Article

The Effect of Laser Micro Grooved Platform Switched Implants and Abutments on Early Crestal Bone Levels and Peri-Implant Soft Tissues Post 1 Year Loading among Diabetic Patients—A Controlled Clinical Trial

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Abstract: *Background and Objectives:* The study aimed to compare the mean crestal bone level (CBL) and peri-implant soft tissue parameters in laser micro-grooved (LMG) platform switched implants and abutments (I&A) post 1 year of functional loading among non-diabetic and type II diabetic individuals. *Materials and methods:* Patients with an edentulous site having minimum bone height and width of $\geq$13 mm and $\geq$6 mm, respectively, were divided into two groups: (i) Non-diabetic-8 (control) and (ii) diabetic-8 (test). LMG Implants were placed and loaded immediately with a provisional prosthesis. Mean crestal bone level (MCBL) was evaluated radiographically at baseline and at 1 year. Peri-implant attachment level (PIAL) and relative position of the gingival margin (R-PGM) were recorded. Implant stability quotient (ISQ) level and implant survival rate (ISR) were evaluated at 1 year. *Results:* Early MCBL within the groups 1 year postloading was similar both mesially and distally (control—0.00 to 0.16 mm and 0.00 to 0.17 mm, respectively; test—0.00 to 0.21 mm and 0.00 to 0.22 mm, respectively) with statistical significance ($p \leq 0.003$, $p \leq 0.001$ and $p \leq 0.001$, $p \leq 0.001$, respectively). However, intergroup comparison showed no significant difference statistically in the MCBL in 1 year post functional loading. The peri-implant soft tissue parameters showed no significant difference between the groups. ISQ level between both groups did not reveal any significant changes ($p \leq 0.92$), and ISR was 100%. *Conclusions:* LMG Implants resulted in minimal and comparable early crestal bone loss and soft tissue changes post 1 year of functional loading in moderately controlled diabetic and non-diabetic individuals, suggesting that this could be a reliable system for use in systemically compromised individuals.

Keywords: diabetes mellitus; laser micro-grooved implants; laser micro-grooved abutments; mean crestal bone level measurements; relative position of the gingival margin

1. Introduction

The survival of dental implants is determined by factors such as implant design, surface properties, and surgical protocol [1]. Initially successful osseointegration determines implant stability, and later the bone remodeling associated with prosthetic loading and

crown placement determines the implant survival rate (ISR). In addition, patient-related local and systemic conditions such as diabetes mellitus are vital modifiers of implant survival and success rates [2].

Chronic hyperglycemia is linked to poor wound healing, altered bone metabolism, and hyperinflammatory responses [3]. In the presence of elevated glycemic levels, there is decreased collagen production during callus formation, resulting in apoptosis of bone lining cells and increased osteoclastic activity, which interferes with osseointegration [4]. In addition, autoimmune reactions are induced in tissues with increased osteoclastic activity, resulting in bone resorption and associated interference with osteoblastic activity. The sensitivity of the parathyroid glands is altered, resulting in an imbalance in calcium and phosphorus homeostasis, which has a negative impact on cellular functions and the extracellular matrix of the bone [5]. Thus, the glycemic status affects the implant survival rate by altering the bone metabolism throughout the healing phase. Therefore, there is a need to understand and address the significance of this potential risk factor in dental implants-related research [6].

Studies have shown that the implant stability parameters, marginal bone loss around implants, soft tissue inflammation, and bleeding on probing increased in proportion to increasing HbA1c levels [7]. Oates et al. reported that moderately controlled diabetics demonstrate a significant difference in mean crestal bone loss but with better implant survival rate [8]. Multiple studies have evidenced that individuals with moderately and poorly controlled type II diabetes have an excellent overall implant survival rate with greater crestal bone loss when compared to non-diabetic individuals. With better implant survival rate in diabetics, optimizing implant designs aimed at reducing crestal bone loss could significantly improve their quality of life [8–10]. Very limited studies have evaluated the potential effect of implant designs on reducing crestal bone loss in diabetic individuals and specifically on moderately controlled diabetic individuals, and this needs further exploration.

Implant design modifications aim at reducing crestal bone loss and achieving better osseointegration, and recently laser-ablated micro-channels or micro-grooves placed within the implant collar have been shown to limit apical migration of the junctional epithelium and prevent crestal bone loss [11]. Histological studies have further suggested that a laser micro-grooved (LMG) implant surface allowed direct contact of a stable connective tissue with an intact biological seal, giving a cold welding effect to the implant collar, and a more robust perpendicular collagen fiber attachment preventing epithelial down growth and crestal bone resorption post 1 year of loading [12–14]. In a recently conducted microbiological and longitudinal study, reduced microbial load was observed at 18 months with minimal crestal bone loss at 3 years in healthy individuals. These results were attributed to laser micro-grooving along with platform switching characteristics [15,16]. Thus, the studies have emphasized that the LMG implant design modifications provided an effective result in the reduction of crestal bone loss in normal healthy individuals. Therefore, the special characteristics, features, and one-year follow-up capabilities of LMG implants when compared to non-diabetic and well-controlled individuals could be used as an advantage to prevent crestal bone loss in diabetic individuals.

No prior studies have evidenced the use of LMG platform switched implants and abutments in preventing crestal bone loss in moderately controlled diabetic patients. Considering the advantages of LMG platform switched implants and abutments, this study hypothesized that the abovementioned precisely designed implants and abutments could have a positive influence on minimizing the mean crestal bone loss and peri-implant attachment loss in moderately controlled diabetic individuals at 1 year post functional loading comparable to that of non-diabetic individuals.

2. Materials and Methods

2.1. Trial Design

This prospective clinical trial was based on a cohort of patients seeking an implant-supported restoration at the Department of Periodontology, SRM Dental College, Ramapuram, Chennai-89 between January 2020 to October 2021. The study was declared to the Institutional Scientific Committee and Ethical Review Board and prior approval was obtained (IRB APPROVAL no. SRMDC/IRB/2019/MDS/No.503). The clinical trial registry number is as follows: REF/2019/12/030040[DE].

2.2. Participants

The participants of the present study were included based on the study group allocation (Figure 1), and were either systemically healthy (control group) or type II diabetic (test group). The general inclusion criteria included male or female patients aged ≥30 to 60 years, with mandibular premolars and molar edentulous sites with sufficient bone height (at least 13 mm) and sufficient bone width (at least 6 mm). The test group, which included diabetic patients with HbA1c levels of 8.1 to 10 (moderately controlled diabetic individuals) was recruited for the study. The study sample was calculated based on results obtained from a study by Aguilar-Salvatierra et al. in 2016 [17]. Taking a 20% dropout rate into consideration, the sample size was increased to 20 edentulous sites that require implant placements with 10 (LMG) platform switched implants and abutments to be placed in each group.

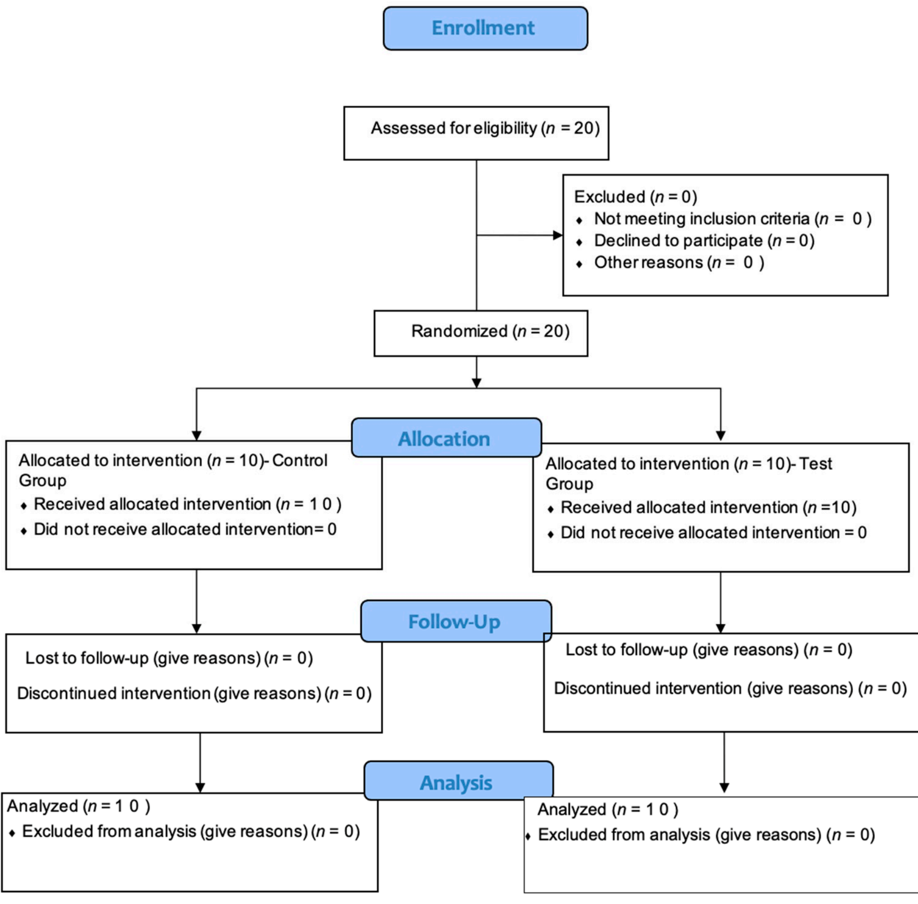

Figure 1. Consort flow diagram.

2.3. Outcome Measures

The primary objective of this study was to evaluate and compare the MCBL changes radiographically (mesially and distally) at baseline (immediately after restoration) and 1 year post functional loading in both non-diabetic (control group) and diabetic (test group) sites, and additionally also to assess and compare the implant survival rates 1 year post functional loading between both non-diabetic (control group) and diabetic (test group) sites.

The secondary objective of this study was to evaluate and compare the relative position of gingival margin (R-PGM) on LMG platform switched implants with LMG abutments among non-diabetic (control group) and diabetic (test group) patients at baseline, 6 months, and 1 year post functional loading. Additionally, we aimed to assess the implant stability quotient (ISQ) values using resonance frequency analysis and compare it between non-diabetic (control group) and diabetic (test group) patients prior to prosthetic restoration.

Hypotheses: considering the advantages of laser micro-grooved platform switched implants and abutments, this study hypothesized that the abovementioned precisely designed implants and abutments could have a positive influence on minimizing the mean crestal bone loss and peri-implant attachment loss in moderately controlled diabetic individuals 1 year post functional loading comparable to that of non-diabetic individuals.

2.3.1. Clinical Parameters to Be Evaluated Include

Full mouth plaque scores (FMPS) [15]: evaluated at baseline (before implant placement) and 1 year post functional loading.

Full mouth Bleeding scores (FMBS) [15]: evaluated at baseline (before implant placement) and 1 year post functional loading.

Periodontal probing depth (PPD) [15]: evaluated at baseline (before implant placement) and 1 year post functional loading.

Clinical attachment level [16]: evaluated at baseline (before implant placement) and 1 year post functional loading.

2.3.2. Site-Specific Parameters to Be Evaluated

Site-specific plaque scores (S-SPS) [15]: evaluated immediately post restoration (baseline), 6 months, and 1 year post functional loading.

Site-specific bleeding scores (S-SBS) [16]: evaluated immediately post restoration (baseline), 6 months, and 1 year post functional loading.

Peri—Implant sulcus depth (PISD) [16]: evaluated immediately post restoration (baseline), 6 months, and 1 year post functional loading.

Peri—Implant abutment attachment level (PIAL) [15]: evaluated immediately post restoration (baseline), 6 months, and 1 year post functional loading.

Relative position of gingival margin (R-PGM) [16]: evaluated immediately post restoration (baseline), 6 months, and 1 year post functional loading.

ISQ [17–19]: evaluated immediately after implant placement for restoration.

Radiographic parameters to be evaluated using RVG [16]:

MCBL changes of mesial and distal aspect (M-MCBL and D-MCBL): evaluated immediately post restoration (baseline) and 1 year post functional loading.

ISR [6]: evaluated 1 year post functional loading.

2.4. Randomization

Patients who fulfilled the inclusion and exclusion criteria were enrolled for this controlled clinical trial. In order to rule out examiner bias, a single calibrated examiner performed all of the clinical parameters. Routine preliminary phase assessments and treatment were performed. Patients who had fulfilled the abovementioned inclusion and exclusion criteria, and who were willing to participate in the study, were selected and divided into two groups. A total of 20 edentulous sites requiring implant placement were recruited for this study. All of the surgical procedures were performed by a single well-experienced

operator. The radiographic parameters were assessed by a separate calibrated examiner who was unaware of the recruitment and the clinical parameters assessed.

2.5. Interventions

A single calibrated operator who was blinded about the diabetic or non-diabetic sites performed the surgical procedures. After administration of local anesthetics (2% lignocaine with 1:80,000 adrenaline) patients were instructed to rinse with 0.12% chlorhexidine solution for 30 s. The implant sites were prepared under local anesthesia, and a mid-crestal incision followed by minimal flap elevation and a pilot drill were driven initially with 950 rpms and 35 Ncm. An implant surgical kit was used to prepare the osteotomy sites and place the implants. Once the final osteotomy was carried out, the implants were driven with an insertion torque of more than 35 Ncm using the implant drive (Figure 2). ISQ was measured using resonance frequency analysis (RFA) (Penguin^{RFA} unit, Integration Diagnostics, Goteborg, Sweden), performed by placing a transducer on the implant collar and the reading of 60 (osseointergration diagnostics) to confirm the primary stability [17–19]. When the reading of the ISQ limit touched 60 and above, it indicated immediate loading. All of the implants were placed equi-crestally. This was followed by the placement of a prosthetic abutment and was restored immediately with functional loading (Figure 3). The same surgical protocol was followed for both the control and test groups. All patients received postoperative instructions to rinse with 0.2% chlorhexidine digluconate twice daily for a two-week period. Analgesic medication (ibuprofen, 500 mg) was prescribed.

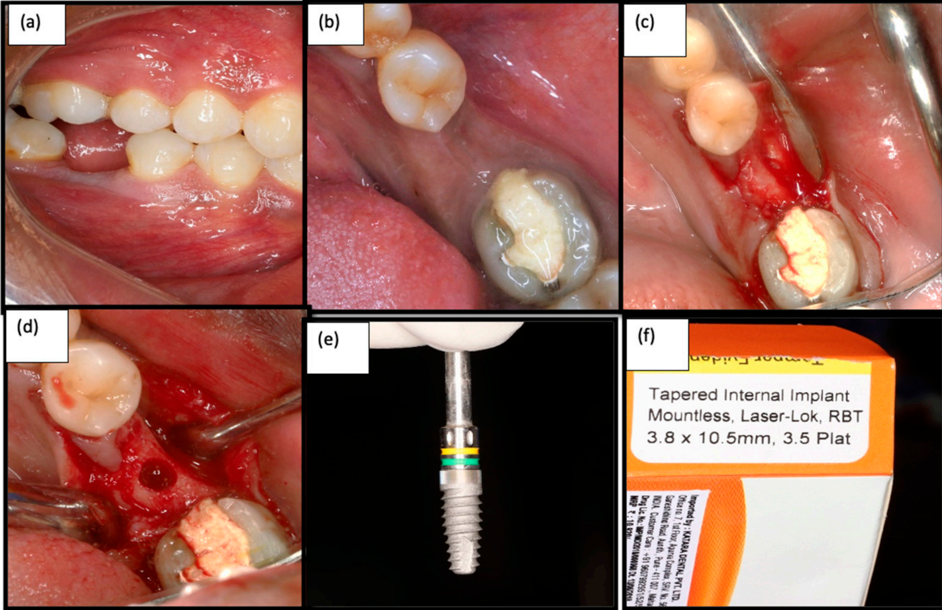

Figure 2. (**a**) Isolated edentulous site—Buccal view; (**b**): Intra oral photograph of the edentulous site—Occlusal view (**c**): Crestal incision given and flap elevation performed (**d**) final osteotomy site preparation done for 3.8 mm; (**e**,**f**) Laser micro-grooved Implants (Laser lok®).

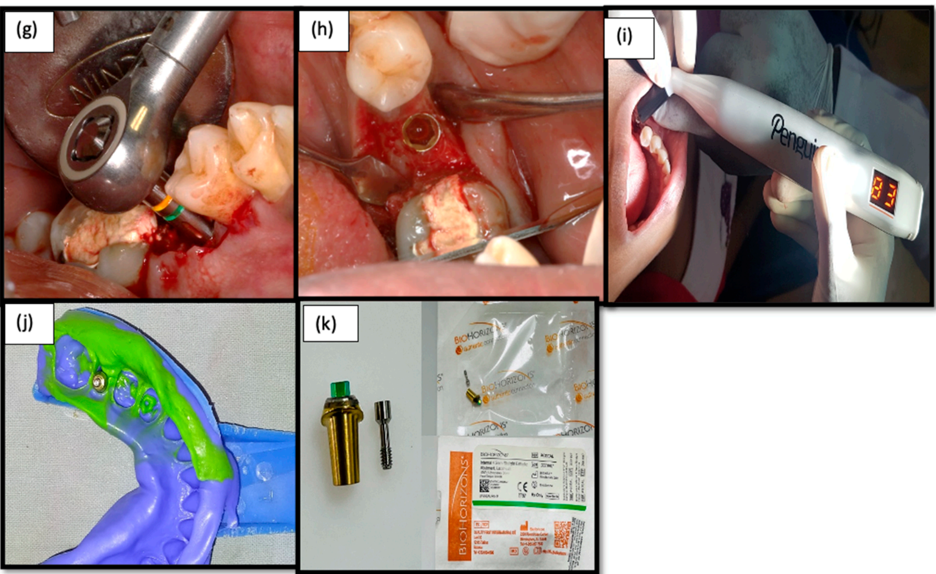

Figure 3. (**g**) Implant driven in using a Bio-horizon torque wrench; (**h**) Laser Micro-grooved implant placed and cover screw given; (**i**) Resonance frequency analysis for prosthetic loading; (**j**) Putty impression taken for prosthetic restoration; (**k**) Laser micro-grooved abutment – Laser Lok®.

2.6. Statistical Analysis

The collected data were analyzed with IBM SPSS Statistics for Windows, Version 23.0. (Armonk, NY: IBM Corp). To find the significant difference between the bivariate samples among the paired groups (baseline and 1 year), a Wilcoxon signed rank test was used. To find the significant difference between the bivariate samples among independent groups (baseline and 1 year) a Mann–Whitney U test was used. To compare the continuous variables between two groups, i.e., intergroup comparison of FMPS, FMBS, PPD, CAL, S-SPS, S-SBS, PISD, PIAL, RPGM, MCBL, ISR, and ISQ, Student's t-test was used.

Intragroup comparison of all of the clinical and radiographic parameters within the control and the test groups were carried out using a paired t-test. In both the above statistical tools the probability value 0.05 was considered a significant level.

3. Results

The mean age of the participants was 38.62 $\pm$ 5.06 years and 42.62 $\pm$ 5.34 years in the control and the test groups, respectively. Gender distribution in the control and the test groups was 5 male /5 female and 4 male/6 female participants, respectively. The groups did not differ based on age and gender distribution (p = 0.46 and p = 0.17, respectively).

Table 1: FMPS and FMBS showed a statistically significant reduction from 15% and 11% at baseline to 10% and 8% at 1 year post functional loading in the control and the test groups, respectively ($p \leq 0.001$). Intergroup comparison of FMPS and FMBS showed no significant difference between the groups at both time points (p = 0.52). PPD in both the control and the test groups showed a minimal reduction from 2.94 to 2.90 mm and from 2.88 to 2.63 mm, respectively, from baseline to 1 year post functional loading with no significant difference (p = 0.90). No attachment loss was observed in both the control and the test groups throughout the study period. Intergroup comparison of PPD between the control and test groups at both time points showed no statistical significance (p = 0.43 and p = 0.39, respectively).

Table 1. Intergroup and intragroup comparison of clinical parameters at different timepoints.

Clinical Parameter		Control Group (Group 1)	Test Group (Group 2)	p Value
PPD	Baseline	2.94 ± 0.16	2.88 ± 0.12	0.43
	1 Year	2.90 ± 0.10	2.63 ± 0.14	0.39
p value		0.90	0.66	
CAL	Baseline	0.00	0.00	-
	1 Year	0.00	0.00	-
p value		-	-	-
Full mouth plaque scores (FMPS) (%)	Baseline	15.74 ± 2.26	15.15 ± 1.85	0.52
	1 Year	10.62 ± 1.38	10.70 ± 1.30	0.32
p value		≤0.001 **	≤0.001 **	
Full mouth bleeding scores (FMBS) (%)	Baseline	11.80 ± 1.20	11.49 ± 1.51	0.51
	1 Year	8.61 ± 0.39	8.53 ± 1.47	0.86
p value		≤0.015 *	≤0.015 *	

CAL: Clinical attachment loss; ** highly significant, * significant.

Table 2: Intragroup comparison of SS-PS in both the control and test groups showed a similar reduction in plaque deposition, i.e., 9% at baseline, 8% at 6 months, and 6% at 1 year post functional loading. There was a statistically significant reduction in the plaque score from baseline to 1 year post functional loading, i.e., 9% to 6%, $p \leq 0.05$, in both groups (Table 3). A similar reduction in the SS-BS was observed in the control and the test groups from baseline to 1 year post functional loading, i.e., 9%, 8%, and 6%, and 9%, 7%, and 6%, respectively. A significant reduction was observed at 1 year from baseline in both the control and the test groups, $p \leq 0.05$ (Table 3).

Table 2. Intergroup comparison of site-specific clinical parameters at different timepoints.

Clinical Parameter		Control Group (Group 1)	Test Group (Group 2)	p Value
S-SPS	Baseline	9.37 ± 6.63	9.37 ± 6.63	1.0
	6 months	8.30 ± 6.70	8.70 ± 6.30	0.90
	1 year	6.25 ± 4.75	6.25 ± 4.75	1.0
S-SBS	Baseline	9.37 ± 6.63	9.37 ± 6.63	1.0
	6 months	8.50 ± 6.50	7.50 ± 5.50	0.66
	1 year	6.37 ± 4.63	6.25 ± 4.75	0.77
PISD	Baseline	2.11 ± 0.58	2.03 ± 0.56	0.80
	6 months	2.09 ± 0.11	2.04 ± 0.13	0.67
	1 year	2.06 ± 0.55	2.07 ± 0.50	0.73
PIAL	Baseline	0.00	0.00	-
	6 months	0.00	0.00	-
	1 year	0.00	0.00	-
R-PGM	Baseline	3.37 ± 0.20	3.53 ± 0.30	≤0.263
	6 months	3.37 ± 0.20	3.53 ± 0.30	≤0.263
	1 year	3.37 ± 0.20	3.53 ± 0.30	≤0.263

S-SPS- Site-Specific Plaque Scores, S-SBS- Site-Specific Bleeding Scores, PISD- Peri-Implant Sulcus. Depth, PIAL- Peri-Implant Clinical Attachment Level, R-PGM- Relative Position of the Gingival Margin.

Table 3. Intragroup comparison of clinical parameters between different timelines.

Clinical Parameter	Timeline	Control Group (Group 1) *p* Value	Test Group (Group 2) *p* Value
Site-Specific Plaque Scores (SSPS)%	Baseline to 6 months	0.598	0.69
	Baseline to 1 year	**≤0.05** **	**≤0.05** **
	6 Months to 1 year	0.56	0.59
Site-Specific Bleeding Scores (SSBS)%	Baseline to 6 months	0.54	0.59
	Baseline to 1 year	**≤0.05** *	**≤0.05** *
	6 Months to 1 year	0.84	0.61
Peri-Implant Sulcus Depth (PISD) (mm)	Baseline to 6 months	0.70	0.89
	Baseline to 1 year	0.89	0.82
	6 Months to 1 year	0.93	0.85
Peri-Implant Clinical Attachment Level (PIAL) (mm)	Baseline to 6 months	-	-
	Baseline to 1 year	-	-
	6 Months to 1 year	-	-
Relative Position of the Gingival Margin (R-PGM) (mm)	Baseline to 6 months	1.0	1.0
	Baseline to 1 year	1.0	1.0
	6 Months to 1 year	1.0	1.0

* significant. ** highly significant.

Table 3: Intergroup comparison of SS-PS between the control and test groups revealed almost similar plaque scores at all time intervals, i.e., baseline (9% vs. 9%), 6 months (8% vs. 8%), and 1 year (6% vs. 6%) with no statistical significance. Likewise, the SS-BS between the control and test groups revealed almost the same bleeding scores at all time intervals, i.e., baseline (9% vs. 9%), 6 months (8% vs. 7%), and 1 year post functional loading (6% vs. 6%) with no statistical significance.

Table 4: Intragroup assessment of PISD in the control and test groups revealed minimal reduction in the sulcus depth from baseline to 1 year post loading with no significant changes at any time points. Intergroup comparison of the PISD between the control and the test groups at baseline, 6 months, and 1 year post functional loading revealed almost similar probing depths at all time intervals, i.e., baseline (2.11 vs. 2.03 mm), 6 months (2.09 vs. 2.04 mm) and 1 year post functional loading (2.06 vs. 2.07 mm) with no significant difference between the groups. No peri-implant attachment loss was observed in both groups throughout the study period.

The R-PGM in the control and test groups revealed no changes in relation to the restored implant crown at any of the evaluated time points (3.37 ± 0.20 mm and 3.53 ± 0.30 mm, respectively). Furthermore, comparison of the R-PGM between the control and test groups at all three timepoints revealed no changes in the position of the gingival margin, i.e., 3.37 vs. 3.53 mm, respectively, with no difference between the groups statistically ($p = 1$).

Table 4. Intra- and intergroup comparison of crestal bone loss.

Radiographic Parameter	Timeline	Control Group (Group 1)	Test Group (Group 2)	*p* Value
M-MCBL	Baseline	0.00	0.00	-
	1 Year	0.16 ± 0.15	0.21 ± 0.03	0.40
p Value		0.003 *	≤0.001 **	
D-MCBL	Baseline	0.00	0.00	-
	1 Year	0.17 ± 0.16	0.22 ± 0.04	0.48
p Value		≤0.001 **	≤0.001 **	

* significant. ** highly significant.

Intragroup comparison of MCBL in the control group in both the mesial and distal aspects showed radiographically minimal early crestal bone loss from baseline to 1 year post functional loading, i.e., 0.00 to 0.16 mm in the mesial aspect and 0.00 to 0.17 mm in the distal aspect with statistical significance ($p \leq 0.003$ and $p \leq 0.001$, respectively). Similarly, radiographically minimal early crestal bone loss was observed in the test group, i.e., 0.00 to 0.21 mm in the mesial aspect and 0.00 to 0.22 mm in the distal aspect from baseline to 1 year post loading with a significant difference statistically ($p \leq 0.001$ and $p \leq 0.001$, respectively); see Table 4. No statistically significant difference was observed between both groups in the M-MCBL and D-MCBL at 1 year post functional loading (Figure 4).

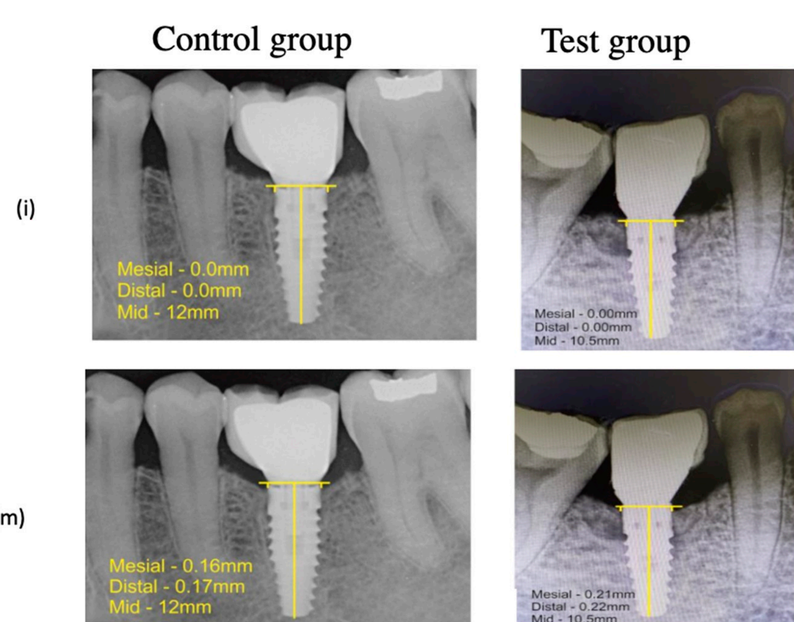

Figure 4. Measurement of Mean Crestal Bone Level—(**i**) Immediately Post functional Loading, (**m**) 1 year Post functional Loading.

ISR between the control and test groups after 1 year revealed 100% survival of all of the implants with no failures in both groups. ISQ between the control and test groups showed similar implant stability quotients of 74.50 ISQ (control group) vs. 74.25 ISQ (test group), which was greater than the permissible limits, indicative that all implants were ready for immediate loading protocol. The ISQ levels between the groups did not reveal any significant changes ($p = 0.92$).

4. Discussion

Titanium alloys have superior mechanical properties, corrosion resistance, and biocompatibility, favoring their wide use in the manufacturing of medical devices. The bioactivity of these alloys is attributed to the presence of a dense and coherent film of a nanometric thick passivation layer composed of TiO2 [20,21]. Newer metal ingots have been introduced in the field of implant dentistry; however, they are at a more novice stage requiring more research before they can be incorporated into clinical practice. Extensive research aiming at micro-structural modification of dental implants such as titanium nanotubes and laser-based technologies have been shown to improve peri-implant parameters and consequently implant survival rate [22,23].

Diabetes mellitus was previously recognized as a relative risk factor for dental implants' survival [24]. However, currently, there is a change in the paradigm and studies indicate that diabetes patients benefit from oral rehabilitation with dental implants [10]. In this study it was hypothesized that in moderately controlled diabetics prone to compromised peri-implant soft and hard tissue healing, surface-modified implant design with Laser-Lok technology could be beneficial. This design allows cells such as osteoblasts and fibroblasts to link and organize themselves optimally in the laser micro-channels, creating a biological seal along the abutment per se and osseointegration along the implant collar with a cold welding effect, which collectively contributes to minimal inflammatory infiltration at the crest module of the implant along with a soft tissue seal, thereby minimizing microbial colonization, contributing to better implant stability, maintaining peri-implant health, and minimizing early crestal bone loss [25–27].

Katyayan et al. suggested that despite the metabolic disparities evident in diabetic patients, an early loading regimen might be successful. Likewise, Al Amri et al. [28] reported identical clinical and radiographic outcomes in terms of soft tissue conditions, crestal bone levels, and implant success rates in type II diabetic and non-diabetic patients based on the early loading strategy. Therefore, in this study immediate implant loading in both groups was chosen, as it could not only enhance successful function but also help in better metabolic outcomes.

The PPD in both the control and the test groups reduced evenly and remained at comparable levels at 1 year post functional loading with no significant difference. Likewise, no CAL was observed in both groups. The results indicate that the patients did not experience any active periodontal disease during the study period. The S-SPS on the abutment surfaces in both the control and the test groups were almost similar from baseline to 6 months and from 6 months to 1 year post functional loading with no statistical difference. The plaque accumulation on the abutment surface significantly reduced from baseline to 1 year in both groups with statistical significance. The similar reduction in plaque accumulation in both the control and the test groups could be attributed to the similar implant and abutment design.

Likewise, almost similar bleeding scores were observed from baseline to 6 months and from 6 months to 1 year post functional loading with no statistical significance in both groups. The bleeding scores significantly reduced from baseline to 1 year in both groups with statistical significance. Intergroup comparison revealed similar bleeding scores at all three time intervals, with no difference between the groups statistically. The similar percentages of S-SPS and S-SBS indicate that LMG on the implants surface results in an early connective tissue attachment seal, allowing for a good mucosal barrier in the collar region, thereby reducing plaque accumulation and reducing gingival inflammation.

Intra- and intergroup assessments of PISD in the control and the test groups revealed minimal reduction or almost similar probing depth measurements with no significant difference between the time points. No peri-implant attachment loss was observed in both the control and test groups. These findings further underscore the previous findings that Laser-Lok implants with LMG abutments established a better mucosal barrier in the collar area around the implant–abutment connection, which was almost on par with the soft tissue gingival fiber attachment in the natural tooth, creating a dense soft tissue

barrier [29,30]. The resistance to probing attributed to the perpendicular orientation of collagen fibers in the connective tissue to the abutment surface was consistent with the findings of minimum peri-implant sulcus depth and no peri-implant attachment loss. Ferraris et al. have demonstrated that the micromachined surfaces with horizontal grooves 3 or 10 mm deep on implant surfaces interfere with epithelial downgrowth through a contact guidance mechanism [31,32]. Furthermore, Nevins et al. histologically evidenced that connective tissue fibers were perpendicularly oriented to the implant surface and prevent the apical migration of gingival epithelial cells and fibroblasts [11].

The relative position of the gingival margin did not change in relation to the implant crown at all of the three time points, both within and between both groups, indicating that the gingival margin was maintained coronally at all time intervals with a defined and stable biologic width.

The MCBL at both the mesial and distal aspects immediately after restoration and at 1 year post functional loading in both groups showed no evidence of bone loss. Intragroup evaluation showed a statistically significant minimal crestal bone loss in the mesial and distal aspects of both the control and test groups from baseline to 1 year post functional loading. Mean mesial and distal crestal bone level measurements between the groups at 1 year post functional loading revealed minimal early crestal bone loss with no statistical significance. Both groups had minimal early crestal bone level changes, which was much lesser than Albrekktson's criteria [33] of permissible early crestal bone loss measurements, with 100% implant survival rate.

A level of 100% ISR [34–36] and similar implant stability were observed in both groups [17–19]. The ISQ was greater than the acceptable levels, and thus an immediate loading protocol was followed in both the diabetic and non-diabetic patients. There have been few investigations on immediate functional loading of single-tooth implants. Published results, on the other hand, showed that immediate functional loading of implants with the traditional placement approach and appropriate primary stability could be a viable therapy option. The possibility of rehabilitating the patient's function and aesthetics in a very short period of time was without doubt attributed to the use of LMG implant surfaces and thus it was proposed that Laser-Lok technology tended to improve hard and soft tissue integration, which might be beneficial to immediate loading. In the present study, an interesting parameter for the immediate loading protocol, the ISQ, was hypothesized and was found to enhance primary stability for immediate functional loading.

Future studies comparing well-controlled, moderately- controlled, and poorly-controlled diabetic patients should be carried out, so as to evaluate if Laser-Lok technology could benefit systemically compromised patients. The small sample size of this study is a limitation and hence further studies based on the present results could be formulated to validate the findings with larger sample sizes involving more prospective parameters.

5. Conclusions

To summarize, the study findings indicated that Laser-Lok implants [33] with laser micro-grooved platform switched abutments reduced plaque accumulation 1 year post functional loading in both diabetic and non-diabetic patients, thereby reducing inflammation. The mean crestal bone level changes were very minimal and comparable in both diabetic and non-diabetic patients, in turn maintaining peri-implant sulcus depth and the relative position of the gingival margin. The study suggested that moderately controlled diabetic patients were no longer a contraindication for dental implant survival rate and stability. The additional value of microtexturing on implant and abutment surfaces could be used as an advantage in diabetic individuals to overcome the pathological changes associated with metabolic changes. Furthermore, in an immediate implant loading protocol, laser micro-grooved implants and abutments might mitigate or eliminate the peri-implant mean crestal bone loss in moderately controlled diabetic individuals.

The present study was the first to evaluate clinical and radiographic parameters in laser micro-grooved implants and abutments loaded immediately in moderately controlled

diabetic patients. However, in the future, studies comparing well-controlled, moderately-controlled, and poorly-controlled diabetic patients rehabilitated with Laser-Lok implants and abutments could be carried out to take complete advantage of this novel technology. The small sample size was a limitation, and thus further studies with larger sample sizes involving more prospective parameters should be designed.

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Oral Health of Patients Undergoing Percutaneous Coronary Intervention—A Possible Link between Periodontal Disease and In-Stent Restenosis

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Abstract: ***Introduction:*** There is a well-documented association between coronary artery disease (CHD) and periodontal disease (PD) mediated by common inflammatory pathways. This association, however, has not been investigated extensively in the special context of in-stent restenosis. This study aimed to investigate the periodontal status of patients undergoing percutaneous coronary intervention (PCI) for restenotic lesions. ***Methods and Results***: We enrolled 90 patients undergoing percutaneous coronary intervention and 90 age- and gender-matched healthy controls in the present study. All subjects received a full-mouth examination by a periodontist. Plaque index, periodontal status, and tooth loss were determined. The periodontal state was significantly worse ($p < 0.0001$) in the PCI group, and each periodontal stage increased the odds of belonging to the PCI group. This effect of PD was independent of diabetes mellitus, another strong risk factor for CAD. The PCI group was further divided into two subgroups: PCI for restenotic lesions ($n = 39$) and PCI for de novo lesions ($n = 51$). Baseline clinical and procedural characteristics were comparable between the two PCI subgroups. A significant ($p < 0.001$) association was found between the PCI subgroup and the severity of periodontal disease, with the incidence of severe PD reaching 64.1%. ***Conclusions:*** Patients undergoing PCI for in-stent restenosis exhibit more severe forms of periodontal disease not only as compared to healthy controls but also as compared to patients stented for de novo lesions. The potential causality between PD and restenosis must be studied in larger prospective studies.

Keywords: in-stent restenosis; percutaneous coronary intervention; periodontal disease

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1. Introduction

Coronary artery disease (CAD) remains the leading cause of morbidity and mortality worldwide. Over the past 40 years, percutaneous coronary intervention (PCI) and stent implantation have revolutionized the field of coronary artery disease (CAD) treatment. Owing to its importance, interventional cardiology remains a field of intensive research and development [1]. Despite technical advances and the development of newer-generation drug-eluting stents (DES), in-stent restenosis (ISR) remains the Achilles heel of interventional cardiology. ISR rates range from 3 to 30% depending on the type of stent used and the clinical setting [2]. Risk factors for ISR are diverse and include patient, lesion, and procedural characteristics. The most important patient characteristics and systemic disease states associated with ISR are age, female gender, diabetes, and chronic kidney disease [3].

Periodontal disease (PD) is defined as a bacterially induced, chronic inflammation of the supporting tissues of the teeth [4]. It is among the most common diseases worldwide, with up to 11% prevalence in its severe form [5]. PD and cardiovascular diseases are both related to several common risk factors, and an increasing number of epidemiological studies seem to support the hypothesis of a possible association between periodontal disease and atherosclerosis [6–8]. The role of inflammatory processes is well documented in the pathogenesis of both atherosclerosis and periodontal disease, most likely proving to be the link between the two diseases. Infected periodontal pockets are typical reservoirs of such organisms and their toxins and degradation products, which can cause inflammatory, immunological, and humoral activities, enter the bloodstream, and contribute to atherogenesis and thromboembolic events [9].

There are several possible pathways in which infectious agents may affect atherosclerotic processes. In periodontitis, bacterial plaque impairs the periodontal epithelium and allows the entry of oral pathogens and their harmful elements (endotoxins and exotoxins) into the bloodstream. Direct invasion of the vessel wall by oral pathogens can trigger an inflammatory response that leads to endothelial dysfunction. This induces infiltration of inflammatory cells and vascular smooth muscle cell proliferation, which constitute the pathogenesis of atherosclerosis [10].

Although the exact pathophysiological pathways leading to de novo coronary artery stenosis, bare metal stent restenosis (BMS), and DES restenosis are not the same, inflammation plays an important role in both [11]. This raises the possibility that periodontal disease linked to a systemic inflammatory response may be associated with not only de novo coronary stenosis but in-stent restenosis as well. A possible association beyond clinical interest may have therapeutic implications as well. From the periodontal point of view, it is also necessary to consider the updates in the non-invasive treatment of periodontitis and in the maintenance of the periodontal patient over the years, which can lead to the maintenance of a healthy condition or to a considerable slowdown in the progression of the periodontal pathology, which consistently results in a lower concentration of inflammatory tissue mediators frequently involved in these processes [12,13].

The current study aimed to ascertain any possible connections between periodontal disease and cardiovascular status in PCI patients. We also hypothesized that periodontal disease might be more prominent in patients undergoing PCI for restenosis, thereby linking periodontal status to the development and progression of restenosis.

2. Materials and Methods

2.1. Ethics Statement

The study was approved by the Human Ethics Review Board of the University of Szeged (approval No. 127/2015-SZTE), and the study design conformed to the Declaration of Helsinki in all respects. Written informed consent was obtained from all participants.

2.2. Protocol and Study Participants

The hospital-based case-control study was conducted between 2016 and 2017. Participants (n = 90) were selected from patients who underwent PCI at the Invasive Cardiology Unit of the Internal Medicine Department, University of Szeged. Of the 90 patients, 51 underwent coronary intervention for de novo lesions and 39 for in-stent restenosis. The healthy control group (n = 90) consisted of people attending mandatory lung screening in the same city and period. Participation was voluntary in both groups; the volunteers did not receive any kind of compensation and were free to quit at any time. PCI and control groups were gender- and age-matched.

The indication for percutaneous coronary intervention was set up by an interventional cardiologist before the beginning of this study in all cases according to local practice based on ESC guidelines [14]. Patients with restenosis were eligible for the study if they were diagnosed with in-stent restenosis (defined as re-narrowing of $\geq$50% of the vessel diameter inside or immediately adjacent to the stent) by an experienced cardiologist requiring

coronary intervention [11]. Medical information of each group was extracted from patient files and hospital records. Laboratory values were acquired as a part of the general post-PCI workup. A questionnaire collected demographic and tobacco use data. Participants were grouped as smokers and non-smokers based on their self-reported tobacco use.

Patients with non-cardiovascular diseases influencing periodontal status, such as excessive alcohol consumption, drug abuse, estrogen deficiency, and local or systemic inflammatory conditions, were excluded from the study. Critically ill patients and patients with less than four teeth were also excluded from the study.

The clinical staging of periodontal disease is currently a subject of debate, even though the progression of the disease is well-established in pathological terms [15]. In the present study, the staging proposed by Fernandes and colleagues was used [16]. It requires the following indicators to be recorded: the number of missing teeth (excluding third molars), plaque index [PI, also known as the Silness-Löe Index (0–3)], bleeding on probing (BOP; the presence or absence of bleeding within 15 s after probing), probing pocket depth (PPD; in millimeters), and clinical attachment level (CAL; to describe the position of the soft tissue in relation to the cemento–enamel junction). This protocol has already been used by this research group in several publications [17–19]. According to the above, a patient's periodontal status is reported as belonging to one of four subgroups: (1) healthy, (2) early, (3) moderate, or (4) severe periodontal disease.

2.3. Clinical Examination

Each subject received a full mouth periodontal examination within 48 h after the percutaneous coronary intervention, performed by an expert in the field. PPD, CAL, and BOP were examined with Williams probes (Hu-Friedy Manufacturing Co., Chicago, IL, USA) at six sites per tooth (mesiobuccal, buccal, distobuccal, disto-lingual, lingual, mesio-lingual).

2.4. Statistical Analysis

Statistical analyses were performed in SPSS 21.0 (IBM, Armonk, NY, USA). Continuous variables were described as means and standard deviations; categorical variables were characterized with frequencies unless otherwise stated. For the comparison of specific parameters between the groups, either one-way ANOVA (with Tukey's HSD for the pairwise comparisons), Kruskal–Wallis ANOVA (with Mann–Whitney U for the pairwise comparisons), or the chi-square test was used, depending on the characteristics of the dataset. The normality of the datasets was determined with the Shapiro–Wilk test, and Levene's test was used to test for the homogeneity assumption. The general significance limit was set at $p = 0.05$ but was corrected for multiple comparisons with the Bonferroni correction where necessary. For hypothesis testing, logistic and multinomial logistic regression analyses and the chi-square test was used.

3. Results

The clinical characteristics of the PCI and healthy control groups are presented in Table 1, with the results of the between-groups comparisons for the different parameters. Figure 1 shows the distribution of periodontitis severity categories in the three groups (PCI patients with restenotic lesions, PCI patients with de novo lesions, and controls). The chi-square test indicated a significant association between group and periodontal status (χ^2 = 35,207, df = 6, $p < 0.001$). That is, whether a subject belonged to the PCI or the healthy control group was significantly associated with their periodontal status. We also found a significant difference in the number of teeth ($p < 0.001$) but not in plaque index between the two groups.

Table 1. Clinical and periodontal characteristics of patients undergoing PCI versus controls.

Parameter	PCI Patients $n = 90$	Controls $n = 90$	Sig. (p)
Age	63.2 ± 10.0	67.3 ± 9.9	0.89
Gender (male)	63 (70)	63 (70)	1
Diabetes mellitus	29 (32)	4 (4)	<0.0001
Hypertension	82 (92)	12 (13)	<0.0001
Hyperlipidemia	67 (72)	0 (0)	<0.0001
Chronic renal disease	10 (11)	0 (0)	<0.0001
Active smoker	16 (18)	24 (27)	0.21
Former smoker	18 (20)	13 (14)	0.43
Number of teeth	14.08 ± 6.99	19.71 ± 5.73	<0.0001
Plaque index (0–3)	2 (1–3)	2 (1–2)	0.40
Periodontal status (1–4)	3 (2–4)	2 (2–3)	<0.0001

Significance limit: $p = 0.05$. Numerical values are presented as average ± standard deviation. Categorical values are presented as n (%). Plaque index and periodontal status as median (25–75th quartile); PCI—percutaneous coronary intervention.

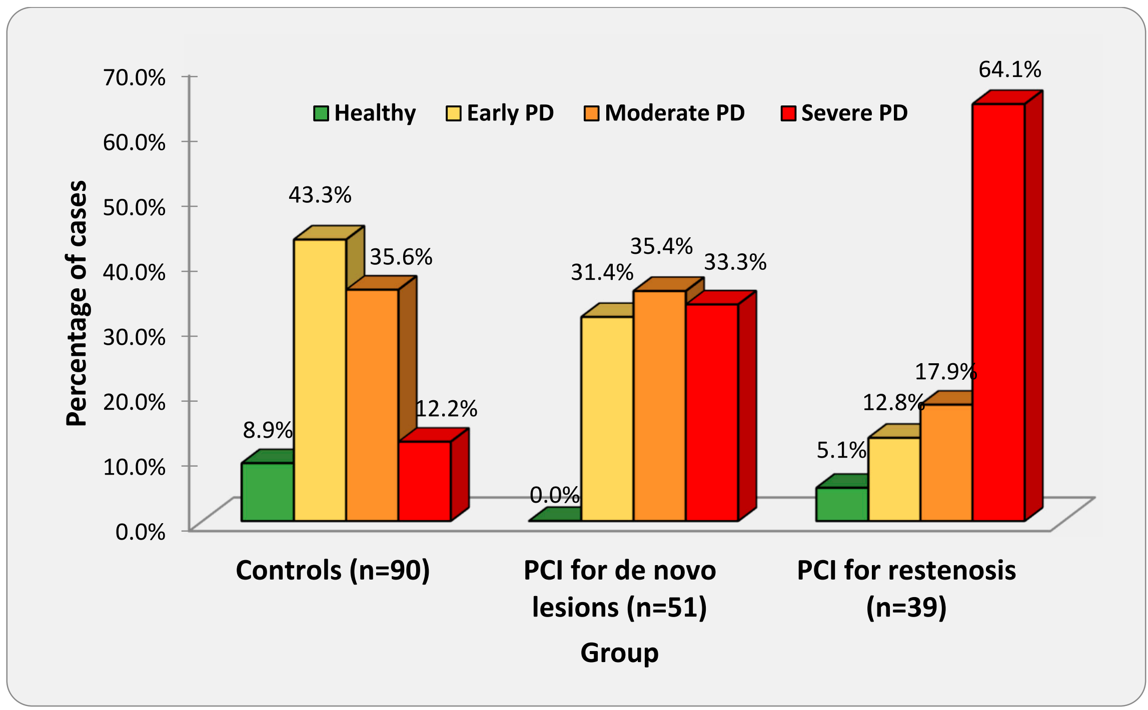

Figure 1. Periodontal state of patients undergoing PCI for restenotic lesions versus de novo lesions and controls.

To examine this question further, a logistic regression model was built. In this model, background variables potentially interfering with systemic health in themselves and/or interfering with the health of the periodontal tissues (periodontal status, diabetes mellitus, smoking, plaque index) were included as factors, and the group (patient or control) was the dependent variable. With this analysis, we sought to determine which factors had a significant association with a subject belonging to the PCI group. The analysis indicated that diabetes mellitus ($p < 0.001$) and periodontal status ($p < 0.05$) were such factors. As for diabetes mellitus, we hypothesized that this factor could influence periodontal health (as often reported in the literature), but this could not be verified in this sample. The chi-square test indicated no significant association ($\chi^2 = 3.049$, df = 3, $p = 0.384$), so we concluded that in this sample, periodontal health was not influenced by the presence or absence of

diabetes. In other words, the two factors exerted their significant effect independently. Regarding periodontal status, we also sought to determine if belonging to the patient group was significantly associated with the severity (stage) of periodontal disease. For this, we used multinomial regression analysis with the group as the target variable and periodontal status as the independent variable. The multinomial regression analysis confirmed the significant effect of periodontal status on whether a given subject belonged to the patient or control group ($p < 0.001$). The odds ratios were as follows: ORstage2: 2.154 (95%CI: 0.42–11.08), ORstage3: 3.13 (95% CI: 0.61–16.04), ORstage4: 15.27 (95%CI: 2.83–82.42). Each periodontal stage increased the odds of belonging to the patient group, but the difference was significant only in the most severe stage (stage 4, $p < 0.01$). In other words, if one had stage 4 periodontal disease, that person had a significantly higher chance of being found in the group of cardiovascular patients at an odds ratio of 15.27.

Within the PCI patient group, two subgroups were differentiated according to the lesion treated during PCI. Fifty-one patients underwent PCI for de novo lesions and 39 for in-stent restenotic lesions. Comparative descriptive data of these groups are given in Table 2, with the results of the between-groups comparisons for the different parameters. We found no significant differences between the two groups regarding baseline clinical and procedural characteristics. Although the sample size was not sufficient for any deeper comparison of these groups, we still wanted to know if the severity of periodontal disease might be associated with restenosis. The frequencies of the four stages of PD by patient subgroup are presented in Figure 1. The χ^2 test indicated a significant association between the patient subgroup and the severity of PD ($\chi^2 = 13.77$, df = 3, $p < 0.01$). Figure 1 shows that the incidence of the individual stages is rather disproportionate in the restenosis group: 64.1% of all the cases fall in stage 4. In the de novo stenosis group, the cases were much more evenly distributed across the categories, even if no healthy case was observed. There was no significant difference between the two groups regarding the number of teeth or plaque index.

Table 2. Clinical and periodontal characteristics of patients undergoing PCI for restenotic lesions versus de novo lesions Significance limit: $p = 0.05$. Numerical values are presented as average ± standard deviation or median (25–75th quartile) as appropriate. Categorical values are presented as n (%). ACE—angiotensin-converting enzyme; ACS—acute coronary syndrome; ARB—angiotensin II receptor blocker; BMI—body mass index; BUN—blood urea nitrogen; CKD—chronic kidney disease; LAD—left anterior descending artery; EF— ejection fraction; LCX—left circumflex artery; LMT—left main trunk; LV—left ventricular; MI—myocardial infarction; MPV—mean platelet volume; (N)OAC—(new) oral anticoagulant; PCI—percutaneous coronary intervention; RCA—right coronary artery; WBC—white blood cell; *in 7 cases DEB was used during PCI for restenosis.

Parameter	PCI Patients with Restenotic Lesions ($n = 39$)	PCI Patients with De Novo Lesions ($n = 51$)	Sig. (p)
Demography			
Age (years)	63.7 ± 8.9	62.8 ± 10.9	0.67
Gender (male)	27 (69)	36 (70.6)	1.00
BMI	30 ± 5.5	29.4 ± 4.7	0.77
Active smoker	6 (15)	10 (20)	0.06
Former smoker	9 (23)	9 (18)	0.43
Medical history			
Hypertension	35 (95)	47 (92)	0.83
Diabetes mellitus	14 (36)	15 (29)	0.64
CKD	5 (13)	5 (10)	0.74
Hyperlipidemia	32 (82)	35 (69)	0.14
Prior PCI	39 (100)	27 (52.9)	NA
LV-EF	55.4 ± 14.5	56.2 ± 10.9	0.99

Table 2. *Cont.*

Parameter	PCI Patients with Restenotic Lesions (n = 39)	PCI Patients with De Novo Lesions (n = 51)	Sig. (p)
Medications			
Aspirin	38 (97)	50 (98)	0.84
P2Y12 inhibitor	39 (100)	51 (100)	1
(N)OAC	9 (23)	8 (16)	0.72
Beta-blocker	34 (87)	47 (92)	0.44
ACE inhibitor/ARB	35 (90)	47 (92)	0.69
Statin	36 (92)	48 (94)	0.73
Ca-channel blocker	7 (18)	16 (31)	0.15
PPI	35(90)	43 (84)	0.45
Laboratory data			
WBC ($\times 10^3/\mu$L)	7.6 ± 1.9	7.9 ± 1.7	0.2
Hemoglobin (g/dL)	125.3 ± 27	131.8 ± 12.9	0.65
Thrombocyte ($\times 10^3/\mu$L)	208.6 ± 67.2	219.3 ± 52.1	0.18
MPV (fL)	10.9 ± 0.7	10.7 ± 1	0.33
Creatinine (µmol)	91 ± 38.9	87.9± 27.1	0.78
Procedural data			
LM/LAD	19 (40)	26 (42)	0.84
LCX	9 (18)	19 (31)	0.13
RCA	20 (42)	17 (27)	0.22
Multivessel PCI	16 (41)	20 (39)	0.86
Stent length	46.6 ± 29.2	40.2 ± 26.9	0.42
Stent diameter	3.1 ± 0.4	2.98 ± 0.47	0.16
DES*	32 (82)	51 (100)	NA
Periodontal condition			
Number of teeth	13.8 ± 7	14.3 ± 7.1	0.74
Plaque index (0–3)	2 (1–3)	2 (1–3)	0.83
Periodontal status (1–4)	4 (3–4)	3 (2–4)	<0.01

4. Discussion

The present study investigated the periodontal status of patients undergoing percutaneous coronary intervention. Patients undergoing PCI for significant coronary artery lesions were more likely to have moderate or severe periodontitis than healthy controls. We also found significant differences in the periodontal health indices between patients undergoing PCI for restenosis and de novo lesions. Namely, severe forms of PD were more often found among patients with restenosis. Our results raise the possibility of an association between in-stent restenosis and periodontal disease.

4.1. Periodontal Disease and CAD

The link between periodontal disease and angiographically verified coronary disease has been well established in stable coronary artery disease and acute coronary syndromes [20–23]. Nonetheless, it is still a matter of debate whether PD is an independent risk factor for CAD or whether the association is based on an abundance of shared risk factors, including, amongst others: age, gender, smoking, diabetes mellitus, hypertension, obesity, low socioeconomic status, and stress [24]. In our age- and gender-matched case-control study, we were not only able to provide further evidence for a strong association between PD and CAD, but we also showed that this association is independent of diabetes mellitus (one of the most prominent shared risk factors for both CAD and PD).

While there was a clear difference in PD status, no meaningful difference was found in plaque index between patients undergoing PCI and healthy controls. The relationship between oral hygiene, as represented by PI, and periodontal disease is well established [25].

Furthermore, hospitalization itself (for the intervention) might negatively affect oral self-care and PI [26,27]. In this study, we found no such effect, so it is unlikely that the difference in PD status was mediated by poor oral hygiene.

4.2. Periodontal Disease and Restenosis

Periodontal disease in the special subset of CAD patients with restenosis has not been studied extensively. Fukushima et al. found an association between PD at baseline and increased risk of future major adverse cardiac events (MACE) in CAD patients who underwent PCI with a drug-eluting stent (DES) for de novo coronary lesions [28]. In this study, however, MACE was driven by non-target lesion myocardial infarction and death and was not powered to investigate differences in restenosis occurrence. Wu Y et al. found a positive, independent correlation between triglyceride index (a marker of insulin resistance), oral infections, and in-stent restenosis in the subgroup of acute coronary syndrome patients 24 months after DES implantation [29].

The pathophysiology of restenosis differs greatly from that of de novo coronary artery disease [11]. Restenosis is a local response to vascular injury caused by the coronary intervention itself in the form of balloon dilatation and/or stent implantation. Damage to the arterial wall triggers an inflammatory reaction which plays a key role in the activation, migration, and proliferation of endothelial, smooth muscle cells, and macrophages [30]. This, in turn, leads not only to reendothelization but potentially neointimal hyperplasia and, in the long run, neoatherosclerosis, thus re-narrowing the stented lumen [31]. Our findings raise the novel possibility of chronic periodontal disease playing a role in this specific pathologic subset, leading to restenosis progression.

Although the exact mechanisms are unknown, several pathways have been suggested by which periodontal disease may contribute to the progression of in-stent restenosis [29]. The most plausible explanation would be through the common inflammatory pathway already well recognized in connecting CAD progression and PD [9]. Indeed, markers of inflammation that are elevated in PD, such as CRP, matrix metalloproteinase 2, and TNF-α, can also increase the risk of restenosis [32]. PD is also closely associated with endothelial dysfunction, a known risk factor of ISR [33]. Endothelial dysfunction in PD may occur as a result of direct vessel wall invasion by oral pathogens triggering an inflammatory response [34] or possibly through high levels of circulating trimethylamine N-oxide, a harmful oral microbiota-generated metabolite [35]. Platelets play an important role not only in hemostasis but also in inflammation. Periodontal pathogens, such as Porphyromonas gingivalis, activate platelets and cause platelet aggregation through HgP44 (hemagglutinin domain protein) [36]. Activated platelets, in turn, can contribute not only to atherosclerosis but also to the progression of restenosis [37]. Furthermore, Porphyromonas gingivalis may influence gene expression in vascular smooth cells leading to increased proliferation and may also induce migration [38]. Smooth muscle cell proliferation and migration play an important role in the pathogenesis of neointimal hyperplasia leading to ISR [30]. Finally, it is important to note that as with PD and atherosclerosis, PD and in-stent restenosis also have been associated with common underlying medical conditions, such as age, gender, diabetes mellitus, chronic kidney disease, and multivessel coronary disease. [3,24] Thus, the question of causality, whether PD is an independent risk factor of in-stent restenosis or the association is based on common risk factors, is a possible debate as with PD and atherosclerosis [24].

4.3. Clinical Implications

Current guidelines advise the use of local antiproliferative agents via drug-eluting stents and balloons in the prevention and treatment of in-stent restenosis. They also stress the importance of intravascular imaging to recognize possible underlying mechanical substrates and advise interventional treatment algorithms based on these results [39]. However, besides local drug- and mechanical therapy, considerable research is directed toward preventing restenosis via a systemic approach using anti-inflammatory drugs. Evidence on

the effect of periodontal therapy on surrogate risk factors of systemic inflammation (IL6, CRP, TNF-alpha) is well established [23,40,41]. This raises the intriguing possibility of systemic inflammation inhibition by periodontal therapy playing a role in a multidisciplinary approach to the prevention of in-stent restenosis. Further prospective randomized clinical trials would be needed to prove this possible effect. On a more general level, our findings call attention to the importance of screening for poor oral hygiene and already-developed periodontal disease in the high cardiovascular-risk group of patients undergoing coronary interventions, especially those with restenosis.

4.4. Study Limitations

All limitations inherent by the nature of cross-sectional, case-control studies apply to our study as well, and as such, it cannot confirm causality. Patient enrollment was skewed towards including patients undergoing PCI for restenosis. Thus, investigator bias leading to unrecognized confounding factors potentially influencing results cannot be ruled out. Patients undergoing PCI for restenosis were compared to healthy individuals and other PCI patients with comparable clinical and procedural characteristics. Therefore, lesion-specific and procedural aspects of the initial coronary intervention, which may also influence the development of restenosis, could not be considered. There were no significant differences in baseline clinical characteristics between the in-stent restenosis and de novo lesion PCI groups. This, however, does not rule out the effect of possible common underlying medical conditions as a formal multivariate analysis could not be performed due to the small number of patients.

5. Conclusions

In conclusion, our study shows that patients undergoing PCI for restenotic lesions exhibit more severe forms of periodontal disease not only as compared to healthy controls but also as compared to patients stented for de novo lesions. The potential causality between PD and restenosis needs to be studied in larger prospective studies. Nonetheless, these facts further support the importance of periodontal screening and periodontal care in PCI patients, which may play a role in hindering future cardiovascular events, including restenosis.

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ROLE OF CATHEPSIN IN ORAL DISEASES – A REVIEW:

ABSTRACT:
Cathepsins are a class of globular lysosomal proteases that are known to be responsible for protein degradation. They have many biological roles besides proteolysis, such as apoptosis wound healing angiogenesis, proenzymatic activation, bone remodeling, and resorption. The role of cathepsins in pathogenesizing systemic diseases such as cancer, bronchial asthma, atherosclerosis, neurological disorders, rheumatoid arthritis and osteoarthritis has been involved. This review emphasizes the role of cathepsins in multiple oral diseases such as periodontitis, odontogenic cysts, ameloblastoma, tumors of the salivary glands and malignant melanoma.

KEYWORDS: Cathepsin, Oral disease, Proteases, cancer

1. INTRODUCTION:

Proteases are the type of enzymes that catalyze hydrolysis of peptide bonds and aid in the digestion of proteins. These enzymes are pervasive in nature, and control many biological processes[1]. They control blood clotting, cell proliferation, angiogenesis, wound repair, necrosis, and apoptosis[1]. There are 84 families of proteases that are categorized by their catalytic activity into serine, aspartic, cysteine, and metalloproteases[1]. The name cathepsin, which is derived from the Greek kathepsein (to digest), was proposed for the protease that was active in a slightly acidic environment[2].Later, Rich Willstaetter and Eugen Bamann proposed the terminology "cathepsin" in 1929 to describe the tissue proteolytic activity of leucocytes at a slightly acidic pH[1]. Cathepsins are graded as:

1. Serine proteases: cathepsins A and G;
2. Aspartic proteases: D and E cathepsins; and lysosomal cysteine cathepsin
3. There are 11 human cysteine cathepsins, i.e., the cathepsins B, C, F, H, K, L, O, S, V, X and W, existing at the sequence level; this was confirmed by a bioinformatic analysis of the draft sequence of the human genome[2].

Such enzymes play a key role in multiple physiological processes such as apoptosis, antigen presentation, activation of proenzymes, wound healing, bone remodeling, neuropeptide, and storage of hormones[1]. The expression of cathepsins is regulated by endogenous inhibitors such as cystatins, serpins, and thyropins to preserve tissue homeostasis[2]. The release of inactive cathepsins and the presence of endogenous inhibitors help to maintain physiological equilibrium[1]. Numerous ailments such as atherosclerosis, osteoporosis,

arthritis, neurological disorders and cancer have been connected with their dysregulation[1]. Cathepsins are also produced by pathogenic bacteria which act as virulence factor and result in development of diseases[1].

2. STUCTURE OF CATHEPSIN:

The crystal structure of papain, a cysteine protease from Carica papaya, was among the first dozen protein crystal structures to be determined. Together with actinidin, these two structures provided the first insight into their three-dimensional (3D) structure. Later developments enabled the isolation of cysteine cathepsins, such as cathepsin B, H, L, S, X, C, from various tissues, while the rest of the cathepsins were expressed in various expression systems[2].

3. CATHEPSIN STRUCTURE ACTIVATION AND INHIBITION:

The cathepsins are produced as inactive zymogen; the prodomain found in zymogen blocks the active sites, preventing hydrolysis of the substrates[3]. Zymogen change is necessary for activation, in which the prodomain is removed[3]. The signal peptide present in inactive state is cleared inside the endoplasmic reticulum and these proenzymes are glycosylated, which are then carried to Golgi apparatus[2]. Phosphorylation of mannose residues occurs in Golgi apparatus, and mannose 6 phosphate is produced which reaches lysosomes through mannose 6 receptor pathway[3]. Acidification occurs in late endodosome resulting in disassociation of prodomain from active site and becoming active in cathepsins[3]. Therefore, Prodomain serves as an autoinhibitor for cathepsins[3]. The entire activation takes place through autocatalytic or transactivation mechanisms in the lysosomes[3]. Activation process is enhanced by acidic condition and presence of glycosaminoglycans. Proteolytic activation is mediated by metalloproteinases in the matrix[3].
Cathepsin activity can be controlled mainly by distortion and blockage of active site by inhibition[1]. Endogenous inhibitors including cystatins, thyropins, and serpins suppress cathepsin[1]. Cystatins form the largest group of endogenous cathepsin inhibitors that primarily target cysteine proteases[4]. Intracellular cystatins inhibit cysteine proteases by slightly blocking the active center by noncovalent interaction[4].
The cystatine family is further divided into:

- cystatin type 1 (stefins),
- cystatin type 2 (cystatins), and
- cystatin type 3 (kinogens).

The serpins can inhibit serine proteases and cysteine proteases[4]. Its inhibitory mechanism is by active site degradation[4]. Thyropins suppress the proteases in cysteine. There are no known endogenous aspartate protease inhibitors till date[4].

4. CATHEPSIN LOCALIZATION AND FUNCTION:

CATHEPSIN B:

Cathepsin B is the first identified member of what has become known as the broad family of lysosomal cysteine peptidases[5].This is the most widely expressed cathepsin encoded by the CTSB gene mapped by chromosome 8p22[1]. It is present at high levels in gingival crevicular fluid and macrophages[1]. Cathepsin B was immuno-localized to granular duct cells and gingival fibroblasts in the submandibular gland[1]. This is active primarily in cellular processes such as proteolysis, antigen synthesis, and apoptosis[1]. Cathepsin B is an essential activator of trypsin in acute pancreatitis[3]. It also causes collagen degradation and other non-collagenous matrix proteins, and thus plays a key role in resorption lacunae formation in deciduous teeth[6]. It can be seen in the human dentin- pulp complex [1]. Cathepsin B was identified also in ameloblasts[1].

CATHEPSIN C:

Cathepsin C (CTSC), also known as dipeptidyl peptidase I, is encoded by the CTSC gene located on chromosome 11q14[7]. It is an exogenous salivary peptidase that extracts dipeptides away from N-terminals peptides[7]. In inflammatory cells, it plays a major role in activating platelet factor XIII and various serine proteases[7]. Increased levels of enzymatically active serine-cysteine cathepsin-C were expressed by dermal / stromal fibroblasts and bone marrow-derived cells, which controlled the complexity of infiltrating immune cells in neoplastic skin, angiogenic vasculature development and overt squamous cell carcinoma growth[8].

CATHEPSIN D:

Cathepsin-D is a proteinase that induces collagenolytic activity, resorption of the bone and is closely involved in tumor progression biological process[9]. Cathepsin-D was present in many normal tissues, including epithelium, fibroblast, and macrophages[9]. Cathepsin-D 's physiological function is thought to be involved in the self-destruction of senescent or weakened epithelial cells[9]. Cathepsin D is involved in protein, polypeptide hormone and growth factor metabolic degradation[10]. It functions as mitogen in many epithelial tissues and helps in remodeling and renewal of tissue[10]. It is present in the gingival fluid in the oral cavity and immuno-located in rat junctional epithelium and oral mucosa[10].

CATHEPSIN G:

It is mainly formed by neutrophils and plays an important role in the elimination of intracellular pathogens and the breakdown of tissues at inflammatory sites[1]. In addition, it is involved in platelet activation that leads to platelet aggregation and formation of clots[1]. CTSG has been found in other myeloid cells, such as B cells, primary human monocytes, dendritic myeloid cells, dendritic plasmacytoid cells and murine microglia[11]. Cathepsin G has a number of functions. It can clear pathogens, regulate inflammation, stabilise blood pressure, and induce thrombogenesis by altering chemokines, cytokines, cell surface receptors and C components[11]. The concentration and activity of CTSG are increased in the synovial fluids of rheumatoid arthritis (RA) patients[11].

CATHEPSIN K:

This protein, a part of lysosomal cysteine proteases, mainly expressed in osteoclasts and is involved in bone remodelling and resorption. It can break down bone and cartilage through its catabolic action[1]. Cathepsin K found in the odontoclasts in the deciduous tooth is responsible for the extracellular degradation of dentin collagen during physiological root resorption[1]. In rheumatoid arthritis joints, cathepsin K is strongly expressed in osteoclasts, in most epithelial cells, and in synovial fibroblasts[2]. Cathepsin K is the only enzyme that has been unambiguously recorded in mice and humans to play an important role in bone resorption[2].Cathepsin K was also highly displayed in patients suffering from ankylosing spondylitis at bone destruction sites[11]. Cathepsin K contributes both to the erosion of the blood vessels and to plaque destabilization[12]. Cathepsin K inhibitors are useful tools for adipogenesis and analyse the role of cathepsin K in obesity, and may represent potential for future treatment[12]. Cathepsin K has also been suspected in pathogenesis of osteoarthritis[12]. In addition to its expression in breast, lung, melanoma and thyroid cancers, cathepsin K has also been associated with increased invasive potential in prostate tumours[12].

CATHEPSIN L:

Cathepsin L is implicated in the cleavage of a broad variety of compounds, such as fibronectin, collagen and laminin, including the extracellular matrix[10]. Cathepsin L is believed to be involved in intracellular or endocytosed protein turnover, antigen processing and presentation, bone resorption, and various other processes[10].

CATHEPSIN S:

Importantly, cathepsin S is expressed in professional antigen-presenting cells (APCs), such as dendritic cells (DCs) and B-cells[2]. It is a lysosomal cysteine protease capable of degrading extracellular matrix components, such as collagen, elastin, fibronectin, laminin, and proteoglycans, which indicate a pivotal role in homeostasis and repair of tissue[13]. Cathepsin S facilitates cell migration and also controls differentiation of osteoblasts and remodelling of bones[13]. Cathepsin S has been widely involved in health and pathology including including autoimmune disorders, allergic inflammation and asthma, diabetes and obesity, cardiovascular and respiratory disorders, as well as cancer[13].

CATHEPSIN W:

Cathepsin W is mainly found in CD8 + lymphocytes and natural killer (NK) cells[2].

CATHEPSIN L:

Cathepsin L variants localized to the nucleus play a role in the regulation of cell-cycle progression[2].Cathepsin L involved in transduction of cardiac signal[3].

CATHEPSIN V:

- Cathepsin V (also called L2) is strongly homologous to cathepsin L but its expression is limited to thymus and testis in contrast to the omnipresent cathepsin L[2]. Specific expression of cathepsin V in human thymic cortical epithelial cells[14]. In stenotic aortic valves and atherosclerotic plaques, the expression of cathepsin V is increased, indicating a role in the degradation of elastin laminae in diseased blood vessels[14]. Cathepsin V was considered a possible diagnostic marker for colon tumours which was identified as an antigen in breast cancer patients[14].
- In patients with multiple sclerosis, the expression and activity of cathepsin B, cathepsin D, and cathepsin S was increased and correlated with the physiological degradation of myelin basic protein[11].
- Cathepsin B and Cathepsin S also had a potential role in the pathology of grave's disease and myasthenia gravis[11].

5. CATHEPSIN IN ORAL DISEASES:

DENTAL CARIES:

- Dental caries is a microbial disease caused by demineralization of inorganic and dissolution of organic matrix[1]. MMPs are primarily involved in the pathogenesis of caries[1]. Cysteine cathepsins are colocalized and thought to activate the latent MMPs and facilitate the development of caries with MMPs[1]. Compared to sound dentin, cathepsin B demonstrated greater immunoreactivity in carious dentin[1]. Cathepsin B levels were associated with rising depth in carious dentin[1]. In the dentinal fluid, MMP-20, MMP-2, and probably also cathepsin B are present and can contribute to the lesion activity in areas with large dentinal tubules[15].

PERIAPICAL LESIONS:

- As a sequelae of pulpal inflammation periapical lesions form around the tooth apex[1]. They manifest as a result of host immune response against bacteria. It may result in resorption of hard tissues and destruction of periapical tissues[1]. Often, some inflamed tissue factors may contribute to the failure of endodontic therapy[1]. Osteoclasts play a key role in deteriorating the bone matrix in periapical lesions[1]. Cathepsin K is primarily expressed in osteoclasts and involved in bone remodelling and resorption[12]. Cathepsin K was a vital bone-resorbing protease and the race for the treatment of osteoporosis was to produce highly selective cathepsin K inhibitors[12].

ORAL LICHEN PLANUS:

- Oral lichen planus is a chronic T-cell- mediated mucosal disease. In psoriasis, cathepsins K in inhibitor has shown to inhibit TLR-mediated cytokine by dendritic cells[16]. TLR4 and TLR9 induction occur in oral lichen planus, and co-expression of cathepsin K has been seen in some dendritic cells[16]. It is therefore proposed that in oral lichen planus, cathepsin K is involved in dendritic cell upregulation of the activity of cytokines[16]. In OLP lesions, epithelial cells under the influence of the underlying stromal proteases of the connective tissue, secreted by the inflammatory cells stained by Cathepsin B, are more likely to turn into cancer cells[17].

PERI-IMPLANTITIS:

- The peri-implantitis inflammatory process affects the tissues surrounding dental implants, sometimes contributing to implant failure[1]. Yamalik et al . noted that cathepsin K operating levels are higher in peri-implantitis and peri-mucositis compared to healthy peri-implant tissues[1]. Increased RANKL expression stimulated the formation of active osteoclasts leading to increased cathepsin K development leading to bone resorption[1].

PERIODONTITIS:

- Periodontitis is a chronic inflammatory disease that is highly prevalent and is characterised by bone, attachment, and even tooth loss[13]. Cathepsin S expressed in periodontitis stimulates the proliferation and migration of PDL cells and thus wound closure, suggesting that this cysteine protease may play a critical role in the healing and periodontal remodelling[13]. Another member of the cathepsin family, Cathepsin K was also associated with periodontal diseases[13]. Cathepsin S is interestingly capable of degrading Cathepsin K, suggesting complex interactions between both cathepsins[13].
- Cathepsin G also showed increased involvement in adults periodontitis[18]. Through proteolytic activation of latent neutrophil procollagenase (promatrix metalloproteinase 8), this enzyme may break down periodontal tissues directly and indirectly and can contribute to periodontitis[18]. As these enzymes correlate with pocket depth, they can serve as biomarkers of periodontal inflammation[18].

6. PAPILLON – LEFEVRE SYNDROME:

It is an autosomal recessive disorder characterised by severe early-onset periodontitis and palmoplantar hyperkeratosis that results in premature loss of teeth. This condition is responsible for mutations within the CTSC gene[1]. CTSC plays a predominant role in phagocytosis[1]. Immunological findings such as reduced neutrophil, monocyte chemotaxis, impaired phagocytosis, and altered superoxide production are noted in patients affected by this syndrome[1]. The cell ability of polymorphonuclear leukocyte (PMNL) does not remove the Aggregatibacter actinomycetemcomitans that result in periodontitis[1].

GIANT CELL TUMORS:

- The giant cell tumour is a benign bone neoplasm marked by localised osteolysis[1]. Cathepsin K is detected exclusively in osteoclast-like giant cells in giant cell tumours which support the hypothesis that it is the predominant factor in osteolysis[1]. Cathespin K staining patterns were large in giant cell lesions in 85% of peripheral giant cell granulomas, 60% of giant cell tumours, and 57% of core giant cell granulomas[1]. Cathepsin L is other protease present in giant cell lesions and tumors[1]. Cathepsin D plays a role in numerous physiological and pathological procedures, including bone resorption[19]. Cathepsin D has been observed in the giant cells of both CGCG and PGCG lesions[19]. The osteoclastic origin of giant cells in both PGCG and CGCG could be confirmed by the expression of Cathepsin D in giant cells, which is considered a factor involved in bone degradation and one of the enzymes present in osteoclasts[19].
- Cathepsin D plays an indirect role in the degradation of the bone matrix through activation of Cathepsin B and L in osteoclasts[19]. Therefore, a higher Cathepsin D concentration in CGCG giant cells may be considered as a factor in developing more active Cathepsin B and L, and therefore more degradation of the bone[19]. Active cathepsin B and L will prevent further osteolytic activity in the lesion[19].

ODONTOGENIC CYSTS AND TUMORS:

- The staining intensity of cathepsin D between various odontogenic cysts was observed in each layer and stroma / capsular wall[1]. In the epithelial lining and stroma, different staining patterns were observed. The staining severity increased gradually from the dentigerous cyst to the odontogenic keratocyst (OKC) via the radicular cyst[1]. This increasing pattern of expression seemed to correlate with increasing aggression[1]. Intense granular staining has been found in OKC's separation region. This finding indicates that cathepsin B may be one of the essential enzymes in epithelium and connective tissue separation in OKC[1]. Marked staining of the granular cells and spillage in granular cell ameloblastoma compared with others may explain its aggressive behaviour, recurrence, and metastatic potential[1].
- Cathepsin-D is a proteinase that induces collagenolytic activity, bone resorption and is closely involved in the biological tumour progression process that has been documented to be an indicator of aggressive behaviour in human tumours, including oral squamous cell carcinoma, due to its ability to digest the extracellular matrix[9] It is suspected that cathepsin-D physiological function is involved in the self-destruction of senescent or weakened epithelial cells[9].
- Cathepsin D is expressed in the epithelium, connective tissue and stromal cells of odontogenic cyst and tumors[9].

SALIVARY GLAND TUMORS:

- Among head and neck tumours, salivary gland tumours are histologically the most heterogeneous tumours. Higher expression of cathepsin D in neoplasms with malignant salivary gland compared to benign tumours. Intense expression of cathepsin D was observed in mucoepidermoid carcinoma and adenoid cystic carcinoma when compared to pleomorphic adenoma, indicating that it was a marker of invasive potential and aggressive behavior[20].

SJOGREN'S SYNDROME:

- Cathepsin B, cathepsin D, and cathepsin S were present in Sjögren's syndrome and had greater immunoreactivity in patient's acini and tears[11]. The cathepsin S inhibitor was effective in the prevention of salivary and lacrimal autoimmune lesions in patients with Sjögren's syndrome[11].

ORAL CANCER:

- Cancer is a multi-stage phase involving genetically altered changes. Diverse proteases monitor the invasiveness and metastasis. Cathepsins degrade the extracellular matrix and thus interrupt intercellular communication.
- Cathepsin B can contribute to uncontrollable proteolysis and participate in the process of tumor development, invasion, and metastasis in dissolution and remodeling of the connective tissue and basement membrane[1]. The expression of Cathepsin B was associated with positive lymph node metastasis and a higher tumor grade, thereby indicating its role in malignant tongue cancer progression[1].
- On the other hand, although Cathepsin C is up-regulated during carcinogenesis of the pancreatic islet, it lacks functional significance in mediating neoplastic progression in that organ[8]. Since the expression and enzymatic activity of both of both Cathepsin B and Cathepsin C are raised in various tumors[8]. Increased levels of enzymatically active Cathepsin C were expressed by dermal / stromal fibroblasts and bone marrow-derived cells, which regulated the complexity of infiltrating immune cells in neoplastic skin, angiogenic vasculature formation, and overt squamous cell carcinoma growth[8].
- The expression of cathepsin D was observed during the conversion of dysplasia to oral squamous cell carcinoma[17]. The expression associated with invasiveness and progression of cancer. The origin of cathepsin D from the lysosome to the invasive front of the tumour is altered and its expression is associated with abnormalities of the p53 gene[17]. Cancer cells also secrete procathepsin D that acts as a metastases and mitogen stimulating proinvasion[17].
- Majority of cancers including a few dysplastic areas surrounding carcinoma tissue, cathepsin K was found in OTSCC patient samples[21]. In the morphologically normal-looking tongue epithelium, we were not able to detect cathepsin K[21]. Cathepsin K was present in carcinomas, as well as stromal cells[21].
- Overexpression of cathepsin L is more likely to lead to progression of tumor in oral cancer[1]. Cathepsin L expression associated with metastasis of the lymph node and poor prognosis, indicating its function as a potent biomarker for cancer prognosis[1].

4. CONCLUSION:

Cathepsins play a vital role in pathogenesis of both systemic and oral diseases. They can act as biomarkers in various oral diseases. Future research and investigation is needed to have a clear idea about the correct pathogenesis of cathepsins in oral diseases.

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