkeratosis was observed in (28.7%) of the cases compared to (11%) of the control (P= 0.000). Acute inflammatory cells (polymorph numerous) was detected in (11%) of the cases and in (4%) of the control (P= 0.03). The cytological evidences of viral infections (koilocytes) was detected among (10.6%) of the cases and (4%) of the controls (p= 0.02). Fungal infection was detected among (3.2%) of the cases and among (1%) of the controls (p= 0.05). The mean AgNOR dots counts was higher among the cases (3.24) than (1.36) among controls, and this was found to be statistically significant (P= 0.00).

Table 2: Distribution of the cases and controls by cytological atypia, inflammatory cells infiltrates infections and mean NORs count.

Variable	Cases		Controls		*P* value
	NO	%	NO	%	
Cytological Atypia (Dyskaryosis)	25	5%	1	1%	0.02
Keritinization	143	55%	11	11%	0.00
Acute Inflammatory cells	55	11%	4	4%	0.03
Viral infections (HPV)	53	10.6 %	4	4%	0.02
Fungal infections	16	3%	1	1%	0.05
Mean NOR count		3.24		1.36	0.00

Table (3) shows distribution of mean AgNOR count with oral epithelial atypia, keratinization and viral infection cells that observed in cases of qat chewing and this was found to be statistically significant (*P*= 0.00).

Table (4) shows the distribution of viral and fungal infection in case and control detected by cytological method (papanicolaou and grocott stain) and ELISA test, the HPV16 detect by cytological method in 53 of case and in 4 of control, hence, detect by ELISA test in 45 of case and in 2 of control, the cytological method might be detect other virus that act as HPV on the cells, the grocott stain special stain for fungal staining were 16 positve with papanicolaou in case compare to 14 by grocott stain .

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Table 3: Distribution of the study population by mean NOR count and pathology.

Variable		Mean AgNOR counts	Std	*P*.value
Atypia	Present	3.76	0.27	0.00
	Absent	3.25	0.29	
Kiratinization	Present	3.40	0.31	0.00
	Absent	3.22	0.29	
Acute inflammatory cells	Present	3.30	0.31	0.24
	Absent	3.27	0.31	
Viral infection	Present	3.47	0.33	0.00
	Absent	3.25	0.30	
Fungal infection	Present	3.41	0.38	0.06
	Absent	3.27	0.31	

Table 4: Distribution of the viral and fungal infection in case and control by different methods

Cytological (HPV+)		ELISA (HPV+)		Fungi detect by papanicolaou stain		Fungi detect by grocott stain	
Case	Control	Case	Control	Case	Control	Case	Control
53	4	45	2	16	1	14	1

According to the duration of qat chewing by years the participants were classified into four groups. The great majority of the participants were found at duration ranges 6-10 years constituting (50%) individuals followed by duration ranges, 11-20 and 1-5 years, constituting (30%) and (11%) respectively. Most of the cases with atypia, keratinization, acute inflammation, viral infection and fungal infection were found among those qat chewers for a duration range 11-20 years, constituting (11.5%), (30%), 17(11.5), (10%), (5%) of the cases respectively, followed by duration 6-10 years, constituting (3%), (30), (10%), (11.5%), (2%) cases respectively. The mean of NOR count increase with duration of qat chewing and this was found to be statistically significant (P= 0.002) as it appears in Table (5).

Table 5: Distribution of cases by duration of qat use and pathology.

Variable	1-5 years (N=57)	6-10 (N=251)	11-20 (N=148)	21+ (N=43)	*P*.value
Atypia	0(0%)	7(3%)	17(11.5 %)	1(2%)	0.00
Keratinization	12(21%)	76(30%)	44(30%)	11(26%)	0.52
Acute inflammatory cells	3(5%)	26(10%)	17(11.5 %)	9(21%)	0.09
Viral infection	8(14%)	29(12%)	15(10%)	1(2%)	0.25
Fungal infection	0(0%)	4(2%)	7(5%)	5(12%)	0.00
Mean NOR count	3.13±0. 25	3.28±0.3 0	3.32±0.3 4	325±0.2 6	0.02

The participants were divided into two gropes according to time of qat chewing, group one chewing qat from 3 to 5 hours per day, group two chewing qat from 6 to 9 hours per day. The cytological epithelial atypia was detected in (7.5%) of group two and in (3.3%) of group one, as indicated in Table (6).

Table 6: Description of the cases by time of exposure hours/day.

Variable	Tim chewing qat by hours/day		*P*. value
	3-5 hours/day (N=300)	6-9 hours/day (N=199)	
Atypia	10(3%)	15(7.5%)	0.030
Acute Inflammatory cells	34(11%)	21(11%)	0.453
Keratinization	86(29%)	57(29%)	0.539
Viral infection	27(9%)	26(13%)	0.098
Fungal infection	8(30%)	8(4%)	0.357
Mean NOR count	3.26 ±0.29	3.30 ±0.38	0.165

Table (7) shows the description of the cytological results according to the frequency of qat chewing per day. Accordingly, atypia was found in (12%) of those use qat twice a day and in (3.6%) of those use qat once a day.

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Table 7: Description of the cases by Frequency of qat replacement

Variable	Frequency chewing qat per day		*P*. value
	Once (N=416)	Twice (N=83)	
Atypia	15(4%)	10(12%)	0.004
Acute Inflammatory cells	48(12%)	7(8%)	0.270
Keratinization	124(30%)	19(23%)	0.126
Viral infection	43(10%)	10(12%)	0.383
Fungal infection	14(3.5%)	2(2.5%)	0.796
Mean NOR count	3.26 ±0.31	3.31 ±0.31	0.195

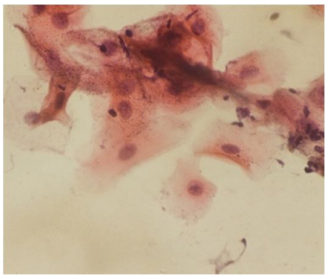
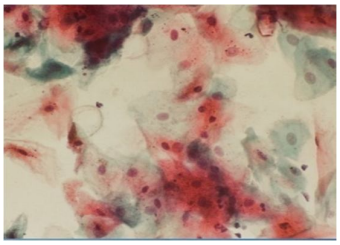
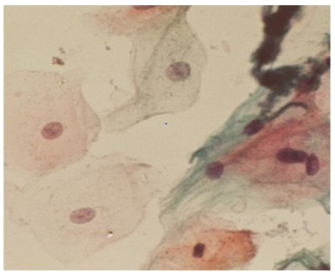


Figure 1: Buccal smears showing atypical celles papanicolau staining x40

Figure 2: The silver nuclear dots. AgNOR staining x40



Figur 3: Buccal smears showing the hyperkeratosis papanicolaou staining x40.



Figur 4: Buccal smears showing the viral infections papanicolaou staining x40

DISCUSSION

The study found a significant correlation between qat usage and oral cytological change when compared to control. In this study, the occurrence of cytological atypia was investigated in 25(5%) smears obtained from qat chewers (case) and in only one(1%) of smears obtained from non-qat chewers (control) and this was found to be statistically significant (P= 0.02). hyperkeratosis found in 143(28.7%) of the case and 4(4%) of the control groups the findings may further suggest that qat components might stimulate the epithelial cells to undergo atypical cellular morphological changes, which may lead to oral precancerous and eventual cancerous lesions as suggested by Ahmed, et al [16], who found atypical cytological changes among qat chewers were 6(4%), and 24 (16%) with hyperkeratosis, and none of the controls showed cytological atypia or hyperkeratosis. Study by Ali et al [17], who reported 22.4% of qat chewers, had oral keratosis, white lesions in the oral mucosa. By Ali et al [18] the use of qat has also been shown to produce a variety of oral mucosal changes, such as acanthosis, hyperkeratosis, and epithelial dysplasia. Also study by Lukandu [19], who found oral cavity, qat chewing has been associated with histopathological changes like hyperkeratosis, epithelial hyperplasia and mild dysplasia. Nevertheless, some studies reported the absence of association between qat use and occurrence of precancerous and cancerous lesions [20], However, these studies investigated the presence of oral cancerous and precancerous by clinical methods; hence, we applied cytological methods. Inflammatory cells infiltrate was identified in 55(11%) of the cases compared with 4(4%) of the control. We think that this variation evidenced the irritation role of qat to stimulate the inflammatory changes (polymorph numerous) such findings were previously studies by AL-Sanbani[21] who found oral white lesions due to qat chewing. By Al-sharabi[22] who revealed that histopathological changes found on oral mucosa included inflammatory cell infiltrate, acanthosis, intercellular edema, epithelial dysplasia and increased amount of collagen fibers. Oral fungal and viral infection was detecte by cytological method in 16(3%), 53(10.6%) of case respectively, associated with qat use due to qat leaves Contaminated with fungi was reported[23]. And the viral infection (HPV) was detect by ELISA test less than detected by papanicolaou stain Because overlap other virus might be detected by this method. AgNOR counts have been of great value in recognizing various benign and malignant lesions, to establish prognoses and to determine the proliferative activity of cells[24]. In this study the mean AgNOR dots counts was higher among cases (3.24) than among controls (1.36) and this was found to be statistically significant (P= 0.000). There is no previous study used AgNOR method to assess the oral mucosal cells when qat users. The mean of AgNOR dots counts have been increase with variable pathology such as (oral epithelial atypia, kiratinization and viral infections) and this was found to be statistically significant (P= 0.002), (P= 0.00), (P= 0.00) respectively. The result found relationships between duration, frequency of qat chewing and oral epithelial atypia was found in 17(11.5%) of those among the duration range 11-20 years, and this was found to be statistically significant (P= 0.000). These findings were similar to study by Al-sharabi [22], who found

white lesions on buccal and gingival at chewing sites were among Yemeni qat chewers who chewed for 3 years or more. Nevertheless, there is study reported no relationship between mucosal changes and duration or frequency of qat use [16]. This might be attributed to the fact that all cases were currently heavy users for more than 3 years; while participants in this study used qat for different periods less and more than 3 years. This study found a relation between epithelial atypia and intensity of qat chewing, and this was found to be statistically significant (P= 0.03). These findings agreed with Al-Sanabani [21]. who found that qat chewing habit caused buccal and gingival white lesion at site of chewing among Yemeni women who chew every day for 3-6 hours over a number of years.

CONCLUSIONS

Qat chewer is a risk factor caused cytological atypia, keratinization and inflammatory ells infiltrate in the oral mucosa, and these change are associated with duration and intensity of qat chewings. Cytology is a screening technique in evaluation of oral mucosal changes and precancerous lesions. ELISA is more sensitive than cytology in detection and confirmation of viral infection. The mean AgNORs count is a useful indicator for cellular proliferation activity in oral epithelium and for prediction of the risk of exposure to certain carcinogenic elements that may induce oral cancer.

REFRENCES

1- Mayne ST, Morse DE, Winn DM. Schottenfeld D, Fraumeni JF. (2006*). Cancers of the oral cavity and pharynx. Cancer epidemiology and prevention*. Oxford University Press. New York.

2- Johnson N.W, Ranasinghe A.W, Warnakulasuriya KAAS. (1993). Potential malignant lesions and conditions of the mouth and oropharynx: Natural History-cellular and molecular markers of risk. *Eur J Cancer Prev* **2:** 31-51.

3- Al-Hebshi N.N, Skaug N. (2005). Khat (Catha edulis) an updated review. *Addict Bio* **10:** 299–307.

4- Kalix P. (1996). Catha edulis, a plant that have amphetamine effects. *Pharm World Sci* **18:**69-73.

5- Ali A.A. (2007). Histopathologic changes in oral mucosa of Yemenis addicted to water-pipe and cigarette smoking in addition to takhzeen al qat. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103:**55-59.

6- Nasr A.H, Khatri M.L, (2005). Head and neck squamous cell carcinoma in Hajja, Yemen. *Saudi Medical Journal* **21**:565-568.

7- Sawair F.A, Al-Mutwakel A, Al-Eryani K. (2007). High relative frequency of oral squamous cell carcinoma in Yemen: Qat and tobacco chewing as its aetiological background. *Int J Environ Health Res* **17:**185-195.

8- Tonnes S.W, Harders S, Charann M, Niess C, Kauert G.F, (2004). Pharmacodnamics of cathinone and norephedrine after the chewing of Qat leaves. *Br J Clin Pharmacol* **56:**125-130.

9- Dimba E, Giertsen B.T, Francis G.W, Johannessee A.C, Vintermyv OK. (2003). Catha edulis (Qat) induced cell death by apoptosis in leukemia cell lines. *Ann N Y Acad Sci* **80:**1010-1020.

10- Goldenberg D, Lee J, Koch W.M. (2004). Habitual risk factors for head and neck cancer. *Otolaryngol Head Neck Surg* **131**:986-993.

11- Diniz-Freitas M, Garcı´a-Garcı´a A, Crespo-Abelleira A, Martins-Carneiro J.L, Ga´ndara-Rey J.M, (2004). Applications of exfoliative cytology in the diagnosis of oral cancer. *Med Oral* **9:**355-361.

12- Navone R, Burlo P, Pich A. (2007). The impact of liquid-based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. *Cytopathology* **18:**356–360.

13- Bancroft J.D, Gamble M. (2006). *Theory and practice of histochemical techniques*. 6th ed. Churchill Livingstone. London.

14- Paiva R.L, Sant'ana Filho M, Bohrer P.L, Lauxen IdaS, Rados P.V. (2004). AgNOR quantification in cells of normal oral mucosa exposed to smoking and alcohol. A cytopathologic study. *Anal Quant Cytol Histol* **26:**175–180.

15- Ahmed H.G, Idris A.M, Ibrahim SO. (2003). Study of oral epithelial atypia among Sudanese tobacco users by exfoliative cytology. *Anticancer Res* **23:**1943–1950.

16- Ahmed H.G, Omer AS, Abd Algaffar SA. **(**2011**).** Cytological study of exfoliative buccal mucosal cells of Qat chewers in Yemen. *Diagnostic Cytopathology* **39(11):**796-800.

17- Ali A.A, AL-Sharabi A.K, Aguirre J.M, Nahas R.2004 A study of 342 oral keratotic white Lesion induced by qat chewing among 2500 Yemeni. *J Oral Pathol Med* **33(6):**368-372.

18- Ali A.A, Al-Sharabi A.K, Aguirre J.M. (2006) Histopathological changes in oral mucosa due to takhzeen al-qat: A study of 70 biopsies. *J Oral Pathol Med* **35:**81–85.

19- Lukandu O.M, Neppelberg E, Vintermyr OK, Johannessen A.C, Costea D.E, (2010). Khat alters the phenotype of in vitro-reconstructed human oral mucosa. *J Dent Res* **89:**270-5.

20- Gorsky M, Epstein J.B, Levi H, Yarom N. (2004). Oral white lesions associated with chewing khat. *Tob Induc Dis* **2:**145–150.

21- AL-Sanabani J.S, (2011). *Dissertation in Oral white lesions due to qat chewing among women in Yemen*. University Berlin. Germany.

22- AL-Sharabi A. (2002). *Oral and para-oral lesions caused by takhazeen alqat (chewing)*. PhD Thesis. University of Khartoum. Sudan.

23- Mahmoud A.L, (2000). Mycotoxin-producing potential of fungi associated with qat (Catha edulis) leaves in Yemen. *Folia Microbiol* **45(5):** 452–456.

24- Orellana-Bustos, A. and Ortega-Pinto, A.V, (2004). Evaluation of keratinization and AgNORs count in exfoliative cytology of normal oral mucosa from smokers and non-smokers. *Med Oral*, **9(3):**197-203.

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