Microbiological and Cytological Response to Dental Implant Healing Abutment

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Abstract

Background and aim is dental implant could induce inflammation due to microbial invasion of pathological type.

15 submerged titanium implants at the second-stage of surgery were used for microbiological and cytological examination.

There was no statistical differences between stage 1 and stage 2 regarding inflammatory response for both microbial and cytological examination.

There were no obvious changes in microbial and cytological data between implant site and adjacent teeth in two stages of the study.

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Clinical article (J Int Dent Med Res 2017; 10(3): pp. 891-898)

Keywords: Dental implant, Peri-implant, Microbiology, cytology.

Received date: 22 July 2017 **Accept date:** 27 September 2017

Introduction

Dental implants have become an important option in treatment plans in dentistry to replace missing teeth.[1] Implant placement procedure can performed using local anesthesia or sedation procedure or under general anesthesia.[2] However; implant failure and peri-implant diseases are still a problem facing implantologists.[3] A successful implant treatment depends on many factors including inflammation of peri-implant tissues, and host-body rejection.[4,5,6,7]

The exposed surface of the implant of the oral cavity gets colonized by different micro-organisms.[8] Many studies indicated that bacterial colonization may occur within 30 minutes after implant placement with different types of microorganisms.[9]

Despite some characteristics of dental implant surface such as roughness, type and shape of the surface of materials used for dental implants affect osseointegration,[10] but these characteristics have some influence on the bacterial colonization or bacterial biofilms formation, as the biofilms on the dental implant surface are the main source of pathogens for peri-implantitis.[11] In addition to that and as a result to the accumulation of plaque on implant surface, dense inflammatory infiltration occurs in connective tissue, which weakens the attachment of gingiva over and around the implant.[12]

Ericsson et al[13] identified two types of cytological entities in the implant crestal region: (a) Plaque-associated inflammatory cell infiltrate (PaICT) and (b) implant-associated inflammatory cell infiltrate.

Apical progression of the plaque is associated with clinical and radiographic manifestations of tissue injury. The density of inflammatory infiltration in mucosa and bone resorption is higher around implants compared to teeth. In addition to that; peri-implant lesions involve the supra-crestal connective tissue and damage to bone marrow.[12]

Periodontal pathogens which are opportunistic like P.gingivalis, Prevotella intermedia, Peptostreptococcus microbes and Fusobacterium nucleate, Actinobacillus, Actinomycetemcomitans, are seemed to be responsible for peri-implantitis in partially

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edentulous patients.[14]

Investigations of the presence of seven pathogenic microorganisms that are responsible for periodontal diseases were done before implant placement and 1 year after loading. They found that in half of individuals with healthy periodontium, there was subgingival biofilm harbor by periodontal pathogens above threshold values[15]while Keller *et al*[16] compared clinical and microbiological features in the peri-implant area of implants carrying either screw retained or cemented supra-structures or investigated the relationship between microflora in the peri-implant area, the inner surface of removable superstructures, and the periodontal microflora within the same subject. They concluded that the leakage of microorganisms through the gap between the superstructure and the abutment plays an important role in the bacterial colonization of the inner surface of crowns and bridges and they found that oral flora affects the implant colonization by different microorganisms.
Cytology represents an aid in the early diagnosis of many diseases but their use in peri-implantitis is not yet commonly used. Very few studies had used cytology in relation to dental implant.[17]

To our knowledge the relation between cytological changes around and inside implant bed with microbiota has not studied in Iraqi patients before.

The aim of this study was to assess the cytological and microbiological changes during and after dental implant in the oral cavity of a sample of Iraqi patients with dental implant.

Materials and methods

2.1 Sampling protocol and clinical criteria:

Ten female patients with total 15 submerged implants were included in this study. They attended Implantology Clinic of the Oral Surgery and Periodontology Department, College of Dentistry, Mustansiriyah University at period between December 2013 to May 2014. Their age ranged from 20-50 years old. The examination of the results continued to August 2016.

All patients gave their consent to use their data for research purposes. The research approved by the scientific committee of Oral Surgery and Periodontology Department, College of Dentistry, Mustansiriyah University.

2.1.1. Inclusion criteria:
Patient with the following inclusion criteria were enrolled in this study: age >18 years, having good oral hygiene, absence of any evident local or systemic diseases and having implant was placed using two stage surgery protocol and it was completely buried under mucosa [had undergo just first stage surgery and with an appropriate healing time of (3-6 months).
A total of 15 titanium implants (Euro technical, Naturactis, Sallanches, France) had been included in the study.

2.1.2. Exclusion criteria:
Patients with Diabetes Mellitus or other systemic disorders as cancer or autoimmunity, pregnancy or lactation, condition that required using systemic/or oral antibiotics within the last 3 months, or systemic anti-inflammatory drugs and smoking. While for the local factors; periodontitis or other oral lesions as well as lack of cooperation, at the time of sampling were all excluded from the study.

2.2 Experimental set:

The second stage surgery was considered as a baseline, all implants were surgically exposed, and healing abutment from the same implant system was used for all patients (Euro teknika, Naturactis, Sallanches, France).
Cytological and bacteriological samples were taken at the second-stage surgery immediately during exposing dental implant and considered as pre-exposing stage (stage1) from 3 sites; from the mucosa above the unexposed implant (A1), from the surface of exposed implant after incision (B1) and from the gingiva around the adjacent tooth which considered as control group (C1). The second samples were taken after 3 weeks of exposing dental implant after total healing of the mucosa (stage 2) from the mucosal tissue around the abutment (A2), from implant bed (B2), and from the gingiva around the adjacent tooth (C2) before taking impression.

2.2.1Cytological sample:
Cytological brush that is used for interdental cleaning of dental plaques was used to scrap the mucosa above dental implant area, and in adjacent area. These samples were considered as mucosal sample while another cytological sample was collected from the abutment bed by scrapping the cover screw after exposing of implant area in the stage 1 of the experiment and from healing abutment screw in the stage 2 of the experiment and this was considered as blood sample.
The sample was spread on clean glass slides,

fixed with 95% ethanol spray and left to dry. After that; the slides were stained by Giemsa stain for 10 minutes, then washed thoroughly by distilled water to remove the excess stain.

A light microscope (Kruss, Germany) was used to examine slides under magnification (x40) for detection of certain inflammatory cells (neutrophils and lymphocytes). The assessment of inflammation was a modification of methods used by Alka *et al.* [27] as follows:

Grade 0: No inflammatory

Grade me (mild): Few neutrophiles and lymphocytes in the background of epithelium or blood cells.

Grade II (moderate): Moderate infiltration of inflammatory cells.

Grade III (severe): Severe infiltration of inflammatory cells.

2.2.2 Bacterial samples:

Sterile disposable swabs with transport media (AFCO transport swab, Jordan, Code No.T1106) were used to take the bacterial samples from patients before exposing the abutment from the same 3 sites that were used for cytological samples. Samples were all swabbed on glass slides heat fixed and gram stained for direct microscopical examination and transferred after a short period of time (less than one hour) to the laboratory to be cultured on blood agar, MacConkey agar, chocolate agar, Colombia blood agar for bacterial diagnosis and on brain heart infusion agar media for cryptoccocus species, Sabaroud dextrose agar for candida species. A set of different media(84plates for aerobic and 84 plates for anaerobic) was cultured to notice biochemical reactions as sugar fermentation like lactose , manitol , urea decomposition, catalase test ,coagulase test ,partial or complete blood haemolysis, IMVIC test ,and sensitivity to specific antibiotics, like vancomycine and clindamycine metronidazole and kanamycine following schemes of diagnostic microbiology for each bacteria[18]

The aerobic set kept at 37C for 24 hours at aerobic conditions. The other set was kept in anaerobic jar with gas pack (CO_2 gas generating box, bioMerieux) to be held for 72 hours in absolute anaerobic condition till the end of 72 hours. Colonies were inspected by microbiologist consultant to diagnose different colonial morphology and, to examine each smears stained with gram stain to show gram reaction and bacterial morphology ,5 separated entities were noticed :Higher bacteria, diphtheroid, yeasts, gram positive cocci and gram negative bacilli. The same experiment was performed in the stage 2 after total healing of the mucosa around the abutment and before taking the impression for the final implantation.

Statistical analysis:

The obtained data was processed using SPSS14, to express the results. Comparison between microbes at the implant site and the control sites done by Pearson correlation test, frequencies and percentages.

Results

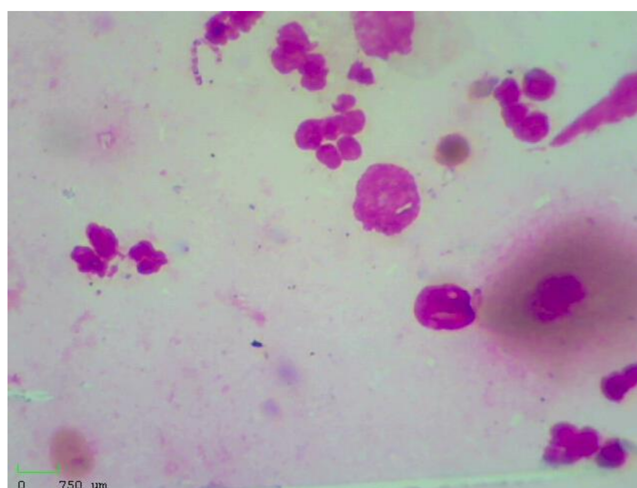

Figure 1. inflammatory cells in oral epithelium.

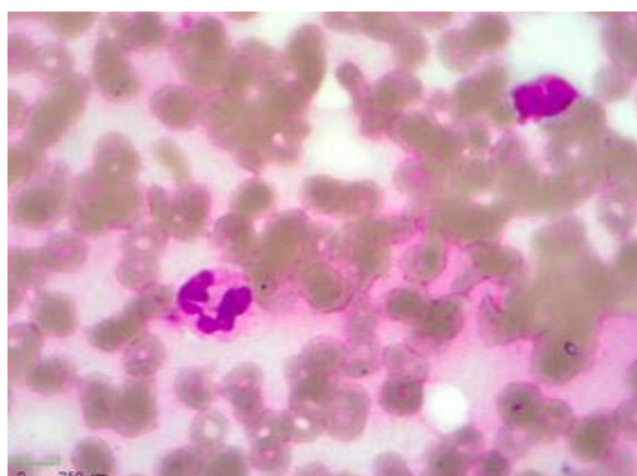

Figure 2. Inflammatory cells in blood sample.

A-Clinical observations: all implant sites were healed without post- surgical complications as perimucitits and perimplantitis. The patients did not suffer from severe pain or trauma that may cause disturbance in ordinary diet intake that may affect the health status of the patients.

B-Cytological results: the data collected from abutment's mucosa (A1, A2) and abutment bed (B1, B2) in addition to adjacent tooth's gingiva (C1, C2) were looking for inflammatory cells distribution in form of neutrophils and lymphocytes. The results are shown in table (1)
Most of the inflammatory reactions were seen in the stage 1(A1) in the wound area over the abutment. Figure (1), (2)

C-Microbiological results:

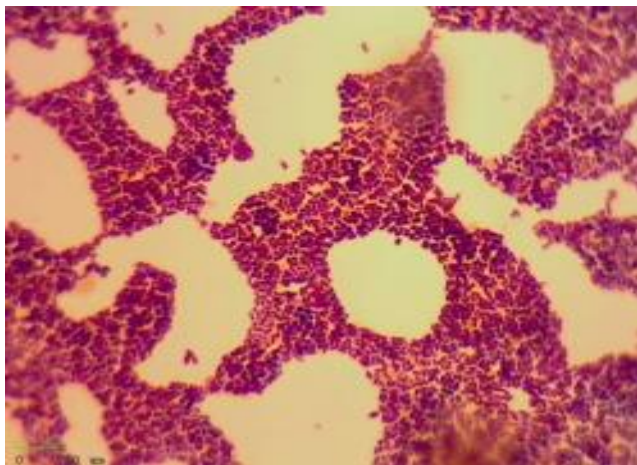

Figure 3. yeast with gram positive cocci.

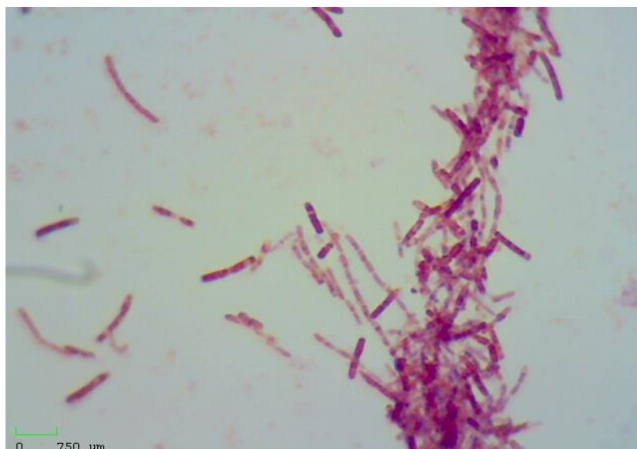

Figure 4. Actinomycete isolated from samples.

During this study; each site was swabbed and studied separately, direct microscopical examination was done for each sample to reveille the type and objective numbers of each microorganism for each gram stained slides.
Most slides showed more than one microorganism in each site to be compared to the adjacent mucosa and blood in implant bed. Basically five groups of microorganisms were found around the implants and the teeth. Those include higher bacteria, diphtheroid, yeast, gram positive cocci and gram negative bacilli in aerobic condition and exclusion of yeast in anaerobic condition.

Higher bacteria in our study showed slight difference between the aerobic and anaerobic cultures and in different observed sites. But mostly the abutment area in the stage 1 showed higher frequency more in the adjacent area.

Yeasts were the major fungi noticed in some sockets and mainly composed of Candida species with or without pseudo hyphae and some sites reviled a heavy growth of cryptococcus neoformans and could be found in both situation aerobic and in microaerophilic status at the implant's site which is a very interesting finding in our study group because it is an opportunistic fungi.

Gram positive cocci were mainly staphylococci species and streptococci species mainly of peptostreptococcus and streptococcus mutants isolated on special media 28. Their frequency was mostly higher in stage 2 in all sites whether aerobic or anaerobic.
Some bacilli were (pleomorphic bacilli as diphthriods) as part of normal flora of the mouth or of lactobacilli species.

As for the gram negative bacilli; the aerobic species were mainly enterobacteriaceae group; lactose fermenter or non-fermenter.
Porphyromonas gingivalis, actinomycete and fusobacterium were the main isolated anaerobic gram negative. Figures (2, 3, 4)

Percentage frequency of these bacteria in the all site's samples is illustrated s in table (2). Strong positive correlation was seen between all types of aerobic and anaerobic microbes mostly in blood samples, mucosal samples and adjacent teeth subsequently, negative correlation seen between different types of microbes was noticed in 4 correlations in all studied groups as shown in table (2).

Discussion

Dental implant as any other manufactured body's implant represents as a forging body that could be exposed to different body's reaction either localized which is characterized by simple inflammatory reaction (peri-implant mucositis and peri-implantitis), or rejection as a sever systemic reaction which could be varied from one patient to another.

In current study there were no obvious

signs of inflammation may be due to short period of time from time of exposing dental implant to the time of impression. Failures of dental implant could be late failures which occur during the first year of implant's loading and attributed to the overloading of the implant placed in a weak bone.[12, 19]

There were some microbial changes between the two stages of the implantation process that could affect the success of implantation. The main changes were in the oral microflora at the abutment area with the adjacent teeth as gram positive cocci (aerobic and anaerobic)and bacilli(aerobic and anaerobic)both showed an increase in the numbers as demonstrated in the table (1). This disagreed with Grover *et al*[20] who compared both stages of the implants but did not find any microorganisms in the studied groups and Leonhardt *et al*[21] who did not find any microbial difference between tooth and implant sulci with 4 mm pocket depth. At the same time, there were no statistically significant differences between adjacent teeth and shallow implant healing abutment bed in terms of microflora.

Higher bacteria count in terms of number of colonies counted was higher in frequency in the mucosa over the implant in stage 1 than stage 2. This may be due to accumulation of dental plaque more frequently on the dental implant than normal teeth surface. This finding was in agreement with Misch[22] who stated that shape, type and design of the implants are the determining factors involved in the development of peri-implantitis.

At the same time; the aerobic type of higher bacteria frequency was more than the frequency of anaerobic type which indicated that implant condition may not enhance the growth of anaerobic type of higher bacteria as oral actinomycetes.

Diphtheroid growth not affected in stage 1 for both aerobic and anaerobic conditions; but in stage 2; there was slight increase for anaerobic condition that may be due to tendency of anaerobic type to grow inside and around implant. Diphtheroid bacilli which are aerobic, non-sporulation, pleomorphic Gram-positive bacilli are usually commensals of the skin and mucous membranes and often found in the healthy mouth and pharynx as anaerobic commensals but some authors suggested that these organisms can cause many infections.[23,24]

Gram positive microorganisms were seen in our study showed increased in stage 2 for both conditions but in aerobic condition were higher and were mainly staphylococci and streptococci. This in agreement with[25] who stated that gram positive cocci were the observed mostly in implant sulci were of less than 5 mm.

Their presence in the abutment area (over and inside) was more in the stage 2 for both aerobic and anaerobic. This could be due to the type and shape of the surface of materials used for dental implants has an influence on bacterial colonization.[11]

The adherence effect of streptococcus on the alloy used in the manufacture of implants was evaluated by Sardin et al,[26] he concluded that the alloy used might be a contributing factor in the attachment of microorganisms and the development of infection around the implants. The alloy used in the fabrication of these implants might have served as a contributing factor in the attachment of Gram-positive anaerobes. From other site[11] found no streptococcus species around and in sulcus of implanted abutments.

Staphylococcus is one of the main microorganisms for peri-implant infections but their presence in the present study may indicate no correlation to periodontal and peri-implant disease.[28]

Also, the amount of gram positive anaerobic species in implant bed was quite similar to adjacent tooth surface in the present study, which supports the findings of other researchers who found that the microbiota of healthy peri-implant tissue and around the normal teeth was similar which is characterized by the presence of small number of gram-positive facultative cocci and rods.[15]

For gram negative bacteria in present study was higher in their frequency in stage 2 for both conditions but for anaerobic condition was higher than aerobic in the 2 stages. This could be due to transition from health to disease (peri-implantitis) due to a shift of the microflora from predominantly gram-positive to gram-negative bacteria.[29]

The absence of inflammation may be associated with absence of spirochete species .This in agreement with Puchades *et al*[30] study that was associated with acute periodontal diseases stated that spirochetes are the main bacteria of the Astra and Branmark implants; but

in the present study they were not found. This could be attributed to the effect of fixture's surface conditions to the microflora of the sulci.

Yeast was the most frequent microorganism in this study in the stage 1 for aerobic condition mainly over the abutment area as compared to stage 2. This may be due decrease in oral hygiene behavior or reduction in body immunity that favorites the growth of yeast.

This agreed with[11] who found that aerobic microbiota was found in all patients, and resolute by its morphological characterization and biochemical activity including fungus (Candida albicans) were isolated.

There was highly significant correlation between different species of microbes available at implant sites in the two stages especially group A. There were only 4 negative correlations between 3 different groups as shown in table (2). In comparing the cytological changes with microbial changes; there were some changes in the microbial growth as a signs of inflammatory response as. This may indicate that the microbial changes related to dental implant do not cause inflammation which could lead to peri-implantitis and this was confirmed by the absence of clinical signs of peri-implantitis.

Conclusions

Dental implant caused certain changes in microbial flora of the oral cavity during the comparism between the implant and normal teeth and in 2 stages of the study. These changes were within the normal range that was confirmed by cytological results which assessed the presence of inflammatory cells.

Acknowledgements

The author would like to thank Mustansiriyah University (WWW.uomstansiriyah .edu.iq) Baghdad-Iraq for their support of the present work.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

groups	Microorgansime types										Inflammatory cells	Inflammatory grade
	G+/ar	G+/an	G-/ar	G-/an	HB/ar	HB/an	DIP/ar	DIP/an	Yeast/ar	Yeast/an		
A1	7 (46.7%)	2 (13.3%)	4 (26.7%)	9 (60%)	4 (26.7%)	1 (6.7%)	4 (26.7%)	2 (13.3%)	8 (53.3%)	N	1(6.7%)	Mild
B1	6 (40%)	3 (20%)	2 (13.3%)	7 (46.7%)	3 (20%)	1 (6.7%)	3 (20%)	4 (26.7%)	8 (53.3%)	N	4(26.7%)	Mild
C1	6 (40%)	3 (20%)	4 (26.7%)	7 (46.7%)	0	1 (6.7%)	1 (6.7%)	1 (6.7%)	9 (60%)	N	2(13.3%)	Mild
A2	9 (60%)	5 (33.3%)	7 (46.7%)	12 (80%)	2 (13%)	0	4 (26.7%)	2 (13.3%)	4 (26.7%)	N	6(40%)	moderate
B2	4 (26.7%)	2 (13.3%)	2 (26.7%)	9 (60%)	2 (13%)	3 (20%)	2 (13.3%)	3 (20%)	4 (26.7%)	N	2	mild
C2	7 (46.7%)	2 (13.3%)	4 (26.7%)	9 (60%)	2 (13%)	1 (6.7%)	4 (26.7%)	2 (13.3%)	8 (53.3%)	N	4(26.7%)	mild

Table 1. Frequency distribution of aerobic and anaerobic micro-organisms types among studied groups in addition to inflammatory cells frequencies (A1,A2)mucosal samples(B1,B2)blood samples(C1,C2) control or adjacent tooth samples.

Group(A)	P value	Sig.	Group(B)	P value	Sig.	Group(C)	P value	Sig.
G+(an)1 vs HB(ar)1	.555	S	G-ve(ar)2 vs G+(an)2	.577	S	G+(an)1 vs G-ve(an)2	.564	S
G+(an)1 vs HB(an)2	.681	HS	G+(an)2 vs D (an)2	.555	S	G-ve(an)2 vs G+(ar)2	.600	S
G+(ar)2 vs Ye(ar)1	.612	S	G+ (ar)1 vs G+(an)2	.577	S	G-ve (ar)1 vs G-ve(an)2	.564	S
G+ (ar)2 vs Ye(an)1	-.533	R S	HB(ar)1 vs D (an)1	.784	Hs	G-ve(ar)2 vs Ye(ar)1	-.535	S
HB(ar)1 vs HB(an)1	.555	S	Hb (ar)1 vs G+(an)1	.583	S			
HB(ar)1 vs HB (ar)2	.555	S	D (ar)2 vs D(an)2	.650	HS			
HB(ar)2 vs Ye (ar)2	.555	S	D (ar)2 vs D (ar)1	.650	HS			
HB (ar)2 vs Ye(ar)2	-.555	S	D (an)2 vs G+(an)2	.555	S			
HB (ar)2 vs Ye(an)2	.681	HS	D (an)2 vs D (ar)1	,659	HS			
D(an)2 vs D(an)2	.535	S	D (an)2 vs HB (ar)2	.650	HS			
D (an)2 vs D(ar)1	.535	S	D(an)1 vs HB(ar)1	.787	HS			
			D (ar)1 vs G+(ar)2	-.650	RS			
			D (ar)1 vs (ar)2	.650	HS			
			D (ar)1 vs D (an)2	.659	HS			
			Ye (ar)2 vs HB (ar)1	.583	S			
			Ye (ar)2 vs G –ve(an)1	.612	S			
			Ye (ar)2 vs G+(an)2	.707	HS			

Table 2. Pearson correlation between groups (A) mucosal samples (B) blood samples (C) control group or adjacent teeth (G-VE: gram negative, G+VE: gram positive, HB: Hylobacteria, D: Diphthroid, Ye: yeast, ar: aerobic, an:aneorbic).

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