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Effect of Iodoform and Different Implant Abutment Connection Designs on the Bacterial Leakage at the Implant-Abutment Interface: An In-Vitro Microbiological Analysis

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ABSTRACT:
AIM: - This in vitro study was conducted to evaluate the effect of iodoform and different implant abutment connection designs on the bacterial leakage at the implant-abutment interface.
MATERIAL AND METHODS: - 40 dental implants with morse taper connection (n=20) and internal hexagon connection (n=20) were investigated. For both the groups, 10 implants were treated with iodoform paste and 10 implants were considered as control group. IA assemblies from all the groups were inoculated with E. faecalis suspension and then immersed in 5Ml contaminated BHI broth for 2 weeks. Later, samples were taken from internal parts of all the implants. Bacterial leakage was determined by observing the turbidity of broth and later colonies were identified by colony forming units CFUs.
RESULTS: - Results showed that control groups of both the connection design had statistically significant more CFU count compared to the iodoform groups. Also, implant samples with internal hexagon connection showed statistically significant high CFU count in comparison to implant samples with morse taper connection.
CONCLUSION: - The findings demonstrated that the iodoform paste-based antimicrobial treatment was ineffective at blocking the microleakage and microbial colonization at the implant-abutment interface in both the internal hexagon and morse taper connections. Though, the colony counts were significantly decreased following the application of iodoform paste on the internal surface of implants.
KEYWORDS: Dental implants, Peri-implantitis, Microleakage, Implant abutment connection

I. INTRODUCTION

The outset of dental implants has significantly spiked dentistry during the last decades. With a success rate of 97% at 10 years and 75% at 20 years, it has now been a reliable treatment for edentulism.[1]Overcoming several variables such as implant materials, surface treatment, surgical protocols, and osseointegrated implants presented high long-term results in partially as well as completely edentulous patients.[3]

However, many shortcomings of implant failures are stated in the literature out of which peri-implantitis is a recent and most prevalent pathology. Peri-implant diseases are characterized into two types: peri-implant mucositis and peri-implantitis. Peri-implant mucositis represents a reversible inflammatory lesion of the soft tissues surrounding endosseous implants without loss of supporting bone or persistent marginal bone loss. Peri-implantitis is distinguished as a site-specific infectious disease that leads to inflammation in the connective tissues and progressive loss of supporting bones around osseointegrated implants.

Dental implants are surrounded by a huge microbiota that contributes significantly to the advancement of the disease and the aetiology of peri-implantitis. Peri-implantitis has exhibited a considerable association with certain pathogens (e.g. anaerobic gram negative periopathogens and opportunistic microorganisms). Various implant structures such as implant surface characteristics, the implant-abutment interface, and the internal compartment of the implants are considered high risk areas for developing bacterial load.[7]

Recent implant systems mainly comprise of two essential parts: an endosteal fixture and an abutment that supports the prosthesis and is attached to the fixture with a screw. Implant abutment interface connection can either be internal or external. Nowadays, internal connection implants are more commonly used than external connection types. In terms of sealing ability, there are two main IAIs available which are the internal morse connection and the butt joint connection. Because of this two-piece implant design, bacterial colonization at the implant-abutment interface is growingly becoming a challenging factor in implant dentistry.[4]

The bacterial leakage did not seem to be prevented even in a good marginal fit of the implant components when observed through a scanning electron microscope.[9] Soft tissue inflammation and loss of supporting bone can be a consequence of the colonization of bacteria inside the implant system and the infiltration of bacteria or their products via the microgap.[10]It has been mentioned that this micro-gap measures approximately 40-60µm; because of this space there is a micro-motion during function which results in increased microbial leakage.[32]

Therefore, for prohibition of microbial leakage and bacterial adhesion through the implant-abutment interface several materials have been discovered in the last decade which has proven to provide better seal at the implant abutment interface. Reportedly, Iodoform paste has given good results for antimicrobial effects against endodontic infections, and more often against facultative and aerobic microorganisms.

The null hypothesis of the current study was that there is no difference in microleakage at the implant abutment interface with or without iodoform and in between two different implant abutment connection designs.

II. MATERIAL AND METHODOLOGY

This in-vitro study was conducted in the Department of Prosthodontics, Crown and Bridge, Mahatma Gandhi Dental College and Hospital, Jaipur, Rajasthan, India. The testing was carried out at Microcheck Labs, Jaipur, Rajasthan.

The study design was approved by the Institutional Ethical Review Committee (Ref. No. MGDCH/IEC/2020-21/T-02) 40 dental implants with two different types of internal connection designs- morse taper and internal hexagon were selected. For morse taper connection, TSIII[SA] Osstem implant with length 11mm and 4.5mm diameter and for internal hexagon connection, BioHorizons implant with 10.5mm length and 3.8mm diameter were used. All implants and prosthetic components were standard manufactured sterile samples. All other materials utilized in the experiment were sterilized inside surgical bags with the use of an autoclave.

Four groups were made with 10 implant samples in each group.

Group 1: Implants with internal hexagonal connection

Group 2: Iodoform coated implants with internal hexagon connection

Group 3: Implants with morse taper connection

Group 4: Iodoform coated implants with morse taper connection

Iodofrom groups: For coating the internal chamber of the implants, an antimicrobial paste named Alvocure, Prevest containing iodoform, eugenol and butamben was used.

The respective titanium alloy abutments on the respective implants were torqued either with 20 Ncm for the internal hexagon implants or with 30 Ncm for the morse taper implants according to manufacturer's instruction.

MICROBIAL SAMPLING:

For this study, Enterococcus faecalis was selected. **Enterococcus faecalis** (EF) is a gram-positive coccus, nonmotile, facultatively anaerobic microbe, a human commensal and an important opportunistic pathogen inhabiting the gastrointestinal tracts, oral cavity and urinary tract, with a size ranging approximately from 1.0 to 1.5 µm. This species is involved in the pathogenesis of secondary endodontic apical lesions and can also be found in root-filled teeth with no apical lesions and also in primary endodontic lesions. **E. faecalis** can survive in extreme environmental conditions (acidic or basic pH, high salt concentration, presence of heavy metals, aerobic and anaerobic). In fact, it fails to grow at 10°C and 45°C, at pH 9.6, 6.5% NaCl in broth, but survives at 60°C for 30 minutes. This bacterium can invade the dentinal tubules for more than 200 µm and is able to survive without the support of other bacteria and can be resistant to a wide range of antibiotics.

Bacterial suspension preparation:-

- A pure culture of E. faecalis (reference strain ATCC 29212) was used. For preparation of the bacterial suspension, the test organism **E. faecalis** was first plated onto Blood agar and then incubated for 24 hours at 37°C. Further, suspension was obtained from this culture by diluting few colonies in brain-heart infusion broth (BHI) to a density of 0.5 McFarland standards ($1x10^8$ colony forming units (CFU)/mL).

Implant Inoculation:-

III. RESULT

All the analysis is performed using SPSS version 23. The descriptive Mean and Standard deviation of the CFU count was estimated. The distribution of the continuous data was assessed using Kolmogorov-Smirnov Test. Considering the statistically significant findings in Kolmogorov-Smirnov Test, which indicates non-normal distribution of the data, we have employed non parametric Kruskal Wallis Test for multiple group comparison. Followed by Man-Whitney U test was used for pairwise comparison. The statistical significance was fixed at $p \leq 0.05$. Kolmogorov-Smirnov Test showed statistically non-significant findings which indicates that the data is non-normally distributed. Therefore, we employed non-parametric test. The Kruskal Wallis Test was used to compare the colony forming unit counts between the four groups. The highest colony forming unit counts was recorded in Group 1(2.74) and least colony forming unit counts was noticed in Group 4 (0.000336). The difference between the groups was statistically significant.

IV. DISCUSSION

Peri-implant bone level changes can be modulated with several factors among which the most important one is the implant abutment connection interface. Micro-movement at the implant abutment level has come up as the main risk factor for mechanical and biologic complications. The IAI microleakage could cause a persistent inflammatory process that ultimately led to the destruction of alveolar bone.

The purpose of this study was to compare and evaluate the antimicrobial effect of iodoform on the microbial leakage in two different implant abutment connection designs (morse taper and internal hexagon) using culture method over a period of 2 weeks. Simultaneously, the effect of different implant abutment connection designs on the microbial leakage at the implant abutment interface was also evaluated. For this purpose, two different types of implant system were chosen- one with morse taper connection design and other with internal hexagon connection. Following that, these implants were tested for microleakage using bacterial contamination. Additionally, the efficiency of iodoform in minimizing bacterial leakage along the implant abutment interface was analysed by coating the internal surface of implants with iodoform pastes. Finally, the results were obtained using culture method by counting the colony forming units of each sample.

The findings demonstrated that the iodoform paste-based antimicrobial treatment was ineffective at blocking the microleakage and microbial colonization at the implant- abutment interface in both the internal hexagon and morse taper connections. Though, the colony counts were significantly decreased following the application of iodoform paste on the internal surface of implants.

Further, the results showed the high CFU count in relation to the implant abutment assemblies with internal hexagonal connection with a mean count of 2.74 when compared to the assemblies with morse taper connection which showed the mean CFU count of 1.60. These results were statistically significant ($p \leq 0.05$). In the groups with iodoform coated IA assemblies, the mean CFU count of internal hexagonal connection assemblies and morse taper connection assemblies were .00086 and .00033 respectively. This shows that there was a significant reduction in the CFU count of the iodoform coated groups of both the connections when compared to the control groups. Though, there was no statistically significant difference seen between both the connections groups with iodoform coated implants. These results demonstrated that the antimicrobial agents (iodoform paste) were effective in minimizing the microbial count levels on the assemblies with both connections and better results were observed with respect to assemblies with morse taper connection.

Tripod et al. conducted a similar study to evaluate the bacterial leakage in internal hexagon and morse taper implant abutment connections. The results revealed that the bacterial contamination occurred in both the connection types with higher count and early contamination with respect to internal hexagon connection implants.[5] D'Ercole et al. studied the microbial leakage in cone morse taper internal connections and in screwed abutment connections over a period of 28 days and concluded that there were lower bacterial infiltration rates of cone morse taper internal connections.[21] Khorshidi et al. compared the bacterial leakage of an 11˚morse taper IAI with that of a butt joint connection. The results showed no case of bacterial leakage in morse taper connection design in 14 days period whereas 70% cases with butt joint connection showed microleakage. This demonstrated that 11˚ morse taper connection has better resistance to microleakage.[4] In a study conducted by Gherlone et al., the internal conical connection revealed a significant reduction of bacterial leakage at 96 hours when the implants were inoculated with E. coli suspension when compared to other internal connections.[24] A cross sectional microbiological analysis was conducted by Canullo et al. in which he observed the bacterial microflora colonizing the implant connections. It demonstrated that only 10% implants with conical connections revealed positive results in relation to rd complex bacteria (P. gingivalis), in comparison to double internal hexagon connections with 68% leakage.[22] Duarte et al. tested the sealing ability of two different materials which are varnish and silicon sealant, at five different implant abutment surfaces. There were no statistically significant differences obtained between the two materials in

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preventing bacterial contamination regardless of the geometric configuration of the implants. Overall, the recent systemic reviews affirms that the morse taper connection appears to be far effective regarding the long time success of dental implants permitting reduced microbial leakage and preventing bone resorption. The morse taper design exhibit superior mechanical stability ensuing in decreased microgaps after implant abutment attachment, which will able to resist bacterial leakage and growth in the inner implant chambers.

V. CONCLUSION

Within the limitations of this study, we can conclude that:

1. The implant abutment assemblies with morse taper connection are more effective in reducing microleakage than those with internal hexagon connection.
2. Iodoform is an effective antibacterial agent in preventing the microbial leakage at the implant abutment interface with better results with respect to morse taper connection than internal hexagon connection.

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