# The Impact of Khat (*Catha edulis*) Chewing Habit on the Epidemiology of Periodontal Diseases of Two Different Groups' Type 2 Diabetic Patients in the Arabian Peninsula: Clinical and Microbiological Comparative Study

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## Abstract

**Background:** Diabetes mellitus is recognized as a systemic disease linked to the progression and severity of periodontal diseases.

**Objective:** The current study aimed to assess the clinical and the microbiological effect of Khat (*Catha edulis*) chewing habit on the epidemiology of periodontal diseases of two different groups' type 2 diabetic patients in the Arabian Peninsula.

**Materials and Methodology:** 400 type 2 diabetic patients (between 20-60 years) were enrolled in the present study. They consisted of 200 Khat chewers and 200 non-Khat chewers. The case history of all patients was recorded. Moreover, plaque index (PLI), gingival index (GI), calculus index (CI), periodontal pocket depth (PPD), and clinical attachment loss (CAL). Sub-gingival plaque samples were obtained for investigation using the anaerobic culture technique. All data was collected then assorted and evaluated statistically by utilizing SPSS and the difference in a ratio calculated by Chi-square test.

During the comparison between the groups in the present study, P-value at < 0.05 (p < 0.05) was considered as statistically significant difference and at <0.001 (p < 0.001) high statistically significant difference.

**Results:** There were significant differences in the prevalence of gingivitis and periodontitis in group I and group II where the prevalence of gingivitis and periodontitis was more in group II than group I. Additionally, there were high significance differences in PLI, GI, CI, PPD, and CAL (p < 0.001). On the other hand, there were statistically significant differences in the comparison of aerobes and anaerobes bacterial colonies/1.5μ accounts between group I and group II.

**Conclusion:** At the end of the present study, we reached that there was a close link between type 2 diabetes millions, the epidemiology of periodontal diseases, and Khat chewing habits of two different groups of type 2 diabetic patients in the Arabian Peninsula.

*Keywords: Arabian Peninsula; Epidemiology of Periodontal Disease; Khat (Catha edulis) Chewing Habit; Type 2 Diabetic Patients*

## Abbreviations

BA: Blood Agar; RA: Rogosa Agar; MSA: Mitis Salivarius Agar; BP: Baird Parker Media; SAB: Sabouraud Agar; WHO: World Health Organization; CPITN: Index of Periodontal Treatment Need; GISPs: Group I Saudi Patients; GIYPs: Group I Yemeni Patients; GIISPs: Group II Saudi Patients; GIIYPs: Group II Yemeni Patients

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## Introduction

Epidemiology is the science that includes the study of determinants and the distribution of events in specific people and the implementation of the results of this study to resolve the medical problems [1]. Periodontal diseases are the most prevalent oral bacterial diseases in the human race as well as their rating is difficult and depends on the average of disease progression, clinical features, age at diagnosis, local factors, and systemic factors that may be risk factors of periodontal diseases [2]. The prevalence of periodontal diseases changes in various areas where there is an increased incidence of prevalent in developing states more than in developed states [3]. According to the previous epidemiological studies, there were differences in severity of periodontal diseases from one area to another where the prevalence of the gingival disease was 100% in people with bad oral hygiene that improved by correction of oral hygiene status [4]. On the other hand, the World Health Organization (WHO) revealed that a prevalence rate of severe periodontal diseases represents about 8-10% of people [5].

Diabetes mellitus (DM) and periodontal diseases are chronic diseases, and there is strong evidence for this correlation appears that diabetic patients are affected by periodontal diseases more than non-diabetic patients [6]. It divides into two types: Insulin-dependent (Type 1) DM and non–insulin-dependent (Type 2) DM. Type 1 occurs due to the destruction of B-cells of the pancreas (insulin-producing cells), but type 2 occurs due to impaired insulin function [7].

Khat (*Catha edulis*) is a widespread plant in East Africa and the South of the Arabian Peninsula as a natural tonic. The consumption of Khat depends on chewing its soft leaves and the small shoots to get euphoria. Most of the Yemeni Khat chewers believe that it is beneficial for the management of mild diseases like colds, headaches, arthritis, depression, and also body pains [8,9]. In a previous study, there were effects of Khat chewing habit on periodontal tissues where pocket depth and gingival recession among Khat chewers patients were more than non-Khat chewers patients [10]. On the contrary, according to a microbiological study, which was carried out on supra and sub-gingival plaque which revealed that Khat chewing habit is not harmful to the periodontal tissues and no significant differences between Khat chewers and non- Khat chewers in oral hygiene status [11].

Finally, there were a few numbers of epidemiological studies that were done to evaluate the prevalence and incidence of periodontal diseases among type 2 diabetic patients, but there are no studies that demonstrate the impact of Khat chewing on the epidemiology and incidence of periodontal disease. Additionally, its impact on the risk of sub-gingival micro-biota among type 2 diabetic patients from South of the Arabian Peninsula. Therefore, our study was designed to clarify these objectives.

## Materials and Methods

### Study design, location and population

The current epidemiological, clinical and microbiological study was conducted on 400 type 2 diabetic patients, including two equal groups, non- Khat chewers (group I) and Khat chewers (group II).The patients were selected randomly according to their Khat chewing history from the outpatient clinics, college of dentistry, King Khalid University and from dental clinics in some hospitals in Jazan region, Saudi Arabia and from dental clinics in some hospitals in Sana'a city, Republic of Yemen within ten months from January 2021 G to October 2021 G. The age of patients extends between 20-60 years, the patients in group I (control group) should not be chewed Khat in the life absolutely, whereas the patients in group II (Khat chewers) should be Khat chewers at least five years and more. All the patients were type 2diabetic patients, didn't get any periodontal treatment. Additionally, they didn't take antimicrobial agents for at least 6months.

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## Ethical considerations

The research proposal and ethical approval were designed according to the instructions and guidelines of The Institutional Review Board (IRB), college of dentistry, King Khalid University. All patients were volunteers and accepted participation in the study before the research started. All the present study steps were done according to the principles of the Helsinki Declaration. The signed informed consent was obtained from all patients after demonstrating the goals of the study.

## Clinical examination

The clinical findings were obtained through case history and clinical examination that was done by the researchers. The patients were interviewed and examined at dental clinics. The case history format included personal data of patients, their objective of the dental clinic visit, duration and daily frequency of Khat chewing, and association poor oral habits like smoking and smokeless tobacco. The epidemiology of periodontal diseases is evaluated by clinical assessment of plaque index (PLI) [12], gingival index (GI) [13], calculus index (CI) [14], periodontal pocket depth (PPD), and clinical attachment loss (CAL) (Figure 1).



*Figure 1: Clinical view of Khat impact on periodontal tissues.*

## Microbiological evaluation

## Microbial samples collection

The microbial samples were obtained from the deepest periodontal pocket using sterilized curettes and put into transport media tubes then the samples were sent to the microbiological laboratory within an hour of collection for investigation.

## Microbiological investigation

The microbiological investigation was done by Matthews and Patel technique to insulate and recognize common oral microorganisms [15]. The microbial samples were cultured where blood agar (BA) was used for the bacterial count, Rogosa agar (RA) for *Lactobacilli*

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count, Mitis Salivarius agar (MSA) for the total count of *Streptococci,* Baird Parker media (BP) for presumptive *Staphylococcus aureus* count, Sabouraud agar (SAB) for *Candida species* count and MacConkey agar for aerobic Gram-negative bacteria count. The incubation of SAB, BP, and BA was aerobically at 37 ◦ C for 48 h, while RA and MSA incubation were under CO2 at 37 ◦ C for 48 h. All the colonies were calculated (colonies/1.5μ) where there was no more identification to the species level. Thus, the Microbiological evaluation in the present study was descriptive quantitative analyses.

### Data collection and analysis

All data of the present study were collected, and ANOVA test was used for the data process. A statistical analysis Chi-square test was used to reveal the statistically significant differences between the two groups in the present study. In the current study, there was one epidemiological index definition of oral hygiene status amount of dental plaque (PLI) was used, and four epidemiological indices definitions of gingival and periodontal destruction (GI, CI, PPD, and CAL) were used. The level of statistical significance was set at $p < 0.05$.

## Results

### The distributions and characteristics of the study patients

Table 1 summarizes the patients' distributions of the present study where there were (70%) males, whereas (30%) females as Saudi and Yemeni patients. The results of the present study revealed that the male patients more consuming of Khat than female patients of both Saudi and Yemeni patients, but Yemeni patients more consuming of Khat than Saudi patients in both genders that may be due to that the agriculture and chewing of Khat are illegal in Saudi Arabia compared to Yemen. Distribution analysis revealed a significant linking between khat chewing habit and male and female patients of the present study ($p < 0.05$).

| Patients' Sub-groups | Genders of patients | GI N(%) | GII N(%) | Chi- Square | |
|---|---|---|---|---|---|
| | | | | Pearson Chi | P-Value |
| Saudi patients (SPs) | Males (Ms) | 40 (10%) | 40(10%) | 0.126 | 0.001** |
| | Females (Fs) | 10(2.5%) | 10(2.5%) | | |
| Yemeni patients (YPs) | Males (Ms) | 100 (25%) | 100(25%) | 2.557 | 0.001** |
| | Females (Fs) | 50(12.5%) | 50(12.5%) | | |

*Table 1:* The patients' distributions.
*N: Number of patients, %: Percentage, **: Highly statistically significant differences.*
*GI: Group I, GII: Group II.*

Additionally, there were differences in the Saudi and Yemeni patients' ages and standard deviation (± SD) of group I and group II among male and female patients. The means ages and standard deviation (± SD) of males and females Saudi patients in group I were 33.93 ± 6.06ys and 29.61 ± 4.2ys more than the mean ages of males and females Yemeni patients 32.22 ± 15.517ys and 21.55 ± 3.461ys, whereas in group II the mean ages and standard deviation (± SD) of male Yemeni patients were 37.00 ± 7.351ys more than the means ages and standard deviation (± SD) of males Saudi patients (35.06 ± 3.01ys) but the mean ages and standard deviation (± SD) of females Saudi patients was 28.3 ± 1.33y more than mean ages and standard deviation (± SD) of females Yemeni patients (22.17 ± 0.83 ys). There were no significant differences in the age of Khat chewers and non-non Khat chewers, although the mean of Khat chewers' ages higher than the mean of non-Khat chewers ages in both males and females ($p > 0.05$) (Table 2 and figure 2).

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| | | | GI | | GII | | ANOVA | |
|---|---|---|---|---|---|---|---|---|
| | | | Range | M& ± SD | Range | M& ± SD | F | P-value |
| Age | Males (Ms) | SPs | 20-55 | 33.93 ± 6.1 | 25- 60 | 35.06 ± 3.01 | 1.408 | 0.103 |
| | | YPs | 20-55 | 32.22 ± 15.5 | 20-60 | 37.00 ± 7.351 | 11.721 | 0.251 |
| | Females (Fs) | SPs | 20- 45 | 29.61 ± 4.2 | 20- 40 | 28.3 ± 1.33 | 1.307 | 0.256 |
| | | YPs | 20-45 | 21.55 ± 3.46 | 20-40 | 22.17 ± 0.83 | 9.391 | 0.740 |

***Table 2:** The patients' ages.*
*GI: Group I, GII: Group II, M: Mean, SD: Standard deviation.*
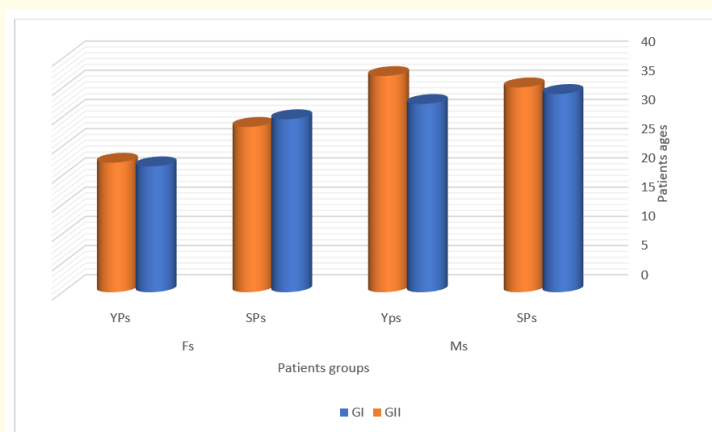


***Figure 2:** The mean of patients ages.*
*GI: Group I, GII: group II, SPs: Saudi patients.YPs: Yemeni patients Ms: Male Patients. Fs: Female Patients.*
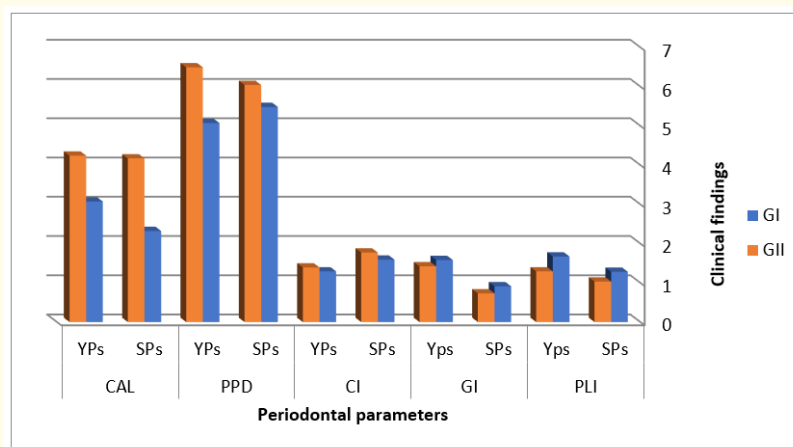
When we requested the patients to clarify the cause of their visit to the clinic of dentistry, almost all of them came due to pain (44,5%) and (32%) suffering from periodontal problems, and (14%) their visit was for a routine checkup and (13.5%) their visit was for other reasons. Furthermore, We found an association between tobacco consuming and Khat chewing among the patients of the group (II), where (60%) of patients were smokers and (30%) smokeless tobacco (snuff) and (10%) used both, which explain the reason of the increased prevalence of periodontitis among Khat chewers more than non-Khat chewers.

## Periodontal findings of the present study

Table 3 and figure 3 elucidate the results of clinical parameters.

| Clinical parameters | | Group I | Group II | ANOVA | |
|---|---|---|---|---|---|
| | | | | F | P value |
| PLI | SPs | 1.28 ± 0.24 | 1.03 ± 0.35 | 2.236 | <0.001 ** |
| | YPs | 1.668 ± 0.504 | 1.294 ± 0.588 | 3.115 | 0.045* |
| GI | SPs | 0.91 ± 0.21 | 0.73 ± 0.11 | 4.185 | <0.001 ** |
| | YPs | 1.584 ± 0.449 | 1.424 ± 0.366 | 6.842 | <0.001** |
| CI | SPs | 1.59 ± 0. 26 | 1.77 ± 0. 31 | 7.264 | <0.005** |
| | YPs | 1.289 ± 0.427 | 1.396 ± 0.584 | 4.2265 | 0.048* |
| PPD | SPs | 5.48 ± 0.41 | 6.04 ± 0.37 | 4.333 | <0.001 ** |
| | YPs | 5.075 ± 1.639 | 6.495 ± 1.663 | 0.058 | 0.043* |
| CAL | SPs | 2.32 ± 0.11 | 4.17 ± 0.24 | 8.468 | <0.001 ** |
| | YPs | 3.065 ± 1.697 | 4.240 ± 1.954 | 0.157 | 0.004 |

***Table 3:** Mean and ± SD of periodontal parameters.*
*PLI: Plaque Index, GI: Gingival Index, CI: Calculus Index, PPD: Periodontal Pocket Depth, CAL: Clinical Attachment Loss. ** :Highly Statistically Significant Differences.*
** Statistically Significant Differences.*

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*Figure 3: The mean of periodonal parameters.*
*GI: Group I, GII: Group II, PLI: Plaque index, GI: Gingival index, CI: Calculus index,*
*PPD: Periodontal pocket depth, CAL: Clinical attachment loss.*

- **Plaque index (PLI):** The mean and standard deviation (± SD) of PLI among Saudi and Yemeni patients (SPs and YPs) for group I and II; (GISPs= 1.28 ± 0.24, GIYPs = 1.668 ± 0.504, GIISPs = 1.03 ± 0.35, GIIYPs = 1.294 ± 0.588). The statistical analysis revealed that the comparison between the mean and standard deviation of PLI was more among Yemeni patients more than Saudi patients in groups I and II with highly statistically significant differences (P ≤ 0.001) among Saudi patients versus statistically significant differences significant (p = 0.045) among Yemeni patients.

- **Gingival index (GI)**: The mean and standard deviation (± SD) of GI among Saudi and Yemeni patients (SPs and YPs) for group I and II; (GISPs= 0.91 ± 0.21, GIYPs = 1.584 ± 0.449, GIISPs = 0.73 ± 0.11, GIIYPs = 1.424 ± 0.366).The statistical comparison between the mean and standard deviation (± SD)of GI of group I compared to group II among Saudi and Yemeni patients revealed that the mean of GI among Yemeni patients was more than the mean of GI among Saudi patients of group I and group II with highly significant statistically differences (p < 0.001).

- **Calculus index (CI)**: The statistical comparison between the mean and standard deviation (± SD) of CI of group I compared to group II among Saudi and Yemeni patients revealed that the mean of CI among Saudi patients was more than the mean of CI among Yemeni patients of group I and group II with highly significant statistical differences (p < 0.005) among Saudi patients and significant statistical differences among Yemeni patients (p = 0.045).

- **Periodontal pocket depth (PPD):**The mean and standard deviation (± SD)of PPD among Saudi and Yemeni patients (SPs and YPs) for group I and II; (GISPs= 5.48 ± 0.41, GIYPs = 5.075 ± 1.639, GIISPs = 6.04 ± 0.37, GIIYPs = 6.495 ± 1.663).The statistical comparison of the mean and standard deviation (± SD)of PPD demonstrated that the mean of PPD among Saudi patients was more than the mean of PPD among Yemeni patients of group I and group II with a highly statistically significant difference among Saudi patients (P <0.001) and statistically significant difference among Yemeni patients when the group I compared to group II (p = 0.043).

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- **Clinical attachment loss (CAL)**: The mean and standard deviation (± SD) of CAL among Saudi and Yemeni patients (SPs and YPs) for group I and II; (GISPs= 2.32 ± 0.11, GIYPs = 3.065 ± 1.697, GIISPs = 4.17 ± 0.24, GIIYPs = 4.240 ± 1.954). The results of the present study exhibited that the mean and standard deviation (± SD)of CAL among Yemeni patients was more than Saudi patients. During the comparison between groups I and II, the statistical differences of the mean and standard deviation (± SD) of CAL were highly statistically significant differences among Saudi patients (p < 0.001) and statistically significant differences among Yemeni patients (p = 0.0040).

A periodontal examination revealed that the severity of periodontal diseases among Yemeni patients was more than the severity of periodontal diseases among Saudi patients. Moreover, Khat chewing habit and type 2 diabetes mellitus both can increase their severity.

In table 4 and figure 4, there were highly statistically significant differences in the prevalence of gingivitis and periodontitis in group I, where the prevalence of gingivitis was more than the prevalence of periodontitis. In this respect, the prevalence of periodontitis among the patients of group II was (77%) more than gingivitis (23%). We found in the present study also that the prevalence of gingivitis and periodontitis in Yemeni patients was more than Saudi patients in both genders in the present study may be due to an increase in Khat chewing duration and daily frequency of Khat chewing habit and low social economy and a shortage of medical care for Yemeni type 2 diabetic patients. Moreover, the prevalence of gingivitis and periodontitis in male patients was more than in female patients (Table 5).

| | | | Gingivitis N (%) | | | | Periodontitis N (%) | | Chi- Square | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | | | | | Pearson Chi | P-Value |
| GI | 137 (68.5%) | SPs | Ms | 27( 13.5% | 63 (31.5%) | SPs | Ms | 11 (5.5%) | 1.943 | 0.004** |
| | | | Fs | 7(3.5%) | | | Fs | 4(2%) | | |
| | | YPs | Ms | 61(30.5%) | | YPs | Ms | 17(8.5%) | | |
| | | | Fs | 42(21%) | | | Fs | 14(7%) | | |
| GII | 46 (23%) | SPs | Ms | 13(6.5%) | 154 (77%) | SPs | Ms | 29(14.5%) | 0.448 | 0.001** |
| | | | Fs | 3(1.5%) | | | Fs | 6(3%) | | |
| | | YPs | Ms | 39(19.5%) | | YPs | Ms | 83(41.5%) | | |
| | | | Fs | 8(4%) | | | Fs | 36(18%) | | |

*Table 4: Prevalence of gingivitis and periodontitis.*
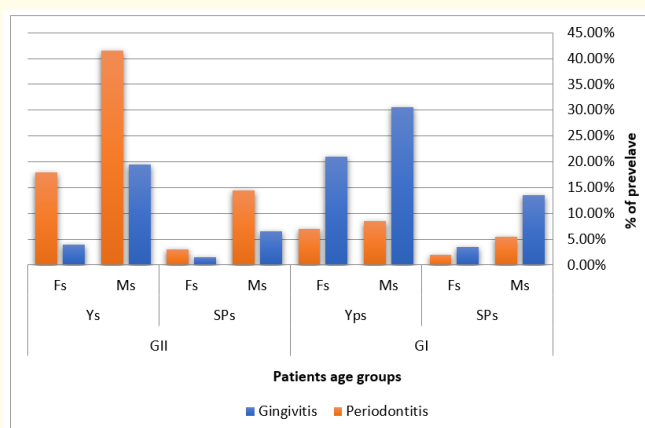


*Figure 4: Prevalence of gingivitis and periodontitis among the participants study.*
*GI: Group I, GII: Group II, Fs: Females, Ms: Males, Ys: Yemeni patients, SPs: Saudi patients.*

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| | | | Male | Female | Chi- Square | |
|---|---|---|---|---|---|---|
| | | | | | Pearson Chi | P-Value |
| Regular | SPs | <10y | 12(30%) | 2(20%) | 0.137 | 0.022* |
| | | >10y | 5(12.5%) | 2(20%) | 0.197 | 0.031* |
| | YPs | <10y | 15(15%) | 10(20%) | 0.382 | 0.041* |
| | | >10y | 60(60%) | 23(46%) | 0.224 | 0.013* |
| Irregular | SPs | <10y | 13(32.5%) | 4(40%) | 4.429 | 0.088 |
| | | >10y | 10(25%) | 2(20%) | 1.338 | 0.067 |
| | YPs | <10y | 10(10%) | 7(14%) | 1.083 | 0.098 |
| | | >10y | 15(15%) | 10(20%) | 2.183 | 0.041* |
| Frequency/Daily | SPs | <3D/W | 34(85%) | 8(80%) | 1.904 | 0.079 |
| | | >3D/W | 6(15%) | 2(20%) | 0.311 | 0.041* |
| | YPs | <3D/W | 16(16%) | 12(24%) | 6.204 | 0.015* |
| | | >3D/W | 84(84% | 38(76%) | 1.608 | 0.09* |

*Table 5: Frequency of Khat chewing habit.*
*SPs: Saudi patients, YPs: Yemeni patients, y: Years, D: Days, W: Weeks.*

## Bacterial findings of the present study

The results of the bacteriologic cultures in the present study are displayed in tables and 6,7 and 8 and figures 5, 6 and 7. There were differences between group I and group II in Facultative anaerobic gram-positive microorganisms, Aerobic and Anaerobic gram-positive microorganisms and Aerobic and Anaerobic gram-negative microorganisms' colonies/1.5μ accounts where the colonies numbers of microorganisms of Saudi Samples (colonies/1.5μ) were more than Yemeni samples. Furthermore, the colonies numbers of microorganisms among Khat chewers more than non-Khat chewers except the colonies numbers of Aerobic and anaerobic gram-positive and gram-negative microorganisms where the colonies numbers of microorganisms among non-Khat chewers were more than Khat chewers.

| | Micro. Findings colonies/1.5μ | Saudi Samples M results ( colonies/1.5μ) (n = 63) | | Yemeni Samples M results (colonies/1.5μ) (n = 55) | | Chi- Square | |
|---|---|---|---|---|---|---|---|
| | | GI (n = 13) | GII (n = 50) | GI (n = 5) | GII (n = 50) | Pearson Chi | P-Value |
| 1 | *Streptococci Viridons* (SV) | 9 (14.2%) | 27 (42.8%) | 0 (0%) | 0 (0%) | 2.211 | 0.003** |
| 2 | *Non-haemolytic streptococci* (NHS) | 0s (0%) | 6 (9.5%) | 0 (0%) | 39 (71%) | 1.111 | 0.001** |
| 3 | *Staphylococci species* (Staph S) | 0 (0%) | 2 (3.2%) | 0 (0%) | 0 (0%) | 7.203 | 0.021* |
| 4 | *Corynebacterium species* (CBS) | 0 (0%) | 3 (4.8%) | 0 (0%) | 0 (0%) | 4.213 | 0.032* |

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|---|---|---|---|---|---|---|---|
| 5 | *Lactobacillus species* (LBS) | 0 (0%) | 1 (1.6%) | 2 (3.6%) | 2 (3.6%) | 2.211 | 0.040 |
| 6 | *Klebsiella pneumoniae* (KP) | 0 (0%) | 2 (3.2%) | 0 (0%) | 2 (3.6%) | 0.131 | 0.663 |
| 7 | *Staphylococcus aureus* (staph A) | 1 (1.6%) | 6 (9.5%) | 0 (0%) | 4 (7.2%) | 0.349 | 0.102 |
| 8 | *β-hemolytic streptococci* (βHS) | 0 (0%) | 1 (1.6%) | 0 (0%) | 1 (1.8%) | 3.001 | 0.277 |
| 9 | *α-hemolytic streptococci* ($\alpha$ HS) | 0 (0%) | 1 (1.6%) | 0 (0%) | 0 (0%) | 0.586 | 0.266 |
| 10 | $\alpha$ -Non-heamolytic streptococci ($\alpha$ NHS) | 0 (0%) | 1 (1.6%) | 0 (0%) | 2 (3.6%) | 1.409 | 0.494 |
| 11 | *Candida albicans* (CA) | 1 (1.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 9.318 | 0.050 |
| 12 | *Streptococcus faecalis* (SF) | 1 (1.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.660 | 0.436 |
| 13 | *Actinomyces* (A) | 1 (1.6%) | 0 (0%) | 3 (5.6%) | 0(0%) | 0.226 | 0.050 |

***Table 6:*** *Facultative anaerobic gram positive (FAnAEG+) microbial findings.*
*n: Number of colonies/1.5μ.*

| | Micro. Findings colonies/1.5μ | Saudi Samples M results (colonies/1.5μ) (n = 16) | | Yemeni Samples M results (colonies/1.5μ) (n = 2) | | Chi- Square | |
|---|---|---|---|---|---|---|---|
| | | GI (n = 6) | GII (n = 10) | GI (n = 2) | GII (n = 0) | Pearson Chi | P-Value |
| 1 | *Staphylococci* (coagulase-Negative) (Staph (C-N)) | 3 (18.7%) | 1 (6.3%) | 0 (0%) | 0 (0%) | 0.233 | 0.002 |
| 2 | *Stomatococcus mucilaginosus* (SM) | 2s (12.5%) | 9 (56.2%) | 0 (0%) | 0 (0%) | 2.288 | 0.001 |
| 3 | *Streptococcus faecalis* (SF) | 1 (6.3%) | 0 (0%) | 0 (0%) | 0(0%) | 0.461 | 0.591 |
| 4 | *Propionibacterium species* (PRS) | 0 (0%) | 0 (0%) | 2 (100%) | 0 (0%) | 0.885 | 0.001 |

***Table 7:*** *Aerobic and Anaerobic gram positive (AE&AnG+) microbial findings.*

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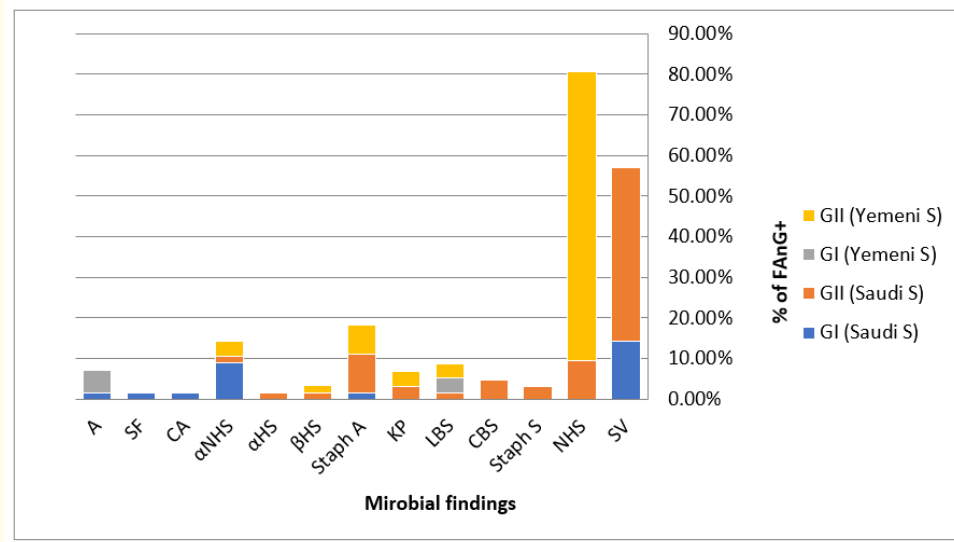***Figure 5:*** *Facultative Anaerobic gram postive (FAnG+)microbial findings.*
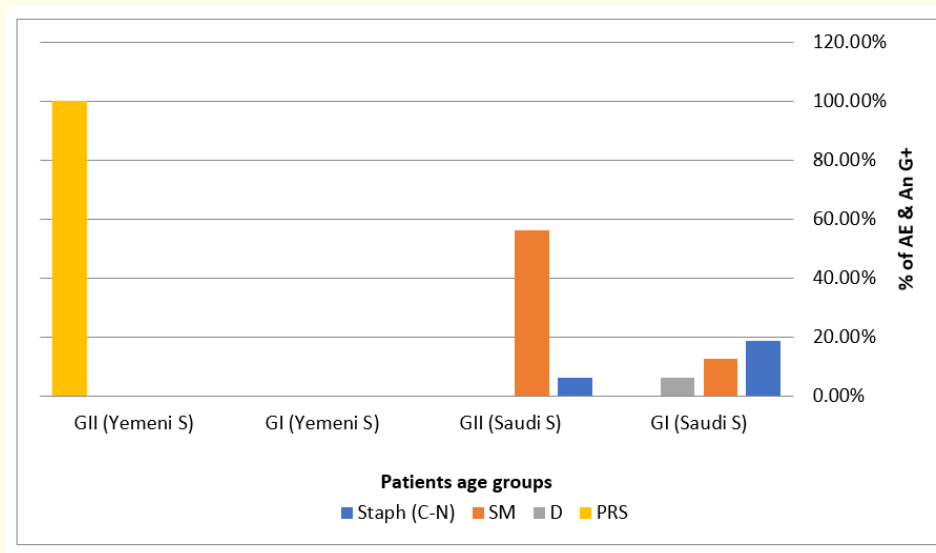


***Figure 6:*** *Aerobic and Anaerobic gram postive (AE&AnG+)microbial findings.*

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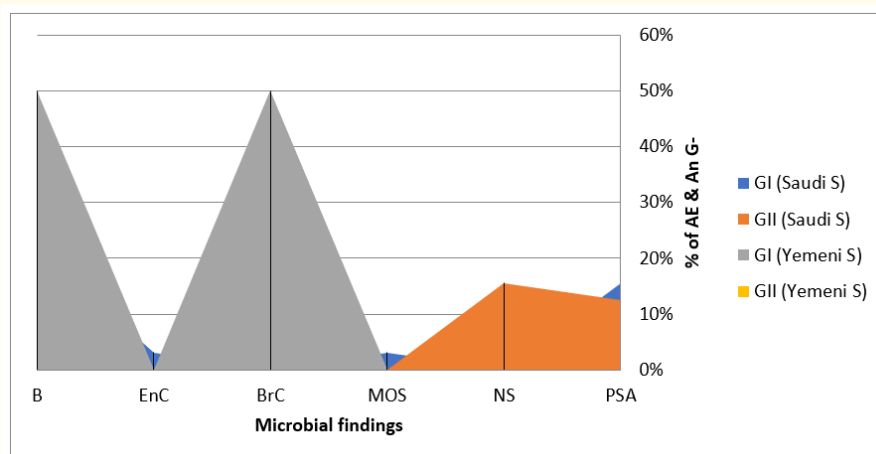
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**Figure 7:** *Aerobic and anaerobic gram negative (AE&AnG-) microbial findings.*

| | Micro. Findings colonies/1.5µ | Saudi Samples M results (colonies/1.5µ) (n = 32) | | Yemeni Samples M results (colonies/1.5µ) (n = 2) | | Chi- Square | |
|---|---|---|---|---|---|---|---|
| | | GI (n = 14) | GII (n = 18) | (n = 2) | GII (n = 0) | Pearson Chi GI | P-Value |
| 1 | *Pseudomonas* (PSA) | 5 (15.6%) | 4 (12.5%) | 0 (0%) | 0 (0%) | 0.609 | 0.002 |
| 2 | *Neasseria species* (NS) | 0 (0%) | 5 (15.6%) | 0 (0%) | 0 (0%) | 0.772 | 0.05 |
| 3 | *Moraxella species* (MOS) | 1 (3.13%) | 0 (0%) | 0 (0%) | 0 (0%) | 0.113 | 0.026 |
| 4 | *Branhamella catarrhalis* (BrC) | 0 (0%) | 0 (0%) | 1 (50%) | 0 (0%) | 2.516 | 0.001 |
| 5 | *Enterobacter cloaca* (EnC) | 1 (3.13%) | 0 (0%) | 0 (0%) | 0(0%) | 0.471 | 0.036 |
| 6 | *Bacteroides* (B) | 7 (22%) | 9 (28.13%) | 1 (50%) | 0 (0%) | 6.615 | 0.003 |

**Table 8 :** *Aerobic and Anaerobic gram negative (AEAnG-) microbial findings.*
*n: Number of colonies/1.5µ.*

The statistical comparison of the microorganisms colonies numbers (colonies/1.5µ) between Saudi Khat chewers and Yemeni Khat chewers revealed that there were highly statistically differences ($p < 0.001$) and statistically differences ($p < 0.05$) in the all numbers microorganisms colonies/1.5µ (Facultative anaerobic gram-positive microorganisms, Aerobic and Anaerobic gram-positive microorganisms, and Aerobic and Anaerobic gram-negative) except *Lactobacillus species* (LBS), *Klebsiella pneumonia*(KP), *Staphylococcus aureus* (staph A), *β-hemolytic streptococci* (βHS), *α-hemolytic streptococci* (αHS), *α-Non-hemolytic streptococci* (α NHS), and *Streptococcus faecalis* (SF), where there were no statistically differences ($p > 0.05$).

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## Discussion

Due to the universal increase in Khat chewers and the associated abnormal changes in dental, periodontal tissues, and oral mucosa. Therefore, arguably the Khat chewing has a risk of oral and systemic health, but the accurate mechanism of how Khat chewing raises the risk of subgingival microbiota and its influence on the epidemiology and incidence of periodontal diseases among Khat chewing type 2 diabetic patients does not fully understand. Furthermore, the lack of epidemiological studies on the impact of Khat chewing on periodontal tissues of type 2diabetic patients in the Arabian Peninsula, therefore this study was designed. As we know that diabetes, mellitus is considered one of the main risk factors of periodontal disease [16]. This hazard effect is threefold in diabetic patients in comparison with non-diabetic patients [17]. In our study, the selection of diabetic patients depended on the relationship between Khat chewing habit and the prevalence of periodontal diseases.

Few studies detected the correlation between diabetes mellitus and oral microbiota among Khat chewers in comparison with more studies that were carried out to assess the correlation between diabetes mellitus and periodontal disease where they found that the periodontal microorganisms in diabetic patients were identical in non-diabetic patients, but *P. gingivalis* was more in diabetic patients than non-diabetic patients [18]. This result agrees with the results of the Japanese study, which was done on diabetic patients with periodontal diseases where they found *P. intermediate* and *P. gingivalis* were more than in diabetic patients with healthy periodontium [19]. According to these studies, there were significant differences in microbial findings between diabetic Saudi patients and diabetic Yemeni patients this may be due to the effect of Khat chewing habit on the periodontal pocket environment.

On the other hand and in the study that was done on diabetic patients aged 19- 25 years, there were no significant differences in oral hygiene status compared to nondiabetic patients, but the gingival inflammation and gingival recession were more in diabetic patients than nondiabetic patients due to the alteration inflammatory response to dental plaque [20]. According to the results of this study, the prevalence and severity of periodontal disease was more in Yemeni diabetic patients than Saudi diabetic patients. These results are in agreement with the results of another study was conducted on diabetic patients [21]. In this case and depending on the present study findings, it may be found that the development of periodontal disease is due to the effect of an increase of Khat chewing habit frequency on the nature of oral microorganisms and host inflammatory reactions in periodontal tissues among diabetic patients.

Khat chewing habit is widespread in some regions in the Arabian Peninsula like Yemen and South of Saudi Arabia as a social habit, and as a result of Khat chewing methods, there are abnormal changes in the digestive system and oral mucosae like gastritis and periodontitis moreover dislocation of the temporomandibular joint and chronic recurrent subluxation [22]. The results of another study revealed that the poor oral hygiene of Khat chewers played an important role in periodontal disease initiation and progression [23]. This result corresponds with the results of the present study, where the prevalence of periodontitis among Khat chewing patients was more than gingivitis. Moreover, the prevalence of gingivitis and periodontitis among Yemeni patients was more than Saudi patients may be due to the most Yemeni patients were uncontrolled diabetics, and most of the Saudi patients were under medical care.

In the present study, the patients said the goal of the visit to the dental clinic was generally due to the pain (44,5%), whereas some of them came as routine dental checkup (14%). Additionally (32%) due to periodontal problems with Khat chewing habit coinciding the results of Humagain M (2011) study where he found (20%) were ordinary dental clinic visit [24].

In the study of Ababneh., *et al*. (2012), there were (76%) suffered from gingivitis, (2.2%) experienced aggressive periodontitis, and (5.5%) experienced chronic periodontitis. Furthermore, they found that the frequency of periodontitis more among males than the female, which was identical with the results of our study where there were (56%) of male Khat chewers affected by periodontitis compared to (21%) of female Khat chewers affected by periodontitis due to the adverse effect of long duration of Khat chewing [25]. Inconsistent

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results were observed in the present study, where the prevalence of periodontitis among Khat chewers was (77%) more than gingivitis (23%). We found in the present study also that the prevalence of gingivitis and periodontitis in Yemeni patients was more than Saudi patients in both genders in the present study may be due to an increase in Khat chewing duration and daily frequency of Khat chewing habit and low social economy and a shortage of medical care for Yemeni type 2 diabetic patients. Moreover, the prevalence of gingivitis and periodontitis in male patients was more than in female patients.

In the current study, PLI and GI in the non-Khat chewers' group were more than the Khat chewers group. The reduction in clinical signs of gingival inflammation is returning to the lowering of plaque accumulation in the patients due to the self-cleaning effect of the Khat chewing habit. The findings of the current study also appeared more calculus accumulation in the Khat chewers group may be due to the effect of Khat chewing on the formation of the mineralization of supragingival plaque, but the mechanism of this effect is still not fully understood.

In the Kenyan study that carried on 131 Mirra (Khat in Kenya) chewers and 199 non-Mirra chewers, there were no significant differences were detected in periodontal status among all patient study groups except the GI in non-Mirra chewers were more than Mirra chewers [11]. This result is not consistent with the results of the current study, where a periodontal examination in the present study revealed that there were statistically significant differences in all clinical periodontal parameters during the comparison between Khat chewers and non-Khat chewers (both Saudis and Yemenis) that may be attributed to the negative effect of type 2 diabetes mellitus on periodontal tissues among the patients of the present study.

On the other hand and according to the previous study that was carried out on Khat chewers patients, there were significantly deeper periodontal pockets on the Khat chewing side more than on the non-Khat chewing side [26]. This result corresponds with the findings of the current study, which exhibited that the PPD and CAL were more in Khat chewing patients than non-Khat chewing patients may be attributed to the trauma during Khat chewing and the defect in host defense among type 2 diabetic patients in the present study.

Khat contents may induce a change in the oral commensal bacteria, which might also be potential factors for periodontal diseases; this could be confirmed by our study findings, which displayed that Khat chewing stimulates the normal flora to become risk factors of periodontal diseases.

In some previous studies, there were adverse effects of smoking and tobacco chewing on periodontal tissues [27]. This result agrees with the clinical finding in the current study where there was a significant risk effect of Khat chewing on sub-gingival microbiota and epidemiology of periodontal diseases because of the association between Khat chewing and smoking among most of the patients in this study due to the effect of smoking on neutrophils functions and their secretions.

This study demonstrated high numbers of Streptococci, whereas the numbers of Staphylococci species, Lactobacilli, Candida, aerobic, and anaerobic Gram-negative bacteria were too depressed in most study samples. This result agrees with other study results reported that Streptococci was detected at all sites in the oral cavity, and Lactobacilli constitute 1% of oral microflora[28]. These results may be due to the patients being Khat chewers, and they were poor oral hygiene as well as were type2 diabetics (immune-compromised patients).

In a chemical study on Khat, which was done by Elhag., *et al.* (1999), there were two antimicrobial agents to several microorganisms like *Mycobacterium sp, Staphylococcus aureus, and Bacillus* subtilis [29]. The results of the present study demonstrated during the comparison of the microorganisms colonies numbers (colonies/1.5μ) between Saudi Khat chewers and Yemeni Khat chewers that there were highly statistically differences ($p < 0.001$) and statistically differences ($p < 0.05$) in all numbers microorganisms colonies/1.5μ (Facultative anaerobic gram-positive microorganisms, Aerobic and Anaerobic gram-positive microorganisms, and Aerobic and Anaerobic gram-

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negative) except *Lactobacillus species* (LBS), *Klebsiella pneumonia* (KP), S*taphylococcus aureus* (staph A), *β-hemolytic streptococci* (βHS), *α-hemolytic streptococci* (αHS), *α-Non-hemolytic streptococci* (α NHS), and *Streptococcus faecalis* (SF), where there were no statistically differences (p > 0.0.5).Thus, some microorganisms were calculated in this study because they are common and can be considered an indicator of periodontal diseases presence and the possible presence of other microorganisms.

The clinical and microbiological findings displayed decreased plaque accumulation, increased destruction of periodontal tissues, and increased the number of virulent pathogens among the patients of group II more than group I. That is maybe due to the self-cleaning effect of Khat chewing, the mechanical impact of Khat chewing on periodontal tissues, and decreased the host defiance of Khat chewers type 2 diabetic patients. Moreover, the adverse effect of smoking and smokeless tobacco, which usually consumed with Khat chewing habit.

This study demonstrates some association factors between Khat chewing habit and periodontal status among type 2 diabetic patients of some areas in Saudi Arabia and the Republic of Yemen. There were some limitations of this study, such as the sample of patients was nonrandom, so it was not representative of patients in other areas, especially in Yemen, where Khat chewing is widespread among most Yemenis. The availability of patients was the main limitation in the present study, where this study was conducted among people with low attendance at the dental clinics (only symptomatic care) as well as chewing of Khat is illegal in Saudi Arabia. Collection of data may be another limitation where we based on the patients' answers in the interview without check of their medical records. There were limitations in the counts of bacteria where the bacterial counts were not accurate per patient due to the nature of the study design.

Despite these restrictions, the present study results reveal that consciousness about the effect of Khat chewing habit on periodontal tissues maybe not be enough among type 2 diabetic patients. The results of our study help to confirm the need for instruction on the relationship between Khat chewing habit and periodontal tissues status among type 2 diabetes for these at risk patients in the areas of this study.

## Conclusion

Although the difficulties during the current study, we reached that unmanaged periodontal diseases among Khat chewers type 2 diabetic patients lead to missing teeth in numerous cases. Also, it has been demonstrated that there is a risk of Khat chewing habit, type 2 diabetes in addition to smoking and smokeless (snuff) tobacco consequently utmost effort should be made in maintaining periodontal health and instructing about it to Saudi and Yemeni peoples to avoid the side effects of these bad oral habits on periodontal tissues especially among diabetic patients who are suffering from a defect in the immune system.

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