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Gingivitis and Toothbrush Contamination

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Abstract

Oral diseases can be controlled by reducing the microbial count in the oral cavity and this can be achieved by maintaining proper oral hygiene. Toothbrushes are the most commonly used oral hygiene aid to prevent the dental and other oral diseases.
To evaluate the association between gingivitis and contamination statues of toothbrush, effectiveness of disinfectants and duration of using the toothbrushes.
The study was conducted on 63 patients and their toothbrushes. Gingival status of participants were evaluated by measuring the gingival index then the patients were asked about the duration of using his/her toothbrush and asked to bring his toothbrush for assessment of contamination status with regards to(Staphylococcus aureus) as well as for evaluation of efficacy of two types of disinfectants. The toothbrush samples were collected and subjected to quantitative assay of microorganisms. The efficacy of chlorhexidine (0.2%) and sodium hypochloride (1%) were assessed at 1, 2 and 3 hours. The data were compared by using student T test, ANOVA and LCD test and t- test. The gingival in dex was higher with patients using the toothbrushes for longer duration, the S.aureus was isolated from all tooth brushes as well as the degree of contamination was increased with increased the duration of use. The chlorhexidine and sodium hypochloride were effective in decontamination of toothbrushes but their efficacy was reduced with increasing the period of using the toothbrush. The gingivitis might be exacerbated by prolong using of toothbrushes, contamination correlated directly with duration of using the toothbrushes and efficacy of disinfectant inversely correlated to duration of use.

Key words: Gingivitis , toothbrush, chlorhexidine, sodium hypochloride.

Introduction:

The main goal of toothbrushing is to remove the dental plaque which is responsible for a number of oral diseases such as tooth decay, gingivitis and periodontitis. The brushing removes the oral biofilm as well as soft debris out of the mouth especially from the tooth and tongue surfaces [1].Toothbrushes play a significant role in disease transmission and increase the risk of infection since they can serve as a reservoir for microorganisms in healthy, oral-diseased and medically ill adults. The care and maintaining of the toothbrush are important for the good oral hygiene and health as well. Although the toothbrush is not the ideal environment for microorganisms' growth, it is capable to obtain life of the microbes [2]. Teethbrushes are sterile before use and got contamination by different bacterial species, viruses and fungi from the mouth and environment immediately after the first use where a biofilm that contain different microbial agent develop in the toothbrushes with using[3]. In addition to oral cavity and its environment, the toothbrushes can become contaminated from hands, aerosol and storage containers. Bacteria which attach to, accumulate, and survive on toothbrushes may be transmitted to the individual causing disease [4].The toothbrushes get contamination within thirty seconds to four minutes after first use and the microorganism remain viable for periods ranging from 24 hours to 7 days[5].The contaminated toothbrushes might play a role in systemic and oral diseases; Injuries to oral tissues are aggravated by the use of contaminated toothbrushes when compared with sterile ones and may even cause septicemias after brushing. Transient

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bacteremia can be induced by tooth brushing, increasing the potential risk of transmission, which may be exacerbated in people with gingivitis and periodontitis [6].The colonization of these pathogenic micro-organisms on toothbrush while being stored in unsanitary conditions represents a potential cause of re-contamination of the oral cavity [7].The tooth brush may get contaminated by Streptococcus, Staphylococcus, and lactobacilli [8]. These bacteria are implicated in the causation of many life threatening diseases such as infective endocarditis besides influencing the occurrence of oral diseases such as dental caries and gingivitis [7- 9]. The toothbrushes are often preserved in bathrooms which are a good place to harbor millions of different pathogenic micro-organisms. This neglect in the proper maintenance of toothbrushes is attributed to lack of public awareness on the possibilities of toothbrush contamination while stored in sanitary settings [10]. Different brushing techniques have been described in the literature, but there is inadequate information about the maintenance of toothbrushes to avoid their contamination with micro-organisms. Hence there is a need for disinfection methods that are rapidly effective, non-toxic and that can be easily implemented. Modern dentistry strongly emphasizes on prevention and biosecurity regarding how toothbrushes should be appropriately stored, used and disinfected. It is essential to decontaminate toothbrushes in order to eliminate pathogenic microorganisms transmitted to used toothbrushes from oral cavity or from other toothbrushes and storage area [11]. Many studies have demonstrated the efficiency of various methods in disinfecting oral microorganisms on toothbrushes such as UV radiation, microwave radiation, ozone inhibition, and chemical disinfection [12]. In spite of novel methods of decontamination, a method that is highly effective and user-friendly is using chemical agents. Caudry and colleagues [13] showed that there was a significant reduction of oral bacteria after immersing toothbrushes in Listerine mouthwash for 20 minutes. Mehta and colleagues [14]demonstrated that there was a significant reduction of oral bacteria after soaking toothbrushes in 0.12% chlorhexidine (CHX) gluconate mouthwash .There were also few studies which reported the efficiency of 0.12% CHX in a different preparation such as mouthwash and spray in disinfection of children's toothbrushes [15, 16].

Aims:-To evaluate the association between gingivitis status, contamination statues, effectiveness of disinfectants and duration of using the toothbrushes.

Methodology:

A total of 63 patients who were attended to dental clinic of AL-Mustansyria from Oct.2013 to Nov.2016 were enrolled in this study, gingival index was assessed clinically and degree of contamination of toothbrushes and efficacy of disinfectants was assessed in microbiology unit according to duration of using the toothbrushes. Patients who were on antibiotics in the last two weeks, using mouth gurgle, used disinfectants or any others methods of sterilization for the toothbrushes were ruled out of study.

Microbiological procedures:

The head of toothbrushes were immersed in peptone(bacterial broth) solution for either (3 or 4 hours) at $37C^0$ then 1 ml from bacterial broth was taken and cultured on selective and nonselective media (nutrient agar, manitol salt agar MSA and blood agar). The culture incubated for 24 hours at 37 C^0, then the Total Colony Forming unit (TCFU) for *S.aureus* on MSA agar was manually counted. By the *S.aureus* was identified by using Gram stain, culture on selective media and biochemical tests including catalase, coagulase, DNase tests.The disinfectants (chlorhexidine 0.2% and $NaHCO_3$) was prepared, numbered as 1, and 2, then the head of toothbrushes of different duration of use was immersed to these solution for either 1, 2, 3 hours then1 ml of theses solution was taken for cultrure in selective media and *S.aureus* was counted by total colony forming method [14].

Ethical issue:

Approval was obtained from concerned authorities, patients name were kept anonymous.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS) version 20 was used for data entry and analysis, mean and standered deviation was used to represent the continuous data. Independent student T test, ANOVA, Last Significant Difference (LCD) and Pearson correlation were used for data analysis. P-value ≤ 0.05 considered significant.

Results:

A total of (63) patients with mean age of 27.9 ±8 years, 30(47.6%) was females and 33(52.4%) was males were included in this study. The mean colony count of S.aureus isolated from toothbrushes which were used for 4 months was more than those used for 2 or less than 2 months and this difference were statistically significant as seen in Table(1). The correlation between colony count and duration of use of toothbrush was positive correlation (r = 0.4). The finding of current study showed that the mean value of gingival index was higher in patients who were used the toothbrush for more than 4 months than those who used brusher for 2-4 months or less than 2 months and this difference was statistically significant as seen in Table (2), even with multiple comparison the same significant difference was reported. The results showed that the mean value of gingival index not differed significantly when compared according to gender and duration of use the tooth brushes as seen in Table (3). When the toothbrushes immersed in chlorhexidine (0.2%) the results showed that the colony count of S.aureus was significantly decreased with time of using the chlorhexidine (0.2%) as well as the colony count was differed significantly according to duration of using the toothbrushes per each hour of using the disinfectant where those which were used for longer duration showed less response to disinfectant agent as seen in Table (4) and Fig. (1).The correlation between reduction of colony count and each of hours of immersion and duration of use of toothbrush at 1 hour of immersion was inversely correlated (r =- 0.6 - 0.3) respectively. When the toothbrushes immersed in sodium hypochloride (NaHco), the results showed that the colony count of staph.aureus was significantly decreased with time of using the agent as well as the colony count was differed significantly according to duration of using the toothbrushes per each hour of using the disinfectant where those which were used for longer duration showed less response to disinfectant agent as seen in Table (5) and Fig.(2). On comparison the effect of disinfectant on toothbrushes the results showed that theineChlorhexidine (Chx) induce more effect than sodium hypochloride (NaHco) but no significant difference was reported between them as seen in Table (6).

Discussion:

Oral cavity is especially colonized by Staphylococcus sp., Streptococcus sp., Neisseria sp., Bacteroides sp., Actinomyces sp., Treponema sp., Mycoplasma sp. These microorganisms can settle the toothbrushes, as well, including the organisms which are not normally associated with the oral flora, like the family of Enterobacteriaceae, [17,18]Although S.aureus belongs to the oral microflora (found in 5% of the analyzed toothbrushes), more attention should be paid due to the possibility for it to cause not only a number of oral diseases, but opportunistic infections as well; therefore, its presence is very important in individuals with impaired health. In study conducted by Pesevska S et al [3] stated; the repeated usage of the toothbrushes in a longer period of time is one of the greatest factors that promote the bacterial survival. After every-day use of the toothbrushes in a period of one month and they found in 55% of the tested samples of toothbrushes only one bacterial species was determined, while in 45% there was contamination with two aerobic bacterial species . With time, conditions for growth of anaerobes

occur gradually, which is proven by the finding of one anaerobic species on the toothbrushes in 5%. Warren D.P and his colleagues [19] found that toothbrushes that are kept in wet conditions, like the bathrooms and non-covered toilets are an origin of fecal bacteria and microbes that are being spread in the air through the aerosols. Wet conditions in the bathrooms may facilitate bacterial growth and crossed contamination when toothbrushes are kept in a bathroom, in a mutual holder without a head cap, either through a direct contact, contaminated fingers and skin commensals, or due to the usage of a mutual toothpaste [20]. Downes J et al and Saravia M.E et al [2,21] demonstrated that the toothbrush contamination in healthy individuals happens early after the first use, and it gets higher with its further usage and these finding in line with our finding in this study. Bunetel et al [22] has found that the toothbrushes in patients with oral diseases are easily being contaminated, and the patients with oral inflammatory diseases responded well to the treatment when they replaced the used toothbrushes with new ones often (i.e. replacing the toothbrush every two weeks) and this finding may explain our finding that gingival index was high with those who used the toothbrushes for longer period where the degree of contamination increased with time and even the response to disinfectant was reduced with time of using the toothbrush . Bello et al [23] discovered that S.aureus, Staphylococcus epidermidis, Escherichia coli and Proteus sp all survived over a period of six days, though there were reductions in total plate counts but negligible after abandoning the toothbrushes so that mean the leaving the toothbrush unused do not eliminate the contaminants and the need for disinfectant or other mode of sterilization are the only way to get rid of contamination. Glass (1992a) [24]found that toothbrushes contaminated with potentially pathogenic bacteria and viruses such as S.aureus, E. coli, Pseudomonas sp and herpes simplex virus from both healthy patients and patients with oral disease and this finding reflect the importance of using the disinfectant for toothbrushes to eliminate the source of infection and protecting the healthy subjects.Our finding demonstrated that chlorhexidine (0.2%) and 1% sodium hypochlorite are effective for decontamination of toothbrush and chlorhexidine had have some superiority on sodium hypochlorite and this finding in agreement with finding of study conducted by Nelson-Filho P et al [6]where they showed that immersion of toothbrushes for (20 hrs.) in 0.12% chlorhexidine gluconate or 1% sodium hypochloride was efficient for disinfection (100% inhibition). We found that the degree of contamination of toothbrushes was increased with increasing the period of use and these finding in line with many recommendation of researchers who reported the importance of changing the toothbrushes after short period of use where the American Dental Association recommends a routine change of toothbrushes every 3 months [25], Glass[27] specifically recommended that healthy patients replace their toothbrush every two weeks. Aouthors[6] reported that patients on Chemotherapy or immune-suppressed patients should change their toothbrushes every three days, and persons submitted to major surgery should change their toothbrushes every day. Many patients, however, reported psychological, economic, and environmental barriers to changing their toothbrushes so frequently. Establishing an easy and effective method for disinfecting a toothbrush would be an important. We isolate the S.aureus from all toothbrushes and this finding is partly in agreement with Samuel O and Abdfeanyi O [28]where they reported that S.aureus was most frequently isolated from the used toothbrushes, with a percentage occurrence of 30% while Enterobacter aerogenes had the lowest percentage occurrence of 6% on the used toothbrushes examined .Sammons et al[29] isolated Staphylococci (48%), Presumptive coliforms (28%) and Pseudomonas (16%) from used toothbrushes while Osho et al [30]isolated Escherichia coli (10%), Enterobacter (10%), S.aureus (20%), Staphylococcus saprophyticus (20%) and Pseudomonas aeruginosa (40%). Malmberg et al [31] isolated Streptococci (50%) and

Staphylococci (86%) from used toothbrushes.

Conclusions and Recommendation:

It was concluded in this study that gingivitis was exacerbated by increasing the duration of using the toothbrushes as well as the contamination of toothbrushes was increased with increasing the duration of use in addition the response to disinfectants was reduced with increasing the duration of use. Toothbrushes must be routinely changed and to be not used more than one month.

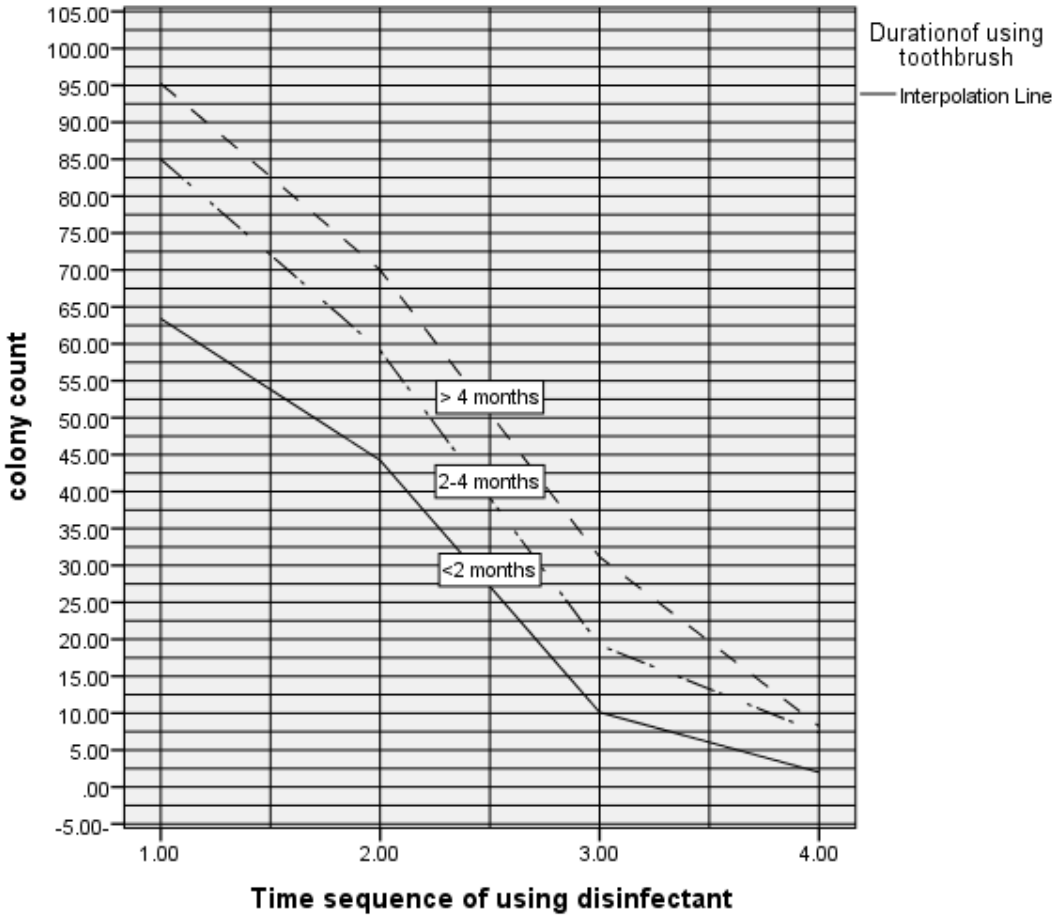

Fig.(1): The mean colony count of staph.aureus according to duration of using the toothbrushes and time sequence of using the chlorhexidine.

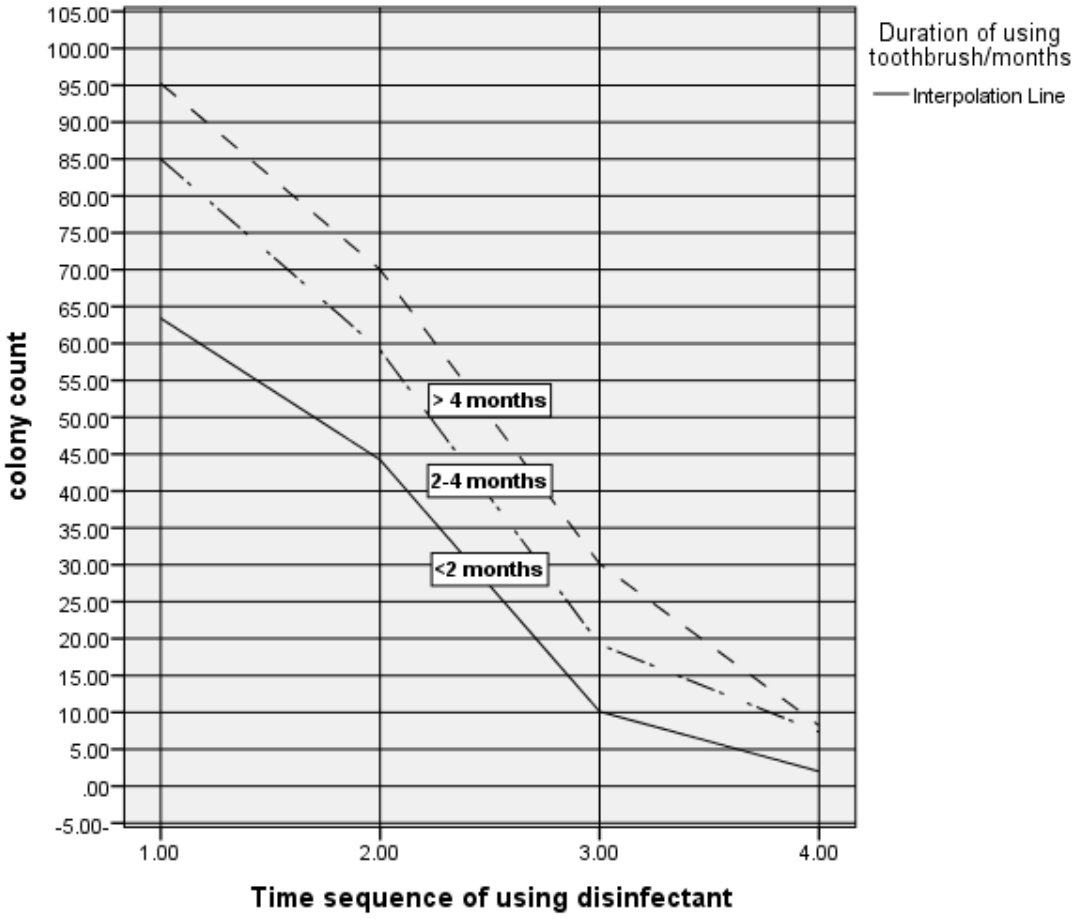

Fig.(2): Mean colony count of staph.aureus according to duration of using the toothbrushes and time sequence of using the Chlorhexidine.

Table (1): The mean colony count of S.aureus isolated from toothbrush with different duration of use.

Duration of using toothbrush	No.	Mean(TCFU/ml)	SD	p-value
< 2 months	19	63.0	2.1	0.001
2 - 4 months	25	85.1	1.9	
> 4 months	19	98.2	7.2	

Table (2): The mean of gingival index with different time of using the toothbrush.

Duration of using toothbrush	No.	Mean(GI)	SD	p-value
< 2 months	19	1.4	0.1	0.01
2-4 months	25	2.3	0.2	
> 4 months	19	3.3	0.2	

Table (3): The mean values of gingival index according to gender and duration of use the toothbrushes.

Duration of using toothbrush		**Gender**	**No.**	**Mean**	**SD**	**p-value**
< 2 months	Gingival index	females	12	1.4	0.1	0.2
		Males	7	1.4	0.2	
2-4 months	Gingival index	females	12	2.4	0.1	0.1
		Males	13	2.3	0.2	
> 4 months	Gingival index	females	6	3.5	0.2	0.3
		Males	13	3.3	0.2	

Table (4): The mean colony count of *S.aureus* according to duration of using the toothbrushes and time sequence of using the chlorhexidine.

Duration of using the toothbrush	**Time sequence of using disinfectant**							
	baseline		**After1 hour**		**After 2 hours**		**After 3 hours**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
< 2months	63.4	2.8	53.0	14.2	10.1	6.8	0.0	0.0
2-4 months	85.1	1.8	62.	7.8	19.4	4.9	3.3	0.8
>4 months	98.8	6.9	70.1	2.2	26.5	3.6	7.2	1.0

(P = 0.001 for all)

Table (5): The mean colony count of S.aureus according to duration of using the toothbrushes and time sequence of using the chlorhexidine.

Duration of using the toothbrush	**Time sequence of using disinfectant**							
	baseline		**After1 hour**		**After 2 hours**		**After 3 hours**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
< 2months	62.5	0.8	40.6	6.60	18.4	5.29	3.3	0.8
2-4 months	85.0	2.1	63.0	9.17	24.9	1.68	7.0	2.0
>4 months	97.7	7.8	74.3	5.01	33.8	7.79	16.5	6.0

P value=0.001 for all

Table (6): The means colony count of staph.aureus according to disinfectant agents and duration of using toothbrushes.

Duration of using the toothbrush		Disinfectant agents			
		Chx		NaHco	
		Count		Count	
		Mean	SD	Mean	SD
< 2 months	baseline	63.4	2.8	62.5	0.8
	1 hour	53.0	9.6	40.7	6.0
	2 hours	10.1	6.8	18.4	5.2
	3 hours	0.0	0.0	3.3	0.8
2-4 months	baseline	85.	1.8	85.0	2.1
	1 hour	62.0	7.8	63	9.1
	2 hours	19.4	4.9	24.9	1.6
	3 hours	3.3	0.8	7.0	2.0
> 4 months	baseline	98.8	6.9	97.7	7.8
	1 hour	70.1	2.2	74.3	5.1
	2 hours	26.5	4.6	33.8	7.7
	3 hours	7.2	1.0	16.5	6.0

(P = ≥ 0.05 NS)

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