Effect of tooth extraction on salivary pH and oral flora

Abstract:

Back ground: Tooth extraction is a surgical procedure to remove tooth from its socket in the alveolar process. Due to stress and anxiety associated with this procedure, salivary secretion could be affected and subsequently this might affect salivary pH. Since oral cavity is inhabitant by various microorganisms, these also could be affected by tooth extraction procedure.

Aims: The aim of this study was to investigate the effect of single tooth extraction on salivary pH and oral microflora and to find out any relationship between these two variants.

Patients and methods: A total of (50) samples of resting saliva were collected in sterilized plastic container from patients admitted to the dental clinic of oral surgery to investigate the effect of tooth extraction on salivary pH. The investigation was carried out using colored paper strip before and after tooth extraction. The same samples were used to detect any changes in the oral flora before and after tooth extraction. All samples were observed by light microscope and biochemical tests were used to identify bacterial species. Direct smears of culture samples were prepared, Gram stain was used to detect *Staphylococcus aureus* while chrom agar media was used for diagnosis of *Candida* species.

Results: The male to female ratio of the participant patients was 2.1:1 and the age range (20-40) years old. The average pH value of resting saliva before and after tooth extraction was 7.28±0.50 and 5.94±0.50, respectively and the differences between the two readings was statistically significant (p= 0.0405). The results also showed that out of 50 samples, 24(12%) isolates were classified as *Staphylococcus* spp. Among them, 12(2.88%) isolates were identified as *Staphylococcus aureus*. The highest percentage of *S. aureus* isolates were found at age group (31-35) years old in regard to other age groups. Few cases 2(6%) of *C. albicans* were identified after tooth extraction as well.

Conclusion: Single tooth extraction decreased salivary pH and slightly altered oral flora with no definitive correlation between these two variants.

Chapter one
Introduction

1. Introduction:

Tooth extraction is a surgical procedure to remove a tooth from its socket within the alveolar process of the upper and the lower jaws. This dental procedure is usually done under local anesthesia or rarely under general anesthesia or sedation. Due to pain associated with needle prick during local anesthesia and expectation of pain during tooth removal, this procedure is usually associated with fear and anxiety. Anxiety is defined as "an emotion characterized by feelings of tension, worried thoughts, and physical changes like increased blood pressure" according to the American psychological association (APA).

Anxiety can be immediately detected by looking at emotional and physiological responses from patients such as increased pulse rate, blood pressure, muscle contractions, sweaty palms and dry mouth (Gordon et al.1998). Saliva has an important role to maintain oral cavity moisture, self-cleansing, antimicrobial substances and neutralizes the acids that are produced by bacterial plaque (Millsop et al, 2017). It has been shown that changes in salivary secretion can affect the composition of saliva including electrolytes, mucus, antibacterial compounds and various enzymes (Thomas et al, 2016). Bicarbonates is one of salivary electrolytes that has a great influence on salivary pH and its concentration is proportional with salivary secretion (Stefani et al, 2014). Thus, salivary pH is dependent on its secretion; the faster the salivary secretion, the higher the salivary pH and vice versa (Rensburg et al.1995). In times of stress and anxiety that can occur due to tooth extraction, there will be a decrease in salivary secretion and the concentration of hydrogen ions (pH) induces its acidity (Foglio-Bonda et al, 2013; Dix et al, 2013). Hence, salivary pH can be used as an indicator for the presence of dental anxiety in patients who will be doing dental care such as tooth extraction beside pulse rate and blood pressure (Mittal et al, 2011).

It is well-known that oral cavity is normally inhabitant by oral microbiota as commensals. These include anaerobes such as bacteriods and prevotella, as well as facultative anaerobes like streptococcus and staphylococcus spp (Osaiyuwu et al, 2010). These microorganisms live in an equilibrium state since the oral cavity provides an ideal conditions for their growth, as the normal temperature of the oral cavity is 37 ºC, the pH of saliva is 6.5-7 with its hydration effect and its action as a medium for transportation of nutrient to the microorganisms (Deo et al, 2019; Lim et al, 2017). However, certain factors may alter the composition of this community or their habitat such as age changes, compromised immune system and hormonal changes, smoking and oral hygiene or dental treatment, and shift to a non-equilibrium state resulting in establishment of new community(Socransky and Haffaji, 2005). Tooth extraction is one of those factors that may permanently change the oral habitat and is expected to influence the composition of oral microbial community. It has been stated that full-mouth tooth extraction altered normal oral microflora, these alterations include the reduction of *A. actinomycetemcomitans* and *P. gingivalis* but to insignificant detection level (Waal et al, 2014). Waal et al attributed their results to the elimination of the sub-gingival area by the full-mouth tooth extraction which decreases the prevalence of A. *actinomycetemcomitans* and *P. gingivalis*.

This research was aimed to study the effect of single- tooth extraction on salivary pH and oral microbiota - particularly *staphylococcus aureus* and to find out any correlation between these two biological parameters. The emphasis on *staphylococcus aureus* as it is the most frequent isolates from the oral cavity and peri-oral region and play an important role in terms of cross-infection and dissemination to other body regions (McCormack et al, 2015).

Chapter two

Patients and Methods

2. Patients and methods:

This study was conducted in the college of dentistry/ University of Babylon during the period from October, 2023 to April, 2024. Patients attended to dental clinic of oral surgery for tooth extraction were recruited in this study. Patients were selected according to the following criteria:

1- Patients with no systemic diseases.
2- Patients not undertaking medications
3- Patients not allergic to ester or amide type local anesthesia.
4- Non- smoker patients.
5- Non- alcoholic patients.
6- Patients with fully erupted permanent dentition.

2.1 Evaluation of salivary pH: After history taking and clinical examination, samples of resting saliva were collected from each patient in a plastic container before (local anesthesia was considered as part of tooth extraction) and after tooth extraction and the salivary pH was measured immediately using a paper strip (Hydrion, USA). The paper strip was submerged in the saliva for 1 min. and then taken out to identify the color changes which referred to the pH value of the saliva. The containers containing salivary samples were perfectly sealed and kept in the fridge at 4 ºC until used for bacteriological tests.

2.2 Bacterial identification:

All the materials and equipment used in the bacteriological study with their manufacturer and country supplier are presented in table 2-1 in the appendix section.

2.2.1 Microscopic examination: All the samples were observed by light microscope . Direct smears of culture samples were prepared, Gram stain was used to detect *Staphylococcus aureus* which produces yellow colonies with yellow zones; whereas other coagulase-negative staphylococci produce small pink or red colonies without changing the color of the medium . If an organism can ferment

mannitol , an acidic byproduct is formed that causes the phenol red in the agar to turn yellow.

2.2.2 Preparation of culture media:

All solid and broth media were prepared according to the manufactures instructions. After being sterilized by autoclaving at 121 ºC for 15 min, the culture media was poured in sterilized plastic petridishes and incubated for 24 h to avoid contamination then stored in a refrigerator at 4 ºC until use.

2.2.3 Mannitol salt agar:

The media was prepared by dissolving 111 g of salt manitol agar powder in 1000 ml of distilled water, and sterilized in the autoclave. All collected samples were initially inoculated on the sterile Manitol salt agar and chromo agar medium and incubated at 37 ^{o}C for 18-24 h in an incubator.

2.2.4 Chrome agar medium:

Soluble chrome agar 47.7 g was dissolved in 1000 ml of distill water and heating to boiling. Then it was poured in 9 cm plastic petridishes. The medium was used for diagnosis of *Candida* species.

2.2.5 Biochemicals tests:

2.2.5.1Gram satin:

The Gram staining method is named after Hans Christian Gram, the Danish bacteriologist who originally devised it in 1844, and is one of the most important staining techniques in microbiology. It is almost always the first test performed for the identification of bacteria. The primary stain of this method is Crystal Violet, which can be sometimes substituted with equally effective Methylene Blue. The microorganisms that retain the crystal violet-iodine complex appear purple brown under microscopic examination.

Stained microorganisms are classified as gram positive, while the unstained are classified as gram negative. In addition, these techniques are useful in the detection

or absence of cell components. Gram's Method uses retained crystal violet dye during solvent treatment to amplify the difference in the microbial cell wall.

The cell walls for gram-positive microorganisms have a higher lipid content than gram-negative cells. First, crystal violet ions penetrate the cell wall of both types of cells. Then, iodine is added to form a complex that makes the dye difficult to remove, in a step referred to as "fixing" the dye. Following iodine, the cells are treated with decolorizer, a mixture of ethanol and acetone, which dissolves the lipid layer from the gram negative cells, and dehydrating the thicker gram-positive cell wall. As a result, the stain leaches from gram-negative cells and is sealed in gram-positive cells. With expedient removal of the decolorizer, cells will remain stained.

The addition of a safranin counterstain to dye the gram negative cells with a pink color for easier observation under a microscope. Thus, gram-positive cells will be stained purple and gram-negative cells will be stained pink.

Reagents Used in Gram Staining

1- Crystal Violet, the primary stain.

2- Iodine, the mordant.

3- A decolorizer made of acetone and alcohol (95%).

4- Safranin, the counterstain.

Procedure of gram stain :

(Gephardt et al, 1981, Feedback from ASMCUE participants, ASMCUE , 2005).

1. Flood air-dried, heat-fixed smear of cells for 1 minute with crystal violet staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.

2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

3. Flood slide with the mordant: Gram's iodine. Wait 1 minute.

4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

5. Flood slide with decolorizing agent. Wait 15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear.

6. Flood slide with counterstain, Safranine. Wait 30 seconds to 1 minute.

7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.

2.2.5.2 Coagulase Test:

A- Free Coagulase Test (Tube Test):

The tube coagulase test is the routine standard test used to identify *S. aureus*. Several colonies of bacterial growth were transferred with a loop to a tube containing at least 5 ml of BHI broth. The seeding tube was covered to prevent evaporation and was incubated at 37˚C overnight. The tube centrifuged, then mixed 0.5 ml of the supernatant with volume of plasma and incubated in the water bath at 37˚C for 4 h. If the plasma coagulates, the organism is coagulase-positive. Some coagulation occurred in 30 minutes or 24 h later. Any degree of coagulation, from a loose clots suspended in plasma to a solid immovable clot, was considered to be a positive result, even if it takes 24 hours to occur (Benson, 2001).

B- Bound Coagulase or Clumping Factor Test (Slide Test):

A drop of sterile distilled water is placed on a clear glass slide. Few bacterial colonies emulsified in the distilled water to give a heavy cell suspension. A drop of defibrinated plasma is added to the mixture. Clumping within 10 sec. indicated the coagulase production (McFadden, 2000).

2.2.6 Preservation of bacterial isolates:

Short Term Storage:

Pure isolates of bacteria were maintained for short period (maximum two weeks) on the surface of nutrient agar (NA) plates. The plates were tightly wrapped with para-film and stored at 4˚C in the refrigerator (Benson, 2001).

2.2.7 Statistical analysis: T-test was used to analyze the differences in the salivary pH before and after tooth extraction. *P* value < 0.05 was considered statistically significant.

Chapter Three

Results

Results:

A total of 25 patients participated in this study, 17 males and 8 females with the male to female ratio was 2.1:1. The age range of the patients was 20-40 years old. The distribution of the patients and their gender according to the age groups is shown in table 3-1.

Table 3-1: Distribution of patients and gender according to age group

Age group	No. patient	Male	Female
20-25	9	8	1
26-30	3	2	1
31-35	6	2	3
36-40	7	5	3
Total	25	17	8

3.1 Evaluation of salivary pH:

The highest pH value of resting saliva before tooth extraction was 8 and the lowest value was 6; whereas the highest pH value after tooth extraction was 7.5 and the lowest pH value was 5. The average pH value before tooth extraction was 7.28 ± 0.50 while the average pH value after tooth extraction was 5.94±0.5.1. The differences between the pH value before and after tooth extraction was statistically significant ($p = 0.040$).

3.2 Bacteriological studies:

The frequency of *S. aureus* and *C. albicans* has been shown in Figure (3-1). As can be seen that *S. SPP* demonstrated the highest microorganism isolated from the salivary samples.

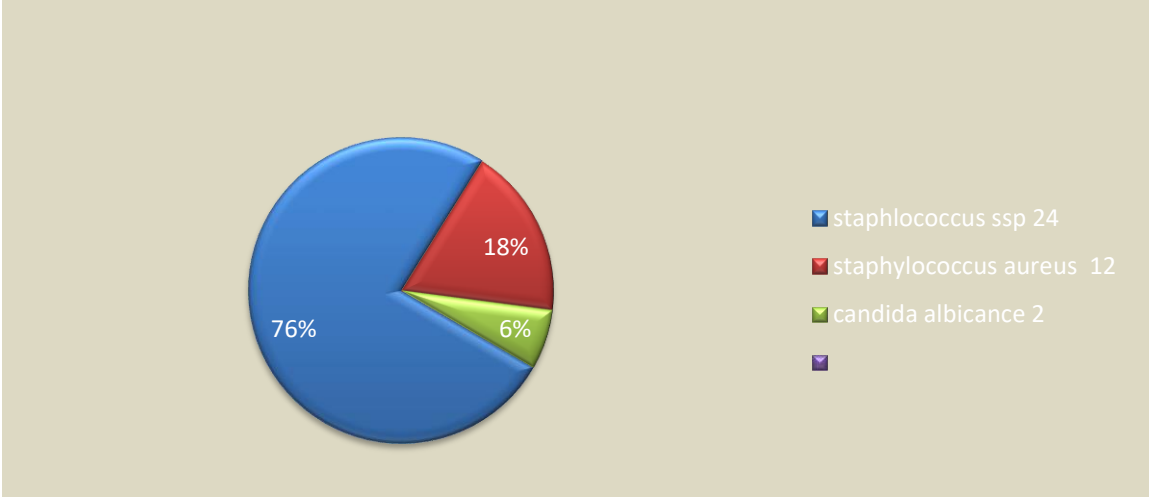

Figure (3- 1): Frequency of *S. aureus* and candida albicance in clinical samples

Table 3-1 shows the distribution of *S. aureus* among age groups. As can be seen that the highest number of *S. aureus* were isolated from age group (31-35) years old.

Table (3-2): Distribution of *S. aureus* isolates among age groups.

Age group	No. microbe 12%
20-25	1(8.3)%
26-30	3(25)%
31-35	6(50)%
36-40	2(16)%
Total	12 (12)%

Figure (3-2) represents the distribution of *S. aureus* among female and male patients. As can be seen that the highest frequency of *S. aureus* were isolated from male patients compared with females.

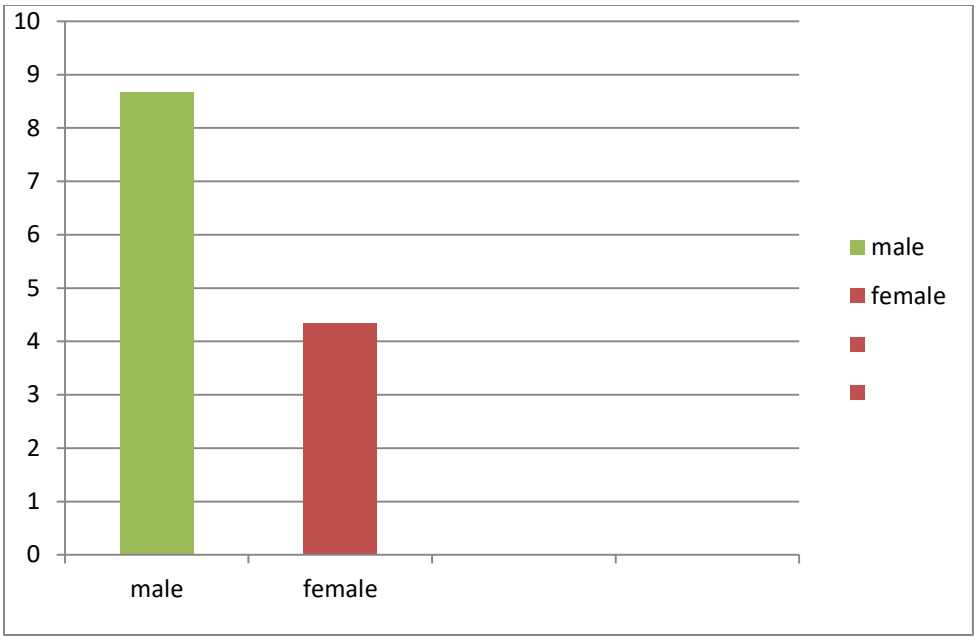

Figure (3-2): Distribution of *S. aureus* isolates among male and female patients.

Chapter four
Discussion

4. Discussion:

Tooth extraction usually associated with stress and anxiety due to pain expectation. Stress has a negative effect on oral physiology and immune system as it reduces the defense mechanism, which render the patient more susceptible to the development of psychosomatic and inflammatory diseases (Castro et al, 2020). Thus, studying the effect of stress and anxiety during tooth extraction is of paramount to avoid such complications. Previous studies discussed either the effect of tooth extraction on salivary pH alone (Stefani et al, 2015) or the effect of multiple-teeth extraction on oral flora (Waal et al, 2014), without discussing this effect on both these biological parameters. This study discussed the effect of single-tooth extraction on salivary pH and oral flora.

The results of this study showed that there was a decrease in salivary pH after single tooth extraction with significant statistical differences between the two groups (before and after tooth extraction). This result agreed with other studies where there was a correlation between dental anxiety and salivary pH (Bira et al, 2014; Stefani et al, 2015) and salivary pH can be used as an indicator of the presence of dental anxiety (Bira, 2008). It had been stated that physiological changes associated with tooth extraction such as dry mouth occurs due to a decrease in salivary flow which causes a decrease in salivary pH , as the latter depends on the salivary secretion (Gordon et al, 1998). The decrease in salivary secretion affects the composition of saliva, particularly salivary electrolytes such as bicarbonates, the concentration of bicarbonates depends on salivary secretion (Stefani et al, 2016). Thus, in case of stress the concentration of bicarbonates decreases and its action as a buffering system decreases raising the concentration of hydrogen ions and enhancing the acidity of saliva (Dix et al, 2013).

The results of bacteriological study revealed that the most common isolates from salivary samples was *S. aureus.* before and after tooth extraction. These results

were expected as this bacteria usually inhabit the oral cavity and peri-oral region (McCormack et al, 2015). Interestingly, the findings of this study showed that the highest percentage of *S. aureus* after tooth extraction was found at the age group (31-35) years old. This could be related to the ability of *S. aureus* to survive in acidic environment (at pH=5.4) compared with other bacterial species (Zohu et al, 2020). However, this result was unexpected as tooth removal would decrease the hard and soft tissue structures of the tooth and thus decreasing the surfaces for bacterial colonization. The highly incidence of *S. aureus* among patients in 30's could be associated with presence of dental fillings materials or dental prosthesis. However, the finding of this study disagree with study of (Abe et al, 2001) where they found that *S. aureus* usually isolates from the oral cavity of elderly patients.

The results of this study revealed that the highest prevalence of *S. aureus* were isolated from male patients compared with females. These results are consistent with that obtained by (John et al, 2014) where (79%) of *S. aureus* were isolated from men and (21%) from women. The results of this study could be related to the higher number of male patients participated in the study compared with female patients. The other possible reason is that female patients are more concern of their oral hygiene and less suffering from periodontal diseases with respect to male patients.

There was no definitive correlation between the decrease of salivary pH and the oral microflora after tooth extraction. Though, few cases (6%) of *C. albicans* were identified in the salivary samples after tooth extraction. This finding might be associated with a decrease in the prevalence of other bacteria in the oral cavity due to a decrease in the salivary pH and the growth of opportunistic pathogens such as *C. albicans* that can grow at varying pH level (Vasconsellos et al, 2014).

Chapter five

Conclusion

5. Conclusion:

Single tooth extraction decreased salivary pH and slightly altered the environment of oral flora by growing few isolates of *C. albicans*. There was no definitive relationship between salivary pH and oral flora after tooth extraction. Further study should be carried out using larger population size and more advanced technique for bacterial isolation such as PCR and more sensitive technique to measure salivary pH.

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Appendix

Appendix:

Table 2-1: Materials and equipment used in bacteriological study and their manufacturer and country supplier of each item.

No.	Materials	Company	Origin
1	Gloves and masks	Top guard	Malaysia
2	Disposable mouth mirror	Mediumion	China
3	Disposable dental probe	Mediumion	China
4	Disposable sterile swab	shanghai snwi medical	China
5	Autoclave	HIRAYAMA	Japan
6	Beakers	Volca	Iraq
7	Centrifuge	Bioneer	Korea
8	Cotton	Dunya	Iraq
9	Disposable Syringe 5ml	Sterile E0.	China
10	Disposable plastic petri dishes	Bio-Hit	Finland
11	Eppendorf tubes	Bioneer	Korea
12	Flask	BBL	USA
13	Incubator	Mammert	Germany
14	Light microscope	Olympus	Japan
15	Oven	Mammert	Germany
16	Plastic containers	Sterile EO.	China

17	Plain tube	CMA	Jordan
18	Refrigerator	Royal	Japan
19	Slides and cover sides	Superestar	India
20	Water bath	GFL	Germany

Table 2-1: Materials and equipment used in bacteriological study and their manufacturer and country supplier of each item.

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