



Integrative Assessments of Clinico—Operative Procedures: Microbial Shifts in Peri—Implant Sulcular Fluid as Predictors of Early Implant Failure

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ABSTRACT

Background: Early implant failure is a serious clinical problem due to the mechanical stability as well as the peri-implant microbial composition. The purpose of this research was to assess the correlations between clinico-operative variables and changes in microbial levels at the peri-implant level as predictors of early implant failure.

Methods: This prospective cohort study (February 2023 to July 2023) included 120 systemically healthy adults who received single tooth implant placement and were enrolled. They were classified into Group A (successful osseointegration, n = 90) and Group B (early implant failure, n = 30). The samples of peri-implant sulcular fluid (PISF) were taken at baseline, Week 2, Week 4, and Week 8. The 16S rRNA gene sequencing was used to measure microbial profiling of peri-implant sulcular fluid to assess the α -diversity and relative abundance of the main

periopathogens such as *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *Fusobacterium nucleatum* (*F. nucleatum*), along with the presence of commensal streptococci ($p < 0.05$).

Results: Group B had lower insertion torque (34.8 ± 6.2 Ncm vs. 38.6 ± 5.1 Ncm, $p = 0.03$) and longer operative time (32.9 ± 6.5 min vs. 27.8 ± 5.4 min, $p = 0.01$) than Group A. The level of relative abundance of periopathogens was greater in Group B, and commensal streptococci were higher in Group A. Week 4 α -Diversity had potential for predicting patients with and without early implant failure

Conclusion: Early implant failure is related to less mechanical stability and dysbiotic peri-implant microbiota. Early monitoring of microbial changes and clinical/operative optimization can enhance the prognosis of implants and direct preventive measures.

Keywords: Dental implants, Early implant failure, Peri-implant sulcular fluid, Microbiota, Insertion torque, Operative time.

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INTRODUCTION

The use of peri-implant rehabilitation has now become the standard care in the replacement of absent teeth with high long term survival rates as well as predictable functional outcomes ¹. Regardless of these benefits, early implant failure (EIF), which is the loss of the osseointegration before or immediately after the prosthetic loading, is a serious clinical problem ². The current evidence indicates that biological, microbial, and operative factors substantially impact the early healing surrounding the implants, and all of them predetermine the stability of the process of the osseointegration ³. The peri-implant sulcular fluid (PISF), which is a reflection of the immediate immunoinflammatory and microbial environment, has become a useful diagnostic medium in evaluating early peri-implant health ⁴. Research has shown that microbial colonies of implant surfaces occur within hours of placement, and alterations toward periopathogenic bacterial communities relate to early inflammatory disintegration and failure ⁵. Nevertheless, the pathways which connect microbial dysbiosis, operative factors, and the postoperative EIF need to be further elaborated ⁶.

Whereas several studies have been conducted to evaluate peri-implantitis and late implant failure, smaller studies have centered on the initial microbial succession that takes place in PISF during the important early healing phase ⁷. The new molecular methods such as 16S rRNA sequencing and metagenomic profiling have shown that successful and early failure of osseointegration is differentiated by distinct microbial signatures ⁸. However, there is still a gap in comprehending the interplay of these microbial changes with the clinico-operative variables, which include flap design, insertion torque, operative length and risk of peri-operative contamination. In addition, the literature on how early microbial deviations can be useful predictors of impending EIF remains unexplored ^{9,10}.

In contrast to the past research that has mainly looked into the parameters and late implant failure, the current study has been unique in combining the early clinico-operative parameters with longitudinal peri-implant sulcular microbiome profiling at the critical period of osseointegration window. This prospective cohort study, to the best of our knowledge, is one of the pioneer studies to determine whether early microbial changes, together with operative factors, can serve as predictive principles of early implant failure.

METHODS

This prospective cohort study (February 2023 to July 2023) included 120 systemically healthy adults that received single tooth implant placement were enrolled at dentistry clinics at SMDC and FMH, Lahore. They were classified into Group A (successful osseointegration, n = 90) and Group B (early

implant failure, n = 30). The written informed consent was then received by all participants before enrolment (Ref: 2023/1124) .

The OpenEpi version 3.0.0 (Atlanta, GA, USA) was used to calculate the required size of the sample, where a 95% confidence level, 80% power, and 5% margin of error were used and the sample size was 120 11. A consecutive sampling method was used to recruit the participants. The inclusion criteria were the participants who are 20-60 years old, placed with single titanium implant, have enough bone volume to place the implant as usual, good oral hygiene, and able to follow-up visits at Baseline, Week 2, Week 4, and Week 8. Patients were not included when they were systemic (i.e., uncontrolled diabetes, immunosuppression), pregnant, smoked, taking antibiotics within three months before surgery, had baseline peri-implant infection, had previous bone grafting or augmentation, or refused to participate.

Eight weeks of postoperative follow-up were conducted on the participants and they were divided into two outcomes-based groups; Group A (Successful Osseointegration, n = 90) and Group B (Early Implant Failure, n = 30). Clinical and radiographic criteria such as mobility of the implants, the existence of pain or suppuration and premature bone loss beyond what would be expected healing thresholds were used to classify it. Peri-implant sulcular fluid (PISF) was taken at Baseline (Day 0), Week 2, Week 4 and Week 8 using sterile periopaper strips that had been placed in the peri-implant sulcus during 30 seconds. The clinical parameters were probing depth (mm), bleeding on probing, insertion torque (Ncm), Implant Stability Quotient (ISQ), operative time (minutes) and flap design (full-thickness or flapless). The standard protocols were used in all procedures to reduce variability and achieve uniformity.

Microbial profiling of PISF samples was done by 16S rRNA gene sequencing. The QIAamp DNA mini kit was used to extract DNA (Qiagen, Germany), and it was sequenced using Illumina MiSeq under V3V4 primers. Quality control was ensured by using reagent blanks, negative controls and duplicate samples. The major microbiological results were α -diversity (Shannon index) and the percentage abundance of major periopathogens, such as *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. nucleatum*. The secondary outcome was a relative abundance of commensal streptococci. Raw sequencing data was filtered, demultiplexed and normalized with QIIME2 software. The relative percentages of microbial abundance were calculated and α -diversity (Shannon index) was directly calculated in the pipeline. To minimize analytical variability, the duplicate measurements were averaged.

The SPSS version 26.0 (IBM Corp., Armonk, NY, USA) was used to conduct statistical analysis. The continuous variables such as age, probing depth, insertion torque, ISQ, operative time, α -diversity, and periopathogen abundance were presented as Mean \pm SD and compared through independent t -tests. Categorical variables were examined employing Chi-square or Fisher exact test; which included gender, bleeding on probing and the flap design. The criterion of statistical significance was $p < 0.05$.

The receiver operating characteristic (ROC) Curve analysis was carried out to determine the discriminatory power of week 4 α -Diversity in predicting between patients with and without early implant failure. To determine the diagnostic accuracy, the area under the curve (AUC) with 95% confidence interval (CI) and standard error (SE) was determined. The statistical significance on AUC was done through tests on whether it was different to 0.5 (no discrimination).

RESULTS

A total of 120 patients were enrolled, with 90 (75.0%) patients attaining successful osseointegration (Group A) and 30 (25.0%) patients exhibiting early implant failure (Group B). The average age of the participants was 44.8 ± 9.6 years, of whom 68 (56.7%) were male, and 52 (43.3%) were female. There was no significant difference between groups in terms of demographics ($p > 0.05$). There were intergroup comparisons in baseline clinical and operative variables. Group B showed a lower mean insertion torque value and a greater period of operation than Group A, whereas the mean baseline probing depth and bleeding indices reported no statistically significant difference ($p > 0.05$). The operation time in the failure group was much longer ($p < 0.05$). **Table 1** demonstrates the comprehensive clinical, demographic, and operative comparisons.

Table 1: Baseline Demographic, Clinical, and Operative Characteristics of Participants (n=120)

Variable	Total (n=120)	Group A: Successful (n=90)	Group B: Early Failure (n=30)	p-value
Age (years), Mean \pm SD	44.8 ± 9.6	44.2 ± 8.9	45.6 ± 10.3	0.58
Male	68 (56.7%)	50 (55.6%)	18 (60.0%)	0.71
Female	52 (43.3%)	40 (44.4%)	12 (40.0%)	
Baseline Probing Depth (mm)	2.86 ± 0.74	2.81 ± 0.70	2.94 ± 0.79	0.39

Bleeding on Probing	32 (26.7%)	22 (24.4%)	10 (33.3%)	0.54
Insertion Torque (Ncm)	37.2 ± 5.8	38.6 ± 5.1	34.8 ± 6.2	0.03*
ISQ (Primary Stability)	69.4 ± 3.7	70.1 ± 3.5	67.8 ± 3.8	0.07
Operative Time (minutes)	29.6 ± 6.2	27.8 ± 5.4	32.9 ± 6.5	0.01*
Flap Design (Full Thickness)	84 (70.0%)	61 (67.8%)	23 (76.7%)	0.48

SD: Standard Deviation, ISQ: Implant Stability Quotient, Ncm: Newton centimeter, χ^2 : Chi-square test, $p < 0.05$ significant.

There was a significant difference in insertion torque and operative duration between groups. Microbial analysis revealed that Group B had always lower levels of α -diversity index at all follow-up intervals. The significant changes were recorded at Week 4 and Week 8 ($p < 0.05$). Besides, periopathogenic species including *P. gingivalis* and *T. forsythia* were more abundant in Group B. **Table 2** represents microbial diversity comparisons.

Table 2: Microbial Diversity Indices (α -Diversity) Across Follow-up Intervals

Timepoint	Group A (Mean ± SD)	Group B (Mean ± SD)	p-value
Baseline (Day 0)	3.42 ± 0.28	3.36 ± 0.31	0.42
Week 2	3.51 ± 0.33	3.39 ± 0.36	0.18
Week 4	3.67 ± 0.29	3.41 ± 0.34	0.01*
Week 8	3.79 ± 0.32	3.46 ± 0.38	0.004*

SD: Standard Deviation, α -diversity: Shannon diversity index, independent t-test was used, $p < 0.05$ significant

The comparative abundance of key periopathogenic organisms was used as a comparison between the groups. Group B demonstrated much more colonization by *P. gingivalis*, *T. denticola*, *F. nucleatum* at Week 8, whereas Group A was more represented by commensal *streptococci*. **Table 3** shows the distribution of periopathogens.

Table 3: Relative Abundance of Key Periopathogens at Week 8

Microorganism	Group A (Mean ± SD)	Group B (Mean ± SD)	p-value
<i>P. gingivalis</i>	6.8 ± 1.9	12.4 ± 3.3	0.001*
<i>T. forsythia</i>	7.3 ± 2.2	11.1 ± 3.0	0.004*
<i>T. denticola</i>	5.1 ± 1.6	9.2 ± 2.8	0.002*
<i>F. nucleatum</i>	10.6 ± 3.1	16.3 ± 4.4	0.006*
Commensal streptococci	32.8 ± 8.7	24.5 ± 7.1	0.03*

P. gingivalis: Porphyromonas gingivalis, *T. forsythia*: Tannerella forsythia, *T. denticola*: Treponema denticola, and *F. nucleatum*: Fusobacterium nucleatum. Values represent mean relative abundance %, independent t-test was used, $p < 0.05$ significant.

Logistic regression was used with multiple variables to identify the independent clinico-operative and microbial predictors of early implant failure. The variables in the model were insertion torque, operative time, implant stability quotient (ISQ), microbial α -diversity (Shannon index) at Week 4, relative abundance of *Porphyromonas gingivalis*, and commensal *streptococci* at Week 8. **Table 4** shows adjusted odds ratios (ORs) and 95% confidence intervals (CIs), and p-values.

Table 4: Multivariable Logistic Regression Analysis for Predictors of Early Implant Failure (n = 120)

Variable	Adjusted OR	95% CI	p-value
Insertion Torque (per 1 Ncm decrease)	1.12	1.03 – 1.23	0.009*
Operative Time (per 1 min increase)	1.09	1.02 – 1.17	0.013*
ISQ (per unit decrease)	1.08	0.98 – 1.19	0.11
α -Diversity (Shannon index, Week 4)	1.46	1.11 – 1.92	0.006*
<i>P. gingivalis</i> abundance (per 1% increase)	1.31	1.12 – 1.54	0.002*
Commensal <i>streptococci</i> (per 1% increase)	0.94	0.90 – 0.99	0.021*

OR : Odds Ratio, CI: Confidence Interval, Ncm: Newton centimeter, min: Minute, ISQ: Implant Stability Quotient, α -Diversity: Alpha Diversity, *P. gingivalis*: Porphyromonas gingivalis, Statistical Models: Nagelkerke $R^2 = 0.48$, Hosmer–Lemeshow test: $p = 0.62$, Correct classification rate = 82.5%, * Statistically significant at $p < 0.05$.

Reduced torque of insertion and increased duration of operation showed an independent correlation with a risk of early implant failure. There also was a significant contribution of microbial parameters to the prediction, with decreasing peri-implant microbial α -diversity at Week 4 and the augmented relative abundance of *P. gingivalis* being the major factor predisposing to early failure. Higher commensal *streptococci*, in contrast, was independently related to the low risk of the implant failure. The model served as an explanation of a large fraction of variance in early implant failures. These results are further strengthened by ROC curve as shown in **Figure 1**.

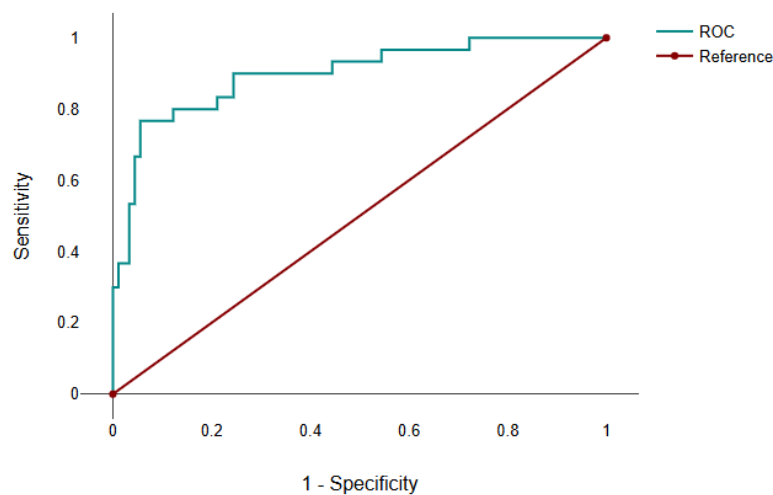


Figure 1: Receiver Operating Characteristic (ROC) curve for Week 4 α -Diversity (Shannon index) predicting early implant failure

Receiver operating characteristic (ROC) curve analysis demonstrated that week 4 α -Diversity had good discriminatory ability for predicting patients with and without early implant failure, with an AUC of 0.894 (95% CI: 0.643–1.000; SE = 0.128; $p = 0.002$).

DISCUSSION

The study examined microbial changes around implants as predictors of implant failure in the first six months. The patients were classified into two groups such as early failure in 30 implants (25%), and successful osseointegration in 90 implants (75%). The failed implants had lesser insertion torque and increased time of operation. The failed implants experienced a significant microbial diversity (α -diversity) reduction after Week 4. The most common periopathogens such as *P. gingivalis*, *T.*

forsythia, *T. denticola*, and *F. nucleatum* were higher in failed implants. In successful implants, there was a greater commensal streptococcus. Week 4 α -diversity could be exploited as a discriminatory marker to differentiate between patients with and without early implant failure. These findings have shown that mechanical stability and early microbial changes play a role in prognosis of implants.

The significance of primary mechanical stability is evident in lower insertion torque in failed implants. Previous experiments have indicated that lack of torque enhances micromotion and causes premature failure¹². Failure implants were linked to longer operative time, which is consistent with the evidence that extended surgery may cause more tissue trauma and local inflammation¹³. There were no significant differences in terms of age, gender, probing depth at baseline, or bleeding on probing indicating that these variables were not significant determinants of early failure in this cohort^{14,15}. Torque and surgical time have a cumulative effect on the early healing and the predisposition towards microbial colonization. This data highlights the significance of optimization of operative technique to increase the stability of implants and minimize the risk of early failure.

Microbial analysis showed that there was significantly lower α -diversity in early failure implants at Week 4 and Week 8. ROC analysis showed that week 4 α -Diversity has potential to differentiate between patients with and without early implant failure (AUC = 0.894). Previous reports have demonstrated that a depleted microbial diversity is associated with peri-implant inflammation and poor osseointegration¹⁶. There were more periopathogens colonizing failed implants, which included *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. nucleatum*¹⁷. This is consistent with the literature that has indicated that periopathogen overgrowth facilitates local inflammation, bone resorption and implant instability¹⁸. On the other hand, commensal *streptococci* were found to be higher in successful implants in line with the literature that reported the preservation of balanced microbiota to defend against early microbial dysbiosis^{19,20}. These findings indicate that early microbial profiling can be an effective predictive tool of implant prognosis, and preventing measures.

The overall findings indicate that the accomplishment of implants is based on both the mechanical and the microbial environment. Reduced insertion torque can result in the occurrence of micromotion which can cause the release of inflammatory cytokines and peri-implant tissue disturbance²¹. Increased duration of surgery can contribute to local stress responses and establish a positive space of pathogenic colonization²². Increased periopathogen load and decreased commensals can hasten the loss and failure of the bones early^{23,24}. These outcomes indicate the possible potential of early microbial surveillance to identify changes that can indicate failure. The maximization of the torque, the reduction of surgical time, and microbial balance may help to enhance the survival of implants and patient outcomes²⁵.

The short eight weeks follow-up and a relatively small sample size of single-tooth implants are the limitations of this study. The immune responses of the hosts were not measured, and PISF samples were examined alone. The research lacked the assessment of multi-implant cases and long-term clinical outcomes. Future research ought to include long-term follow-up, multi-centre trials as well as evaluation of host-microbe interactions. Additional interventions to reduce perio-pathogen or commensal may also improve the prognosis of early implants.

CONCLUSION

Mechanical and microbial factors are contributing factors to early implant failure. Lower insertion torque and longer operating time of implants were important predictors of failure in this study. Microbial analysis demonstrated that the failure group had decreased α -diversity and an increased number of periopathogens, such as *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. nucleatum*, whereas successful implants had increased commensal *streptococci*. These results suggest that early microbial monitoring with optimization of surgical procedures can aid in the prediction and prevention of early implant failure.

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CONFLICT OF INTEREST

None.

ETHICAL APPROVAL

This prospective cohort study (February 2023 to July 2023) included 120 systemically healthy adults enrolled at dentistry clinics at SMDC and FMH, Lahore with classification as into Group A (successful osseointegration, n = 90) and Group B (early implant failure, n = 30) after an informed consent (Ref: 2023/1124).

AUTHORS' CONTRIBUTION

MM: Study design, manuscript drafting, critical revision. **MR:** Statistical analysis, data modeling, results drafting. **M:** Clinical procedures, intraoperative data collection, clinical review. **AS:** Patient

recruitment, screening, follow-up management. **SUF**: Data validation, literature mapping, manuscript verification and correspondence. **ZI**: Project administration, ethical approvals, safety coordination. **SA**: Senior supervision, resource provision, intellectual oversight. All authors read, critically revised, and approved the final manuscript, and agree to be accountable for all aspects of the work.

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