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Oral Immunological Profile Impact on Local and Systemic Disease

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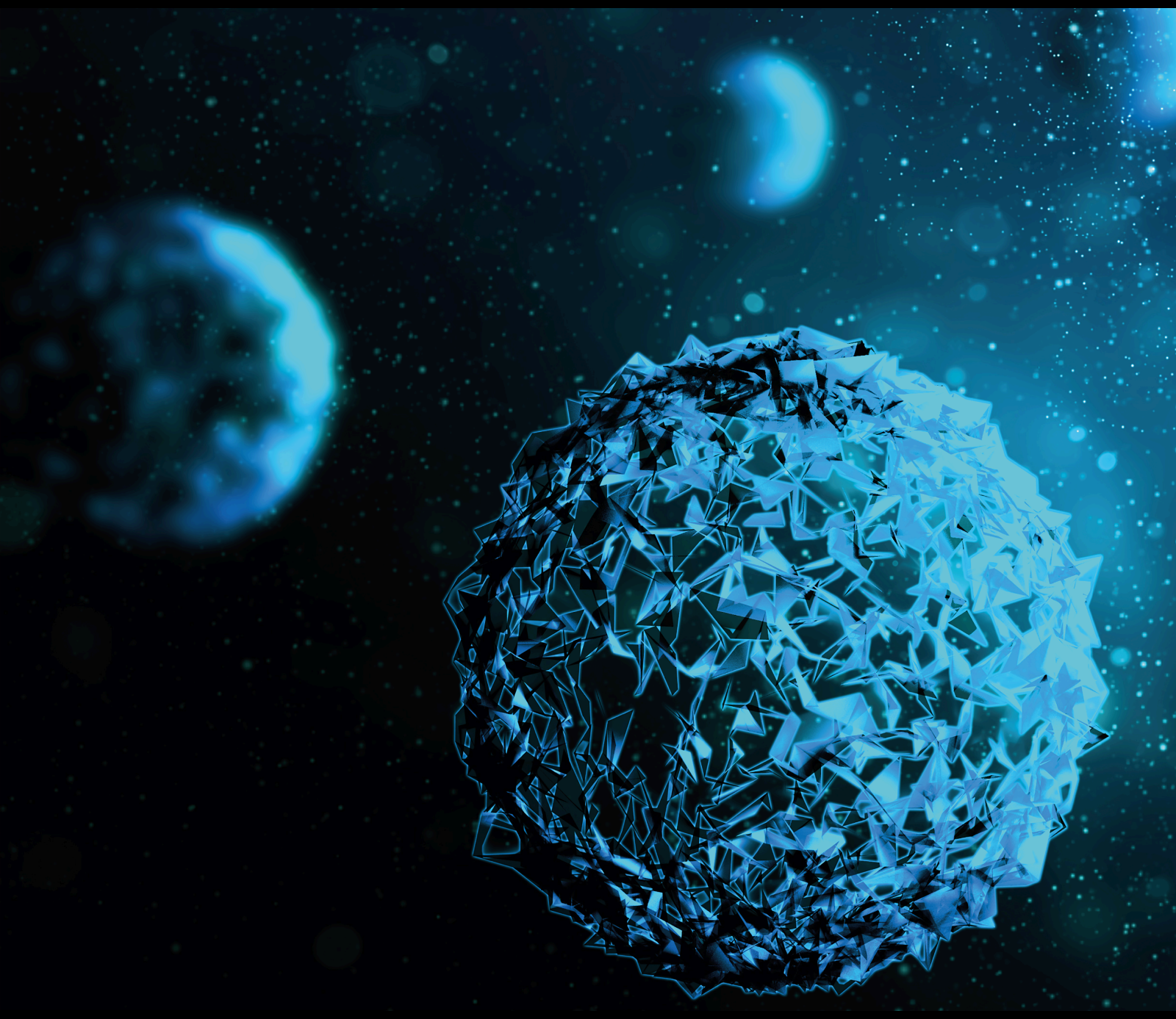

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Editorial Board

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Hindawi
BioMed Research International
Volume 2020, Article ID 5273046, 2 pages
https://doi.org/10.1155/2020/5273046

Editorial

Oral Immunological Profile Impact on Local and Systemic Disease

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Received 15 May 2020; Accepted 15 May 2020; Published 27 May 2020

Recently, the international literature has focused on the potential correlation between the systemic diseases and typical oral clinical conditions, which deeply influence a patient's oral health. In addition, research in recent years has increasingly highlighted on odontology data for general patient health assessment. Therefore, the presence of chronic inflammatory processes at the oral level may prevent some patients from following a particular therapeutic path, such as chemotherapy for the cancer patients.

In this context, many medical specialists are therefore turning to dentists to determine the absence of acute or chronic inflammatory conditions in patients' mouths. Oral health status is an indicator of overall body health, and this can help inform a medical specialist on the most appropriate type of therapy to implement for a patient. Moreover, patients who begin systemic therapy with bisphosphonate drugs are required to have a high oral hygiene level with no infections. The mechanism of action of bisphosphonate drugs means that they impair bone healing and modeling, which in turn leads to increased risk of jaw osteonecrosis following surgical dental procedures. Oral infection increases the risk of patients developing this condition. Regarding diabetes in particular, several published papers have demonstrated how control of periodontal health may be useful in managing the general health of patients. There are also correlations observed between changes in oral inflammatory cytokines and cardiovascular risk. Oncological pathologies, which are often accompanied by a pattern of alterations in gene expression, have also been reported to exhibit oral manifestations with alteration of the oral mucosa. Moreover, these pathologies can be located both at the oral cavity and in other body areas, and for this reason, the first dental visit is crucial to have quick diagnosis.

This special issue aimed to highlight the close relationship between systemic disease and oral pathologies and to investigate the connection between alterations in the oral immunological profile and systemic disease. Researchers were invited to submit original research articles and review articles in this field, with a focus on profiles of oral inflammatory cytokines and gene expression related to systemic and local pathologies. Submissions concerning acute, chronic, and oncological conditions were all welcomed. In order to cover state-of-the-art research and understanding in all relevant disciplines, submissions covering anatomical, histological, and biological features of the oral immunological profile of systemic disease patients, together with bioengineering and tissue engineering research, were all encouraged.

Victor M Martines et al. in their paper aimed to compare variations in quantified tumor necrosis factor-alpha (TNF-α) levels in patients with periodontitis stage 2 grade B (POD2B) and/or type 2 diabetes (T2D) and to identify any relationships between this cytokine and these diseases. Kruskal-Wallis tests was used to identify differences in TNF-α levels, LI, PD, BMI, BG, and HbA1c by group. Differences ($p < 0.001$) were found for LI, PD, BG, and HbA1c. A Spearman test was used to calculate possible correlations between TNF-α levels and LI or PD identified a weak but significant negative correlation of TNF-α with LI (Rho = –0199; $p = 0.012$), and a moderately positive correlation of LI with PD (Rho = 0.509; $p < 0.001$). The authors concluded how no

variation was found between TNF-α levels and the presence of POD2B, POD2B/T2D, or T2D, suggesting the absence of any direct relationship between progression of these diseases and TNF-α levels. However, a correlation was present between low TNF-α concentrations and greater LI.

Diana Peniche Palma et al. compared levels of matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) in gingival crevicular fluid (GCF) from subjects with controlled and noncontrolled type 2 diabetes mellitus (T2D), with and without stage 2 grade B periodontitis (POD2B) versus healthy (H) subjects. The authors found how the highest concentration of MMP-9 corresponded to the H group, while the lowest corresponded to the T2D controlled group. Regarding MPO levels, the highest levels were associated with the T2D controlled with POD2B group and the lowest with the T2D controlled group. Therefore, no apparent relationship between the elevation of MMP-9 and MPO levels was observed among subjects with T2D, with and without POD2B, compared to H subjects.

Colonna et al. tested in an animal model the nerve regeneration technique with a hypoallergenic acellular dermal matrix used to wrap the microsurgical neural suture and investigated how the application of suture material could influence the oral health. The authors stated how no correlation between the material applied and oral status was recorded and, moreover, how the histological and functional assessments showed a functional recovery of the injured nerve in the test groups, stressed by the results of the grasping tests and the meaningful increasing in fiber diameter and higher g-ratio. Moreover, a connective tissue cuff distinguishes the distal portion of the injured nerve. Considering the easy availability and handling of the material used in this study, we can conclude that this experimental technique can be considered as a valid alternative to protect nerves in nerve wrap surgery.

Cervino et al. performed a literature revision of the diabetes and oral health correlations. In their paper a comprehensive review of the literature was conducted according to PRISMA guidelines accessing the NCBI PubMed database. The authors conducted the search of articles in English language. The results of the last 10 years have been considered, which present useful information regarding the oral conditions. A total of 17 relevant studies were included in the review. The study evaluated only papers with specific inclusion criteria regarding oral health. The works initially taken into consideration were 782; subsequently applying the inclusion and exclusion criteria, there were 42 works. After a careful analysis of the work obtained by two academics that have worked separately, there have been 17 studies. The authors concluded how the psychological and psychosocial alterations, certainly present in these patients, are probably due to local and systemic alterations; this is confirmed by the correlation between oral health and quality of life reported by the patients [1–5].

The published papers raised great visibility and specially the one about diabetes and oral health quickly reached about 22 citations in Scopus.

Conflicts of Interest

The authors declare no conflict of interest replacing the publication of this article.

Marco Cicciù
Tolga Tozum
Claudio Stacchi

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Hindawi
BioMed Research International
Volume 2019, Article ID 7984891, 6 pages
https://doi.org/10.1155/2019/7984891

Research Article

Quantification of TNF-α in Patients with Periodontitis and Type 2 Diabetes

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Received 7 June 2019; Accepted 24 June 2019; Published 4 July 2019

Guest Editor: Marco Cicciù

Objective. The present study aimed to compare variations in quantified tumor necrosis factor-alpha (TNF-α) levels in patients with periodontitis stage 2 grade B (POD2B) and/or type 2 diabetes (T2D) and to identify any relationships between this cytokine and these diseases. *Methods*. Levels of the cytokine TNF-α in gingival crevicular fluid in patients with POD2B and/or T2D were evaluated. A total of 160 subjects were distributed into four groups: those with POD2B (n=44); those with T2D (n=37); those with POD2B/T2D (n=40); and healthy subjects (n=39). Glycosylated hemoglobin (HbA1c) and blood glucose (BG) levels were quantified in each subject. Data were collected on body mass index (BMI), loss of insertion (LI), and probe depth (PD). Gingival crevicular fluid samples were collected from the most acutely affected periodontal pocket and gingival sulcus in each subject, and TNF-α was quantified by multiplex analysis. *Results*. Kruskal Wallis tests was used to identify differences in TNF-α levels, LI, PD, BMI, BG, and HbA1c by group. Differences ($p<0.001$) were found for LI, PD, BG, and HbA1c. A Spearman test was used to calculate possible correlations between TNF-α levels and LI or PD identified a weak but significant negative correlation of TNF-α with LI (Rho=-0199; p=0.012), and a moderately positive correlation of LI with PD (Rho=0.509; $p < 0.001$). *Conclusions*. No variation was found between TNF-α levels and the presence of POD2B, POD2B/T2D, or T2D, suggesting the absence of any direct relationship between progression of these diseases and TNF-α levels. However, a correlation was present between low TNF-α concentrations and greater LI.

1. Introduction

Diabetes mellitus is a public health problem in many countries. In medical terms, it encompasses a group of metabolic disorders with heterogeneous clinical and genetic characteristics, which manifest as abnormally high blood glucose levels. These disorders have a profound impact on health in affected individuals, causing high morbidity and mortality rates, and constitute an economic burden on health systems [1, 2]. The International Diabetes Federation states that China, India, the United States of America, Brazil, Russia, and Mexico have the highest number of diabetic patients [3]. In 2012, diabetes was estimated to be the direct cause of 1.5 million deaths, and another 2.2 million deaths were attributable to hyperglycemia. Of these 3.7 million deaths, 43% occur in people less than 70 years old. Type 2 diabetes (T2D) accounts for 90 to 95% of cases worldwide and most commonly occurs in adults but is now increasingly common in children. By 2030, diabetes is projected to be the seventh cause of death worldwide [4, 5].

Periodontitis (POD) is the most common form of periodontal disease. It is more prevalent in adults but can occur

in children. Although multifactorial in origin, three main factors are involved in POD appearance and evolution: accumulation of plaque and calculus; level of bacterial virulence; and cellular immune response [6]. Evolution is slow to moderate, but more rapid periods of destruction can be observed, influenced mainly by systemic or environmental factors that can affect the normal interaction between host and bacteria [7]. Plaque accumulation and host response to it can be affected by local factors such as systemic diseases (*e.g.,* diabetes mellitus, HIV), which can depress host defenses, and the environment (*e.g.,* smoking and stress) [8].

Both T2D and POD influence oral cavity health. Diabetes is a risk factor for gingivitis and POD when related to formation of a more persistent inflammatory infiltrate. An inverse influence may also exist in that POD could be a risk factor for diabetic decompensation [9]. A complex bidirectional relationship between T2D and POD may occur that would create a vicious circle exacerbating both diseases when present simultaneously in the same individual [10–13].

Recent studies have been focused on the role of cytokines in periodontal diseases in diabetic patients. Cytokines are a group of cell regulators vital in the production and activation of effector cells that initiate and regulate different immune and inflammatory responses [14–16]. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory mediator considered to be a soluble mediator released from immunocompetent cells. It exercises a wide range of proinflammatory and immunomodulatory effects in different cell populations, such as stimulating prostaglandin synthesis, promoting tumors in a variety of cancers, producing proteases, and activating osteoclastic function and therefore bone resorption. Its myriad functions suggest that TNF-α plays an important role in mediating the immune-inflammatory responses initiated by infection or other types of damage [17]. During its initial production in the inflammatory response, TNF-α is also vital for maintaining chronic inflammation, angiogenesis, tissue remodeling, tumor growth, and metastasis; TNF-α blockers are therefore effective in treating a variety of acute and chronic inflammatory conditions [18].

An important proinflammatory cytokine and immune response modulator, TNF-α production occurs in response to stimuli from cell types such as macrophages, neutrophils, keratinocytes, adipocytes, fibroblasts, and NK, T and B cells. High serum levels of this cytokine have been detected in patients with POD, suggesting it may be contributing to pathogenesis. Its activation also stimulates bone resorption by induction in osteoclast progenitor proliferation, and production of extracellular matrix metalloproteinases, cytokines, collagenase, and prostaglandins [19–22].

The aim of this study was to compare the variations in quantified TNF-α levels in patients with POD and/or T2D and to identify any relationships between this cytokine and these diseases.

2. Materials and Methods

A cross-sectional study was done and approved by the Institutional Bioethics Committee. Subjects were selected from the Admission Dental Clinic, Faculty of Dentistry, Autonomous University of Yucatan (UADY); after explanation of the procedure, those choosing to participate signed an informed consent.

Presence of T2D was identified based on the 2019 American Diabetes Association parameters [23]: glycosylated hemoglobin (HbA1c) values $\geq$ 6.5% indicate diabetes and those $\leq$ 5.6% are normal; glucose blood (GB) levels (8 to 10 hours) $\geq$ 126 mg/dL indicate diabetes and those $\leq$ 100 mg/dL are normal.

According to specific classification for periodontal and peri-implant diseases and conditions, patients included were targeted with stage 2, grade B POD [24]. Periodontal probing with a calibrated periodontal probe was done (UNC-15, Hu-Friedy, Chicago, IL, USA); all teeth were examined except third molars. Subjects were excluded who had received periodontal treatment, chemotherapy, and antibiotic and/or anti-inflammatory treatment in the six months prior to examination or exhibited systemic diseases other than T2D. From a total of 160 selected subjects, four study groups were formed: group 1 (POD2B, n=44); group 2 (T2D, n=37); group 3 (POD2B/T2D, n=40); and group 4 (control with healthy subjects exhibiting no periodontal disease, n=39). Gingival crevicular fluid (GCF) samples were collected from periodontal pockets with $\geq$ 4 mm depth and $\leq$ 3 mm insertion loss in groups 1 and 3, and from the mesiovestibular gingival sulcus of the first lower molar in groups 2 and 4. Samples were collected by first isolating the tooth with a cotton impeller and removing the supragingival plaque with a curette (Gracey, Hu-Friedy, Chicago, IL, USA), avoiding injury to the marginal gingiva. After slightly drying the crevicular site with air, GCF was obtained by inserting a PerioPaper strip (PerioPaper, ProFlow, Amityville, NY, USA) into the sulcus or periodontal pocket to the point of resistance and leaving it there for thirty seconds. Strips contaminated with saliva or blood were discarded and a new sample taken at a different site. After collection, the PerioPaper strips were immediately placed in sterile Eppendorf vessels and stored at -70°C until analysis. The GCF was extracted by two elution methods in 0.05% PBS-T solution followed by centrifuging at 12,000 g for 5 minutes and at 4°C, until reaching a final elution volume of 80 μL. Of this volume, 40 μL were tested with Luminex platform (Magpix, Millipore, St. Charles, MO, USA) and analyzed with a MILLIPLEX analyst software (ViageneTech, Carlisle, MA, USA). Results were expressed per mL of elution to measure TNF-α levels in the total amount (pg) and concentration volume according to the formula TNF-α (pg)/volume (μL).

Kruskal-Wallis test was applied to identify any differences in the data for TNF-α, loss of insertion (LI, %), probe depth (PD), body mass index (BMI), blood glucose (BG), and glycosylated hemoglobin (HbA1c) by group. A Spearman test was used to evaluate the possible existence of a correlation between TNF-α count and LI or PD (statistical significance $p \leq 0.05$).

3. Results

Analysis of the BMI, BG, and HbA1c data showed 31.87% of the total sample to exhibit glycemic levels outside

Table 1: General characteristics of sample.

GROUP		BMI	BG*	HbA1c*
		Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)
POD2B	n=44	27.8±3.37 (23.31-38.45)	95.43±10.42 (75-123)	5.61±0.61 (4.7-7.4)
T2D	n=37	29.75±5.18 (21.83-51.78)	150.59±72.66 (78-328)	7.6±1.76 (4.8-11.9)
POD2B /T2D	n=40	29.78±5.05 (20.96-44.85)	201.35±93.00 (68-460)	8.21±1.87 (4.9-12.0)
CONTROL	n=39	28.51±5.44 (20.67-44.27)	95.08±14.02 (75-151)	5.59±0.57 (4.0-6.8)

BMI: Body mass index; BG: Blood glucose; HbA1c: Glycosylated hemoglobin; *p=0.001

normal levels (Table 1). In the comparison between groups, BG and HbA1c had different values ($p<0.001$). Paired comparisons for BG identified differences ($p<0.001$) between Control-T2D, Control-POD2B/T2D, POD2B-T2D, and POD2B-POD2B/T2D. For HbA1c, differences ($p<0.001$) were found between POD2B-T2D, POD2B-POD2B/T2D, Control-T2D, and Control-POD2B /T2D. The T2D and POD2B/T2D groups had the highest values (Table 1).

Analysis of periodontal condition identified differences between groups for LI and PD ($p<0.001$), with the highest values in both cases being in POD2B and POD2B/T2D (Table 2). No differences in TNF-α concentration were found between groups. A Spearman test identified a weak but significant negative correlation between TNF-α levels and LI (**Rho=-0.199; $p=0.012$), and a moderate but significant positive correlation between LI and PD (***Rho=0.509; $p<0.001$).

4. Discussion

There are multiple conditions that can affect the periodontal health. Locally, factors such as dental malposition, poorly adjusted restorations, maxillofacial fractures, and uncontrolled use of chlorhexidine-based products have been reported [25, 26]. Likewise, several systemic conditions have been reported that influence the periodontal status of patients, among which T2D, hypertension, hemophilia, leukemia, and certain digestive disorders can be mentioned [27–29]. All these conditions have been studied separately, observing important changes in the evolution of periodontitis and the associated immunological factors.

TNF-α is an important proinflammatory cytokine and modulator of the immune response. It is produced in response to stimuli from cell types such as macrophages, neutrophils, keratinocytes, adipocytes, fibroblasts, and NK, T and B cells. High serum TNF-α levels have been detected in patients with POD since it contributes to disease pathogenesis. This cytokine stimulates bone resorption by inducing osteoclast progenitor proliferation, as well as production of chemokine, extracellular matrix metalloproteinases, cytokines, collagenase, and prostaglandins. Cytokine concentration in GCF has been linked to degree of glycemic control in diabetic patients [19–22].

Studies have shown that diabetic patients with periodontal infection have a higher risk of losing control of their glycemic condition; and this deteriorates over the long term compared to diabetic patients who do not suffer POD [30–32]. In the course of periodontal disease, various proinflammatory mediators occur, such as interleukin- (IL-) 1, IL-6, and IL-8, IFN- γ, CCL5, TNF-α, prostaglandins, and metalloproteinases. These mediators alter the activity of leukocytes and osteoblasts-osteoclasts and promote the tissue remodeling process both locally and systemically. The proinflammatory cytokine TNF-α regulates production of collagenase, prostaglandin E2, molecular adhesion cells, and factors related to bone resorption. Secreted mainly by monocytes and macrophages, elevated TNF-α levels have been observed in chronic gingival inflammation processes and in GCF in patients with POD [9, 33, 34].

No intergroup differences in TNF-α concentration were observed in the present study. This is similar to the lack of differences in TNF-α concentration in the GCF between patients with POD2B, aggressive POD, or systemically healthy patients reported in a Turkish population [35]. However, in the present study, differences were present between groups in terms of LI and PD, with the POD2B and POD2B/T2D groups having the highest values. These results agree with a study of the progression of periodontal lesion in which no differences in TNF-α concentration were observed when comparing active and inactive sites in 56 patients in a Chilean population diagnosed with moderate to advanced chronic POD [36, 37]. They also agree with a study done in Brazil in which no differences in TNF-α concentration was noted between POD2B/T2D and POD2B patients [38, 39].

Increases in TNF-α concentration in patients with POD2B/T2D or POD2B have also been described in a Portuguese population, but with no differences between groups [40, 41], like the present results. A study in Korean patients found no correlation between TNF-α levels and gingival tissues in patients with POD [30, 31]. The lack of difference in the present results may have occurred because 68.13% of the patients exhibited good glycemic control. This can translate into low TNF-α expression in POD2B/T2D patients because hyperglycemia can overregulate levels of TNF-α, and other cytokines such as GM-CSF and IL-6, in both healthy and periodontal affected tissues [42, 43]. This overregulation also enhances epithelial cell stimulation capacity by providing an inflammatory system that must be interrupted for the disease to improve; this interruption can occur when hyperglycemia is controlled or when a periodontal disease enters remission [42].

Reis et al. found no differences in TNF-α levels in patients with or without POD2B, and neither did they observe decreases in TNF-α levels after nonsurgical periodontal treatment [44]. In another study, quantification of cytokine expression in diseased peri-implant tissue found

Table 2: Loss of insertion and probe depth.

GROUP	LI*,**,***	PD*,***	TNF-α**
	Mean±SD (Ranges)	Mean±SD (Range)	Mean±SD (Range)
POD2B	n=44	4.68±1.68 (1.66-10.33)	0.48±0.38 (0.10-0.91)
T2D	n=37	2.48±0.48 (1.00-3.00)	0.56±0.38 (0.10-0.91)
POD2B/T2D	n=40	4.55±1.14 (2.66-6.66)	0.50±0.40 (0.10-1.17)
CONTROL	n=39	2.35±0.40 (1.66-3.00)	0.50±0.39 (0.10-1.00)

LI: Loss of insertion; PD: probe depth; Kruskal Wallis test *p=0.001; Spearman test **,***p=0.001

no differences in TNF-α concentrations between sites with mucositis and peri-implantitis in both GCF and saliva [45]. In a comparison of different treatments in residual pockets using the final concentration of acute-phase proteins, no changes were found in TNF-α concentration between data from the baseline condition, at 14 days and at 6 months [46].

Duarte et al. found significant differences between TNF-α concentration and T2D when comparing healthy and infected sites in patients with and without T2D [40, 47]. This differs from the lack of difference in the present results, perhaps because BG levels in the present study subjects were not very high and 68.13% of the subjects exhibited adequate glycemic control. Expression of TNF-α can be attributed to an increase in RAGE or expression of TLR4, which directly affect the response of epithelial cells and their antagonists inducing the proinflammatory cytokine response, as well as an increase in cell surface receiving capacity [48]. These receptors also work collaboratively to induce expression of these cytokines in oral epithelial cells, although this collaboration is not involved in inducing innate immunity receptors. Indeed, a correlation exists between a lack of control of blood sugar levels and deficiency in the epithelial barrier which translates into an increase in expression of the immune receptors of the innate immune response and an exacerbated inflammatory response [49].

Increases in TNF-α concentrations have been reported after nonsurgical periodontal treatment, with higher levels in healthy subjects than in unhealthy patients [50]. These results coincide with the present results in which higher TNF-α levels were observed in healthy subjects than in the POD2B, POD2B/T2D, and T2D groups. A possible explanation for these reduced inflammatory protein levels in patients with these chronic diseases is that host immune response may be diminished.

Interstudy variation in TNF-α concentrations may be due to several factors including periodontal disease severity, subject age, sample type, population type, and technique details such as storage temperature and pretest storage time [51].

GCF has been widely used as a diagnostic tool for various periodontal diseases. Available evidence indicates that GCF can influence the progression of periodontal diseases when combined with systemic diseases, suggesting that local changes in GCF can be reflected as systemic inflammation through direct expression of circulating inflammatory mediators [52]. The presence of T2D plays a fundamental role in development of chronic POD. Its resistance and control can affect TNF-α concentrations, possibly explaining the contrasts between different studies mentioned previously.

5. Conclusions

The present results indicate that good control of BG in patients with POD2B/T2D can directly influence expression of the TNF-α cytokine; however, low TNF-α concentrations are directly correlated with greater insertion loss and probe depth.

Data Availability

The general and clinical characteristics of sample used to support the findings of this study are included within the article.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

This work was supported partially by UADY-EXB-214, PFCE-UASLP, and PRODEP grants.

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Hindawi
BioMed Research International
Volume 2019, Article ID 5613514, 8 pages
https://doi.org/10.1155/2019/5613514

Research Article

Levels of Myeloperoxidase and Metalloproteinase-9 in Gingival Crevicular Fluid from Diabetic Subjects with and without Stage 2, Grade B Periodontitis

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Received 18 May 2019; Accepted 29 May 2019; Published 2 July 2019

Guest Editor: Marco Cicciù

Objective. The present study aimed to compare levels of matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) in gingival crevicular fluid (GCF) from subjects with controlled and noncontrolled Type 2 Diabetes Mellitus (T2D), with and without stage 2 grade B periodontitis (POD2B) versus healthy (H) subjects. *Methods.* The levels of both enzymes, from 80 GCF samples collected with PerioPaper strips, were analyzed by a Multiplex/Luminex assay. Five groups were formed, all current patients at the Institutional Dentistry Service, and distributed as follows: two groups of diabetics (one controlled and one poorly controlled); two groups with the previous conditions and diagnosed with POD2B; and one H group. *Results.* The highest concentration of MMP-9 corresponded to the H group, while the lowest corresponded to the T2D controlled group. Regarding MPO levels, the highest levels were associated with the T2D controlled with POD2B group and the lowest with the T2D controlled group. *Conclusions.* No apparent relationship between the elevation of MMP-9 and MPO levels was observed among subjects with T2D, with and without POD2B, compared to H subjects.

1. Introduction

The periodontium is a functional unit formed by a group of specialized tissues that surround the teeth. It can be classified, due to its main functions, into two categories: the attachment periodontium, which involves periodontal ligament, cementum, and alveolar bone; the protection periodontium, only formed by the gingiva which is in a close relationship with the gingival sulcus: a "V" shaped, shallow cavity that relies underneath the gingival margin. In health, the gingival sulcus maintains a depth of 0-3 millimeters (mm), measured from the gingival margin to the base of the gingival sulcus, and also contains a low amount of gingival crevicular fluid (GCF) which is an inflammatory exudate that increases its volume when inflammation occurs and also contains a variety of biomarkers that are related to inflammatory processes [1].

The oral cavity is a main source of bacterial biofilm, and the periodontium can be an ideal reservoir for oral pathogens and its proinflammatory products, such as MMP-9 and MPO, since it has an anaerobic environment inside the periodontal sulcus, a vast gingival blood stream that it is connected to the alveolar blood circulation and a rich source of collagen fibers. When bacterial invasion of the gingival sulcus occurs, a periodontal pocket is formed, increasing the depth of the

sulcus to 4 mm or more and causing an augmentation of the production of GCF. If bacterial colonization continues and the hosts defenses cannot overcome it, then a periodontal disease, such as periodontitis (irreversible destruction of the alveolar bone) or gingivitis (reversible swelling of the gingiva), will settle in [2].

Periodontitis is an inflammatory, multifactorial, progressive condition with accumulation of plaque and calculus, characterized by a change in the ecology of the subgingival microbiome: this leads to a slow but progressive destruction of the periodontium [3].

In 2017, Papapanou et al. proposed a new and more specific classification for periodontal disease. This classification involves four stages of periodontitis based on severity (according to the level of interdental clinical attachment loss, radiographic bone loss, and tooth loss), complexity, extent, and distribution. In addition to stages, three grades that reflect biologic features were also established. Since this study targeted patients with stage 2, grade B periodontitis, it is convenient to define this pathology as follows:

(i) Stage 2 periodontitis: clinical loss of attachment (CAL) of 3-4 mm with radiographic bone loss limited to the coronal third (15-33%) but no tooth loss due to periodontitis, maximum probing depth ≤ 5 mm with mostly horizontal bone loss [4].

(ii) Grade B: direct evidence of progression of < 2 mm over 5 years and indirect evidence of progression of 0.25 to 1.0 mm. The destruction is commensurate with biofilm deposits and shows grade modifiers, such as smoking more than 10 cigarettes per day and diagnosis with T2DM, with levels < 7.0% of (HbA1c) [3].

Periodontitis is highly associated with systemic diseases such as T2D, which is a chronic pathology characterized by polyuria (increase of urine production), polydipsia (augmentation of the ingestion of water), and polyphagia (exacerbation of hunger) [5]. Both mentioned pathologies have a bidirectional relationship [6]. T2D is also known for being a chronic disease characterized by sustained hyperglycemia, which results in continuous elevation of systemic glucose. It is known to involve a series of complex processes that include modification of lipid and protein metabolism [7]. Diabetic patients are highly related to microangiopathies, nephropathies, retinopathies, and neuropathies of the peripheral nervous system; therefore, these patients have a higher risk of bacterial infections, specially oral ones, and, as a clear example of this, the incurrence of periodontal disease in T2D patients is highlighted [5].

While developing chronic pathologies, the immune system plays a fundamental role that influences the course of the disease. For instance, T2D patients show a higher production of immunocomplexes and a lower amount of T lymphocytes, and also an imbalance in the lymphocyte population (prevalence of CD8 over CD4 lymphocytes) [5].

A great variety of immune mediators are involved in the evolution of mentioned diseases; as an example of these mediators, matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) are enzymes that produce damage to collagen-rich tissue when overproduced. MMP-9 and MMPO are mainly synthesized by neutrophils, with increased levels during inflammatory processes, leading to overproduction of both proteins [8, 9].

MMP-9 is a gelatinase associated with degradation of gelatin and type IV collagen and is identified in samples of gingival crevicular fluid from patients with periodontitis [10]. In addition, MPO enzymes are capable of enabling the formation of free radicals such as hypochlorous acid, which is a powerful protein oxidant agent. This acid is known for its bactericide properties; when overproduced, occurring as a consequence of abnormal neutrophil apoptosis during an altered inflammatory response, it causes the destruction of surrounding tissue [11, 12].

It was established that as a consequence of hyperglycemia in patients with T2D, there is a lipidic and a proteic exposure to glucose, leading to a nonenzymatic glycation of both lipids and proteins and then to its oxidation, resulting in the production of advanced glycation end-products (AGEs). Once formed, the AGEs start the formation of reactive species of oxygen (RSO), which are responsible for augmenting the respiratory burst among neutrophils, in turn causing the overproduction of enzymes like MMP-9 and MPO [13, 14].

Gingival crevicular fluid is a physiological fluid that also works as an inflammatory exudate that flows from the gingival sulcus or periodontal pocket. Volumes are typically low and generally increase with inflammation in the periodontal tissues [15, 16]. It contains high levels of proteins (cytokines and others) and defensive cells (neutrophils and others), while its volume tends to increase during inflammatory conditions due to major capillary permeability. This fluid has been frequently used for periodontal research because of its accessibility, rich contents, and composition, which can be altered (increasing proinflammatory biomarkers) when another chronic, inflammatory disease is present, such as T2D, obesity, celiac disease, and rheumatoid arthritis [17–20].

Due to the need for more accuracy in periodontal diagnosis, the use of cell and molecular biology, as indicators of health or disease, has increased in the past 20 years. The idea of sampling GCF to target a great variety of biomarkers has become one of the least invasive methods in periodontal research. This method consists in introducing a sterilized PerioPaper strip inside the gingival sulcus, obtaining samples of GCF, which should then be analyzed to determine its content. The former technique was introduced initially by Brill and Krasse in 1958 [21] and also by related studies by Menassa et al. who confirmed the presence of neutrophils, MMPs, and MPOs in this fluid and an increase of volume with the inflammatory process. GCF is an important tool to confirm periodontal diagnosis [22].

Therefore, sampling and quantifying MMP-9 and MPO through GCF with noninvasive techniques could be useful in describing the relationship between the exacerbation of inflammatory conditions as shown locally in POD2B and metabolic ones, such as T2D.

The main purpose of this study was to compare the GCF levels of MMP-9 and MPO in subjects with controlled

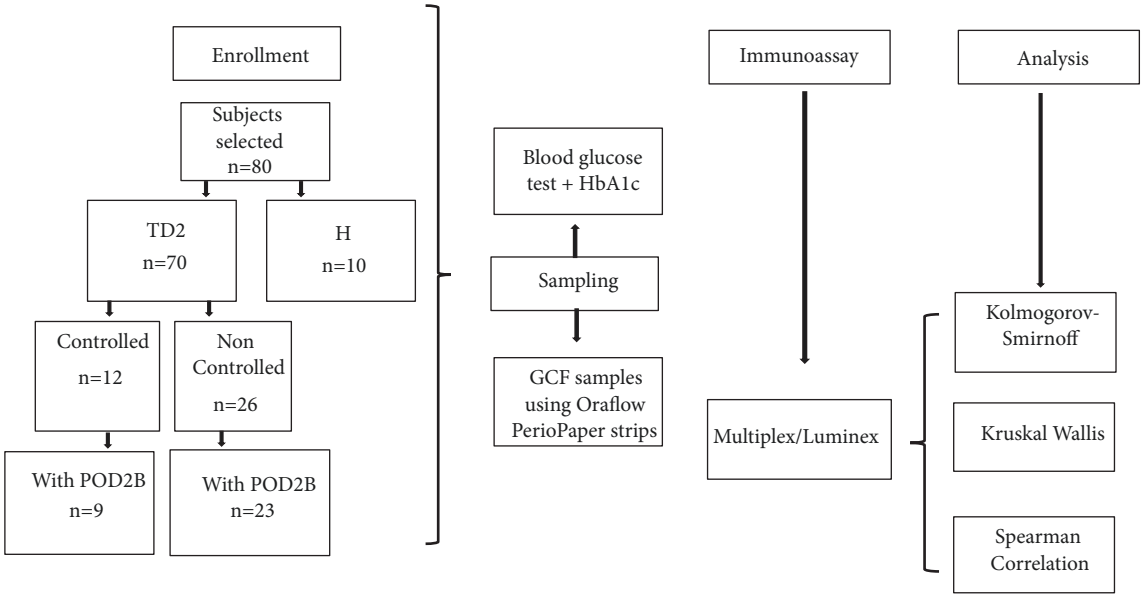

Figure 1: Description of patient's distribution in the groups for analysis.

and noncontrolled T2D, with and without POD2B, versus H subjects.

2. Materials and Methods

2.1. Design Study Population Clinical Examination. The present transversal and analytic study involves a total of 80 patients who were divided into five groups, all of which are current patients in the Faculty of Dentistry, Autonomous University of Yucatan (UADY). We included patients who met the following criteria: female or male patients with an age range of 25-65 years, who also had a former diagnosis of T2D stage 2, grade B periodontitis. We excluded patients who did not consent to participate in this study; patients with any other periodontal disease other than stage 2, grade B periodontitis; patients who sustained any antibiotic therapy within 3 months before the sampling of GCF; pregnant patients; patients with orthodontic or prosthetic therapy; and patients who had any systemic disease apart from T2D.

Out of five groups, two included diabetic subjects (a controlled subgroup and poorly controlled subgroup); the following two groups included patients diagnosed with T2D (also a controlled subgroup plus a poorly controlled subgroup) in addition to POD2B. Finally, one last group was comprised of H with neither an apparent systemic condition nor periodontal disease (Figure 1).

2.2. Type 2 Diabetes and Periodontitis Diagnosis. T2D diagnosis was confirmed via HbA1c (≥ 6.5%) and blood glucose (≥ 200 mg/dL) tests. On the other hand, POD2B was diagnosed using both clinical and radiographic parameters, which included the clinical parameters: probing depth (PD) ≥ 4 mm, CAL, and presence of bleeding while probing (BOP), in addition to persistent halitosis and gingival swelling. Radiographic parameters included a horizontal bone loss pattern [11–13]. Radiographic variables of bone level (BL) were obtained by calculating the difference between 100% and the percentage of bone loss from the cement-enamel junction to the alveolar bone crest [23].

2.3. Periodontal Evaluation. Periodontal probing with a calibrated periodontal probe (UNC15, Hu-Friedy, Chicago, IL, USA) was performed by the same examiner among all 80 subjects, including 6 sites per tooth: three vestibular sites and three palatal/lingual sites (mesial, medium, distal). The tooth with the deepest periodontal pocket from each individual was selected for the GCF sample, for which we used sterile PerioPaper strips (PerioPaper, ProFlow, Amityville, NY, USA). This was performed after isolating the tooth with cotton rolls and removing supragingival plaque/calculus with a McCall curette; one PerioPaper strip was introduced in the gingival sulcus (for the H groups) or the pocket for 30 seconds by a trained periodontist. In POD2B patients, GFC samples from deep probing sites were obtained. Samples from each mesiovestibular site of the first molars were obtained in H volunteers. If there was blood contamination, or a plaque of saliva occurred while sampling, another site was selected [24, 25].

Afterwards, samples were immediately stored at -70°C until analysis. GCF was extracted via 0.05% phosphate buffered saline with Tween 20 (PBS-T) wash solution, which is an optimal formulation of stabilizers, salts, and detergents in removing excess material from membranes or microtiter plate wells without disrupting the antigen/antibody binding reaction, with centrifugation at 12,000 g for 5 min at 4°C to reach a final elution of 80 μL. Samples were processed through a Multiplex panel (Millipore, St. Charles, MO, USA.), according to the manufacturer's instructions. All data were collected through a Luminex platform (MAGPIX, Millipore, St Charles, MO, USA) and analyzed with MILLIPLEX analyst

Table 1: Patient distribution and diagnosis criteria.

		Sex		A∗	LCA∗	BG∗	GH∗
		Female	Male	Mean ± SD [Ranks]	Mean± SD [Ranks]	Average ± SD [Ranks]	Average ± SD [Ranks]
Groups		n=53 66.25%	n=27 33.75%				
n=70 87.50%	T2D/C n=12 15%	n=7 58.33%	n=5 41.66%	54.16 ± 10.81 [34, 5]	1.98 ± 0.48 [1.4, 2.7]	103.16 ± 9.88 [89, 119]	5.85 ± 0.44 [4.8, 6.4]
	T2D/NC n=26 28.75%	n=18 78.26%	n=5 21.73%	51.17 ± 9.84 [32, 65]	2.03 ± 0.38 [1.4, 2.9]	188 ± 89.65 [78, 383]	8.56 ± 1.66 [6.5, 11.5]
	T2D/C/POD2B n=9 11.25%	n=7 77.77%	n=2 22.23%	52.44 ± 9.11 [41, 65]	2.63 ± 0.88 [1.7, 4.5]	129.44 ± 28.76 [90, 189]	6.04 ± 0.47 [4.9, 6.4]
	T2D/NC/POD2B n=23 28.75%	n=17 65.38%	n=9 34.61%	50.65 ± 8.54 [39, 65]	2.85 ± 0.87 [1.6, 5.3]	243.53 ± 94.25 [100, 460]	9.19 ± 1.50 [6.6, 12]
n=10 12.50%	H n=10 12.50%	n=4 40%	n=6 60%	36 ± 14.92 [25, 63]	1.85 ± 0.15 [1.6, 2.1]	91.20 ± 12.31 [76, 114]	5.86 ± 0.63 [5.1, 7.4]

Age [A]. Clinical loss of attachment [CAL]. Blood glucose [BG]. Glycated hemoglobin [GH]. ∗ p= 0.05.

software (ViageneTech, Carlisle, MA, USA). Results were expressed per ml of elution [25–27].

The study protocol was explained to all study participants, who signed informed consent (approved by the Ethics Committee of the CIR-Biomedicas, Autonomous University of Yucatan), according to ethical standards of the Declaration of Helsinki. Adopted in June 1964, it was modified by the World Medical Assembly of Korea in October 2008.

2.4. Statistical Analysis. To process data, an SSPS statistic package was used. Data distribution was assessed by the Kolmogorov-Smirnoff test; all variables (T2D, POD2B, levels of MMP-9 and MPO, and clinical loss of attachment) were analyzed through a Kruskal-Wallis test with the Siegel and Castellan post hoc method, while two of the variables (CAL and levels of MMP-9 and MPO) were compared with a Spearman correlation test, and a statistical significance was set at $p < 0.05$.

3. Results

A total of 80 samples of GCF were collected. The distribution of patients and results from diagnosis criteria are shown in Table 1.

The group with the lowest CAL was the T2D/noncontrolled (NC)/POD2B, with an average of 2.85 mm followed by the T2D/controlled (C)/PC group, with an average of 2.63 mm. The least affected group was the H group, with an average of 1.85 mm, while the T2D/C and T2D/NC groups had 1.98 mm and 2.03 mm of CAL, respectively (Table 1).

Regarding metabolic control, it was determined in terms of blood glucose levels [mg/dL] and GH percentage (%). That being said, we obtained the following results: The average of blood glucose for the group T2D/NC/POD2B was the highest among all with 243.53 mg/dL, as well as the GH results (9.19%). Yet groups with the lowest levels of blood glucose were T2D/C group with 103.16 mg/dL and the H group with 91.20 mg/dL. Both had the lowest levels of GH, with 5.85% and 5.86%, respectively (Table 1).

Comparing the levels of MMP-9 and MPO among patients with T2D with and without POD2B versus H ones, we observed that the highest concentrations of MMP-9 were found in the H group, while the lowest were found in the T2D/C group. Referring to MPOs, the highest concentration was represented by the T2D/C/POD2B group and the lowest by the T2D/C group (Table 2).

After contrasting the quantification of MMP-9 and MPO levels among diabetic patients (controlled and noncontrolled), we found that the highest levels of MMP-9 were associated with the T2D/NC/POD2B group. The highest levels of MPO were found in the T2D/C/POD2B group, while the lowest concentrations for both enzymes were found in the T2D/C group, respectively (Table 3). While associating the averages of CAL and concentration of MMP-9 and MPO, we observed a higher loss of CAL, for the T2D groups with and without POD2B, and higher levels of MMP-9 and MPO when patients had noncontrolled disease. This pattern was not shown for levels of MPO in the T2D/NC/POD2B group, in which there were higher levels for loss of CAL; however, there was not a higher concentration of MPO levels: instead, this was shown in the T2D/C/PC group (Tables 1 and 2).

After analyzing all of the data through the Spearman correlation test, it was determined that the only positive correlation with statistic relevance ($p < 0.05$) was found

Table 2: Comparison between levels of MMP-9 and MPO of diabetic patients with and without POD2B and H ones.

Groups	MMPs-9 Mean ± SD [Ranks]	MPOs Mean ± SD [Ranks]
T2D/C/POD2B	1.70E+06 + 9.82E+05	3.99E+05 + 9.07E+05
n = 9	[2.99E+05 - 2.91E+06]	[6.68E+03 - 2.78E+06]
T2D/NC/POD2B	1.75E+06 + 9.06E+05	1.38E+05 + 2.29E+05
n = 26	[1.38E+04 - 3.05E+06]	[6.68E+03 - 9.56E+05]
T2D/C	1.14E+06 ± 7.22E+05	3.48E+04 ± 5.20E+04
n =12	[3.53E+05 - 2.70E+06]	[6.68E+03 - 1.45E+05]
T2D/NC	1.69E+06 ± 8.59E+05	8.75E+04 ± 9.72E+04
n = 23	[1.25E+05 - 2.87E+06]	[6.68E+03 - 3.25E+05]
H	1.86E+06 ± 7.34E+05	1.62E+05 ± 4.60E+05
n = 10	[7.45E+05 - 2.98E+06]	[6.68E+03 - 1.47E+06]

Matrix metalloproteinases-9 [MMPs-9] p = 0.259.
Myeloperoxidases [MPOs] p = 0.170.

Table 3: Comparison of MMP-9 and MPO levels between diabetic patients controlled and noncontrolled.

Groups	MMPs-9*	MPOs*
	Average ± SD [Ranks]	Average ± SD [Ranks]
T2D/C/POD2B	1.70E+06 + 9.82E+05	3.99E+05 + 9.07E+05
n=9	[2.99E+05 - 2.91E+06]	[6.68E+03 - 2.78E+06]
T2D/NC/POD2B	1.75E+06 + 9.06E+05	1.38E+05 + 2.29E+05
n=26	[1.38E+04 - 3.05E+06]	[6.68E+03 - 9.56E+05]
T2D/C	1.14E+06 ± 7.22E+05	3.48E+04 ± 5.20E+04
n=12	[3.53E+05 - 2.70E+06]	[6.68E+03 - 1.45E+05]
T2D/NC	1.69E+06 ± 8.59E+05	8.75E+04 ± 9.72E+04
n=23	[1.25E+05 - 2.87E+06]	[6.68E+03 - 3.25E+05]

∗Matrix metalloproteinases-9 [MMPs-9] p = 0.259.
∗Matrix myeloperoxidases [MPOs] p = 0.170.

between the elevated loss of CAL and the elevation of MMP-9 and MPO in the H group (Figure 2).

4. Discussion

The current research starts with the bidirectional relationship between T2D and periodontitis (POD). Several authors have confirmed the former statement. There is available data demonstrating the increase in prevalence of chronic and degenerative systemic diseases, such as T2D, when poor oral hygiene is present; some indicators of the oral hygiene decay are the presence of dental caries, periodontitis, and the diminished saliva flow [28].

A systematic review by Chee et al. suggests that patients with T2D are more likely to have POD2B and increased severity of disease [23]. They demonstrated that diabetic subjects receiving periodontal treatment and maintaining control of their metabolic levels showed a reduction of 0.4-0.65% in GH [23]. Conversely, Rajhans et al. observed an abnormal lymphocyte function as a result of the increased levels of glycemia, which leads to the formation of AGEs, RSO, such as MPO, and finally to early cell apoptosis in response to the high levels of MMPs [13]. Preshaw et al. declared that subjects with T2D, especially if they had noncontrolled blood glucose levels, manifest major lymphocyte activity [29]. Shin et al. demonstrated that patients who have both T2D and POD2B also have a diminished chemotaxis and an abnormal apoptosis, retaining lymphocytes on periodontal tissue, leading to tissue damage due to the constant formation of MMPs and RSO [14].

It is important to recognize that systemic pathologies, as well as localized ones, potentiate the loss of CAL. This statement is maintained by Firati et al. who found a positive relationship between T2D and probing depth (*e.g.,* CAL), as well as a proportional increase among them as the disease evolves; the severity of periodontal disease and its effects on alveolar bone increase in function, given the time elapsed from its beginning [30].

Among the different factors associated with T2D and POD2B, the local presence of proinflammatory markers was the main interest for the present study. The most studied biomarkers associated with inflammation are cytokines, which are essential during both pathological and physiological processes. As an example of this, the levels of

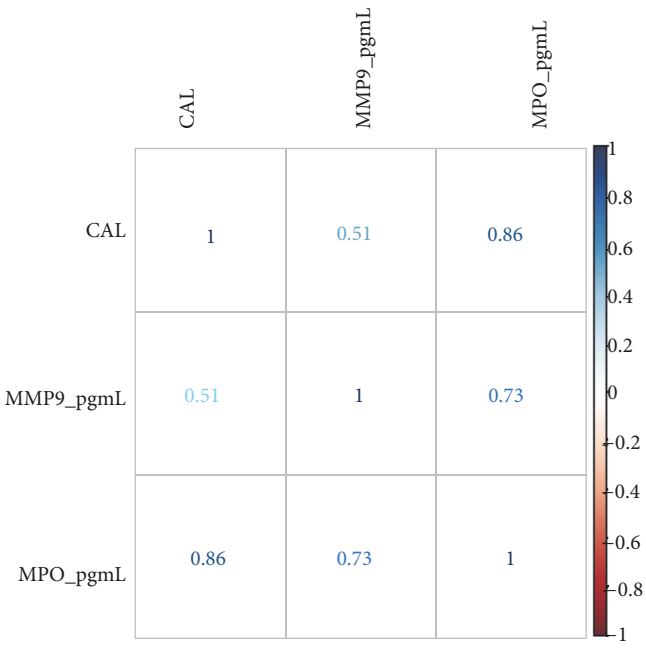

Figure 2: The Spearman correlation test for the H group, associating levels of MMP-9 and MPO with loss of CAL. A positive correlation was found between elevation of loss of CAL and elevation of MMP-9 and MPO levels.

Interleukine-1 (IL-1), which is the main biomarker of gingival inflammation, increase in GCF when inflammation occurs [28].

Some authors suggest that the levels of proinflammatory markers increase when localized and/or systemic inflammatory processes occur. In this context, Sorsa et al. proposed a change in the levels of biomarkers such as MMPs, while analyzing samples of GCF, which were decreased in health and increased in disease [31]. Therefore, knowing their concentration could be potentially useful for an accurate diagnosis.

Taba et al. related the augmentation of levels of MPO enzyme to the progress of periodontal destruction [32]. It was presumed that this elevation was due to abnormalities in neutrophilic apoptosis which lead to the accumulation of MPO and the following destruction of connective tissue, leading to a deplorable periodontal state and also potentizing systemic conditions like T2D. Kumar et al. reported that a high level of blood glucose, generally found in patients with noncontrolled T2D, induces the expression of MMP-9 in macrophages [33].

One study finding is the elevated GCF concentration of MMP-9 for H subjects; however, Pihlstrom et al. analyzed samples of gingival tissue in three groups of patients: an H group, a group of patients with T2D and POD2B, and a final group of patients with only POD2B; the highest levels of MMP-9 were found in the group with simultaneous diseases [34]. The previous statement differs from results of our current work.

Marcaccini et al. compared levels of MMP-8, MMP-9, TIMP-2, and MPO in GCF samples from H patients and patients with POD2B before and after 3 months of nonsurgical periodontal therapy; they also obtained an elevation of levels of all enzymes in the periodontal patients versus the H ones, as well as lower levels after receiving treatment [15]. These results are comparable to this research in which the concentration of MPO was higher in patients with T2D, both controlled and noncontrolled with POD2B, but the levels for MMP-9 in the present study were highest in the H group. In agreement with our results, Maeso et al. obtained the highest levels of MMP-2 in H patients versus the POD2B patients [35]. They suggest that these findings are related to limitations of the ELISA test, which was used to analyze CGF samples, but was not specific to quantify active forms. Instead of using an ELISA test, they suggest using methods such as zymography or the polymerase chain reaction (PCR) [33]. The Luminex immunoassay was chosen for this study and it is based on the ELISA assay. duPont et al. found similarities after comparing cytokine levels that were analyzed with both assays [36]. They did not find many variations, but assumed that small variations were accountable for the commercial kits used. Additionally, Prabhakar et al. also compared both immunoassays, establishing a correlation between them [37].

However, for this research, the patients who had controlled T2D and POD2B showed the highest concentrations of MPO, which were even higher than those obtained by noncontrolled T2D patients with POD2B; it was expected to have an opposite result. Death et al. established that the action of the drugs used to lower blood glucose can increase the activity of biomarkers such as MMP-9 and MPO [38]. This can be explained by the current results. Also, Sato et al., after studying blood samples from controlled T2D and H patients, found lower levels of MPO for the T2D group and higher levels for the H group [39]. They showed that the results can be explained by abnormalities in the formation of this enzyme, which require oxide-dependent microbicide mechanisms, with the initial formation of active oxygen. Markert et al. demonstrated that the accumulation of oxygen was reduced in patients with T2D; for this formation, insulin-dependent enzymes was required, where activity was reduced due to lack of peripheral insulin [40].

During the elaboration of this study some drawbacks arose, such as the limitations of the Multiplex Luminex assay, which did not differ between the active and nonactive forms of the target enzymes; this could be an explanation for the highest levels of MMP-9 on the healthy subjects. In spite of the difficulties, this study can be the start of a series of further studies that will confirm or deny the main role of both MMP-9 and MPO in the progression of chronic-inflammatory processes such as DM2 and periodontitis.

5. Conclusions

As an overall conclusion, no apparent relationship between the elevation of MMP-9 and MPO levels in GCF was observed among subjects with controlled and noncontrolled T2D, with and without POD2B, in comparison to H subjects.

In addition, this research encourages the readers to do a further analysis of proinflammatory biomarkers that are increased in GCF samples. The identification of them could

mean a link between periodontal destruction or systemic decay with the role of unnoticed substances, whose presence causes the exacerbation of the mentioned conditions, which could also be an explanation of why some of the patients with both T2D and periodontitis present extensive attachment loss. Nevertheless, further investigation and randomized control trials are needed to confirm or discard the possible relationship between the elevation of MMP-9 and MPO levels in samples of patients with periodontitis and T2D.

Data Availability

The data used to support the finding of this study are included within the article.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Hindawi
BioMed Research International
Volume 2019, Article ID 4750624, 8 pages
https://doi.org/10.1155/2019/4750624

Research Article

The Use of a Hypoallergenic Dermal Matrix for Wrapping in Peripheral Nerve Lesions Regeneration: Functional and Quantitative Morphological Analysis in an Experimental Animal Model

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Received 5 April 2019; Accepted 20 May 2019; Published 17 June 2019

Guest Editor: Claudio Stacchi

Introduction. The aim of this research was to test, in an animal model, the nerve regeneration technique with a hypoallergenic acellular dermal matrix used to wrap the microsurgical neural suture. *Materials and Methods*. Two groups of rats received the cut of limb right median nerves. The regeneration technique considers for both groups an end-to-end nerve suture. In the experimental group (A) was used also a wrapping protocol by a conduit of collagen matrix currently used in oral surgery. The animals underwent functional grasping tests (at 1, 3, 5, and 7 months) and a histological and quantitative analysis of distal nerve was performed at the end of experimental time. *Result*. After seven months, the grasping test reveals functional recovery in each tested animal; this improvement is more evident in Group A. The fibers appear well organized with restored myelin sheaths in both groups. Group A showed a great quantity of connective tissue surrounding the nerve. The quantitative morphology analysis in both groups shows a similar fibers density, fiber diameter, and myelin thickness. The differences between the groups in axon mean diameter are significant. In Group A M/d, D/d, and g-ratio is significantly higher compared to control group. *Conclusions*. Histological and functional assessments show a functional recovery of the injured nerve in the test groups, stressed by the results of the grasping tests and the meaningful increasing in fiber diameter and higher g-ratio. Moreover, a connective tissue cuff distinguishes the distal portion of the injured nerve. Considering the easy availability and handling of the material used in this study we can conclude that this experimental technique can be considered as a valid alternative to protect nerves in nerve wrap surgery.

1. Introduction

In plastic, otolaryngology, oral, and maxillofacial surgery, nerve repair is required when, for traumatic events, malformations, or oncological diseases, a nerve lesion occurs [1, 2].

The most frequent peripheral nerve injuries are caused by upper limb traumas. Also, craniofacial soft tissue injuries, isolated or in combination, can determinate nerve lesions [3, 4].

The facial nerve trauma causes are classified into accidental and surgical (unavoidable or iatrogenic) [5].

Kretlow et al. report that these injuries are the most common traumatic craniofacial damage (10% of all emergencies) and determine facial palsy stands between 5 and 25% [6].

During craniofacial surgery (pontocerebellar, parotid, and temporal bone) a facial nerve trauma may occur. In 7%-10% of temporal bone fractures facial nerve pathology is present [7].

In pediatric and geriatric patients slips, trips, and falls are the main cause of facial traumas, while violence and motor vehicle accidents are predominant in adults [6, 8, 9].

Loss of sensory and motor function in the anatomic innervated area or development of neuropathic pain is pathognomonic symptoms [10].

A neurorrhaphy is recommended when the nerve recovery does not occur due to nerve gap, neuroma, and scar tissue formation. The objective is to obtain the axons regeneration with minimal loss of fibers at the suture line [11].

Several studies have been carried out to identify the gold standards in the surgery approach of nerve repair according to the different clinical situations to be treated: sutures, glues, grafts, or tubules were considered.

Some surgeons prefer to use collagen sleeves or fibrin glue for facial nerve repair [12].

The clinical observation shows how the best results are achieved when the surgical technique is tensionless, performed with few stitches put in the outset connective sheath. Nerve trunk dissection should be avoided, delicate perineural tissue manipulation is needed, and nerve gaps more than 20 mm require a nerve graft to be repaired [13–17].

However, complications like nerve scarring and neuroma may occur, causing the failure of regenerative procedures in peripheral nerve surgery [13–17].

Latest generation glues can be used as an alternative to sutures, especially in the case of smaller trunk repair. In order to avoid sacrifice of nerves used as grafts, tubules in synthetic materials or autogenous conduits (arteries, veins) can be used [18–20].

In order to maintain distal effector function in case of more proximal trunk lesion some surgical, selective procedures as babysitting and nerve transfers have also been proposed [21].

In this animal model study, a collagen substitute as an alternative for nerve wrapping and repair was tested.

2. Materials and Methods

For the study were selected 16 Wistar adult female rats weighing between 200 and 300 g.

Experimental surgery was carried out at the Microsurgical Laboratory of the Ecole de Chirurgie in Paris (Institutional license from the "Direction Départementale de la Protection des Populations," DDPP number C-75-05-23) according to the French law on experimental animal research (law no. 87–848, October 19, 1987). All the surgeries were carried out by expert surgeons certified by the "Service Protection et Santé Animals du Ministère de l'Agriculture."

The animals, caged separately under a normal light cycle and fed *ad libitum*, were numbered with the international classification and divided into two groups of eight animals (A, B).

8-12 hours before surgery a period of fasting was observed; a suspension of fluid intake for 2-4 hours before anesthesia was practiced. Anesthesia was achieved with intraperitoneal tiletamine and zolazepam (3mg/Kg) and the dissection performed under magnification (2,5-0,4 x Leica microscopes)

The upper arm and axillary region were then shaved and cleansed with antiseptic solution; an incision was performed following the margin of pectoralis muscles to expose the brachial plexus in the axilla.

On the left arm, the median nerve was then identified and transected with a razor before its division into the terminal branches. On the right side, the procedure was repeated, and the proximal stump buried with 7/0 sutures in a subpectoral muscle pocket. Median nerve burying was performed to prevent spontaneous reinnervation, which could produce interference in functional tests. This modification would allow a greater stability in the nontreated median nerve limb and therefore a better evaluation of the functional recovery of the contralateral nerve repair if it occurred.

Different procedures were then applied to each test side (left arm). In Group A, a direct end-to-end suture was performed and a collagen sheath derived from an acellular hypoallergenic dermal matrix (ADM) (OrACELL®), used in dentistry, that retains native growth factors, collagen, and elastin was wrapped around the suture into a protective sleeve 10 mm long (5 mm from each side from the suture). 9/0 nylon sutures were used [23, 24].

In Group B (control) an end-to-end nerve suture was performed. The wounds of surgical accesses were sutured in Nylon 3/0.

After surgery each animal was caged separately and fed *ad libitum*, constantly followed and monitored in consultation with the central veterinary service and, if necessary, treated with analgesics (carprofen sub cut. 4-5 mg/kg once or twice/day).

In both groups, the functional effects of the therapy have been monitored by the modified grasping test shown by Papalia et al., in order to avoid the limitations that occur using the classical grasping test device such as the tendency of test animals to walk on the grid and the wrist flexion while holding the grid bars. [25] At the end of the observation time (7 months), the rats were sacrificed with an intraperitoneal anesthetic overdose and a histologic analysis of the treated area was performed.

2.1. Functional Analysis. During the postoperative period, all animals were tested for flexor digitorum muscle function using the grasping test, from the first month (T_1) and then every 2 months (T_2, T_3), until the sacrifice (T_4) [25, 26]. For measurement the dynamometer (BS-GRIP Grip Meter- 2 biological Instruments, Varese, Italy), consisting of a precision balance connected to a grid for the animal to grasp, was used.

The test was performed by holding the rat by the tail and bringing it closer to the grid allowing it to grasp and pull. The value recorded was the maximum weight that the animal pulled to maintain its grip before loosening it. For each group numerical data detected from T_1 to T_4 are expressed as mean and standard deviation. (SD).

Table 1: Groups A and B grasping test results (grams). Functional results at T_1 - T_4 (T_1 = 1 month, T_2 = 3 months, T_3 = 5 months, and T_4 = 7 months).

	Group A				Group B			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
1	0	90	77	245	32	130	200	224
2	0	110	135	278	0	133	112	100
3	14	82	152	248	20	134	135	170
4	0	100	195	238	70	142	180	200
5	0	80	72	90	40	82	125	224
6	66	126	142	226	34	106	166	208
7	0	132	210	270	24	60	128	202
8	68	180	175	254	44	32	128	186
Average	18,5	112,5	144,75	231,125	33	102,375	146,75	189,25
sd	30,32	33,32	50,33	59,4	20,28	40,44	31,24	40,34

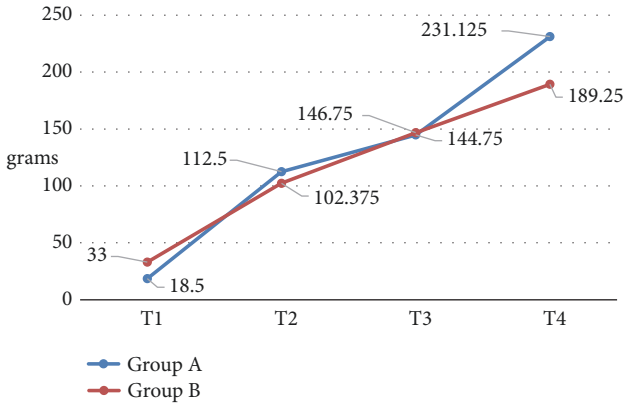

Figure 1: Grasping test results (grams) at T1 - T4 (T_1 = 1 month, T_2 = 3 months, T_3 = 5 months, and T_4 = 7 months).

The difference of rat grip force between groups, in each observation time, was statistically assessed. P<0,05 was considered statistically significant (Table 1, Figure 1).

2.2. Histology and Quantitative Analysis. Distally to the repair site, a 20 mm segment of left median nerve was taken, including in Group A the collagen wrapping.

The samples were immediately fixed in glutaraldehyde 2.5% in 0.1M PBS (pH 7.4) for five-six hours. Following postfixation in osmium tetroxide 2% for two hours they were dehydrated in ethanol (from 30% to 100%).

The samples were then washed in propylene oxide and embedded in resin (equal parts of Araldite M and Harter, which contained 0.5% of dibutyl phthalate plasticizer and 1-2% of accelerator 964).

For high-resolution optical microscopy, semithin transversal (thickness=2.5 μm) sections were cut, starting from the distal stump of each sample, using an Ultracut UCT ultramicrotome (Leica Microsystems). Sections were stained with toluidine blue (1%) and analyzed with a DM4000B microscope equipped with digital camera DFC320 and IMG50 Image Manager System (Leica).

One section for each animal sample was randomly chosen and the cross-sectional area was examined (Figures 3-4).

Then, 14 fields in each section were selected using a systematic random sampling protocol for stereological and morphometrical analysis.

To avoid edge effect and distortions, a procedure with two-dimensional dissector based on the choice of the fibers in sections upper part, was applied.

For each observation field (Oa) randomly selected, the number of fibers (Nf) was manually counted and their density/mm^2 (De) was calculated according to the size of the observation field (De=Nf x1000/Oa) (Table 2).

The areas of fibers and axons were measured, allowing the calculation of internal diameter of myelin = axon diameter (d) and external diameter of myelin = fiber diameter (D) and myelin thickness (M) as well the ratio M/d; D/d; and d/D (g-ratio) was calculated [22] (Table 2, Figure 2).

Then for each sample and for the two groups means and SD of quantitative parameter was made, and data were analyzed. The differences for values of P<0,05 were considered statistically significative.

Both statistical analyses were performed using SPSS 17.0 for Window package and Platform Prism Software package (GraphPad, La Jolla, CA, USA).

3. Results and Discussion

3.1. Results

3.1.1. Functional Analysis. The grasping test result in each group of rats shows that to maintain its grip in T_0, Group A rats exert an inferior average strength compared to the one registered in Group B (18,5± 30,32 grams vs. 33±20,28 grams). No grip is shown in 63,5% of first group test animals versus 12% of control group.

In T_1 and T_2 Group A test animals exert superior average strength compared to Group B (112,5±33,32 grams vs. 102,37±40,44 grams and 144,75±50,33 grams vs. 146,75±31,24 grams). In T_1 only one test animal keeps exerting force=0. From T_2 is registered a functional recovery in every test

Table 2: Quantitative analysis data: average and sd in each sample and in Groups A, B of Axon diameter (d), fibre diameter (D), myelin thickness (M)(μm), and M/d; D/d; and d/D ratio.

	Group A							Group B						
	Density	Axon Diameter (d)	Fiber Diameter (D)	Myelin thickness (M)	M/d	D/d	d/D (g-ratio)	Density	Axon Diameter (d)	Fiber Diameter (D)	Myelin thickness (M)	M/d	D/d	d/D (g-ratio)
1	33317	2,86	3,87	0,50	0,19	1,38	0,74	39519	2,49	3,47	0,49	0,21	1,42	0,71
sd	7440	1,44	1,77	0,23	0,08	0,16	0,1	10478	0,92	1,14	0,16	0,05	0,11	0,06
2	3931	2,53	3,43	0,45	0,19	1,38	0,73	43631	2,31	3,32	0,51	0,23	1,45	0,69
sd	9487	1,09	1,29	0,13	0,05	0,11	0,05	9476	0,88	1,14	0,17	0,06	0,11	0,05
3	49522	2,37	3,21	0,42	0,19	1,38	0,73	46178	2,36	3,25	0,44	0,20	1,41	0,72
sd	12800	0,89	1,06	0,12	0,06	0,11	0,06	9438	0,98	1,18	0,13	0,06	0,12	0,06
4	34597	2,82	3,69	0,43	0,17	1,34	0,75	39968	2,57	3,63	0,53	0,23	1,45	0,70
sd	9076	1,19	1,37	0,12	0,05	0,10	0,06	12026	1,09	1,36	0,17	0,08	0,16	0,07
5	42993	2,26	3,08	0,41	0,20	1,40	0,72	31847	2,58	3,63	0,53	0,22	1,45	0,70
sd	11114	1,07	1,25	0,12	0,06	0,12	0,06	7626	1,10	1,34	0,16	0,07	0,13	0,06
6	38216	2,90	3,93	0,51	0,19	1,38	0,73	43631	2,15	3,09	0,47	0,24	1,48	0,68
sd	6369	1,02	1,24	0,15	0,05	0,11	0,05	7050	0,93	1,12	0,14	0,08	0,16	0,07
7	44585	2,23	2,94	0,36	0,18	1,35	0,75	41998	2,23	3,24	0,51	0,25	1,50	0,67
sd	10070	1,04	1,20	0,11	0,05	0,11	0,06	9391	0,93	1,14	0,15	0,09	0,19	0,07
8	26671	3,47	4,77	0,65	0,20	1,41	0,72	41003	2,28	3,34	0,53	0,26	1,53	0,66
sd	8494	1,54	1,89	0,21	0,06	0,13	0,06	12190	1,16	1,43	0,17	0,10	0,20	0,08
AVERAGE	38652	2,68	3,62	0,47	0,19	1,38	0,73	40972	2,37	3,37	0,50	0,23	1,46	0,69
SD	7184	0,42	0,59	0,09	0,01	0,02	0,01	4287	0,16	0,19	0,03	0,02	0,04	0,02

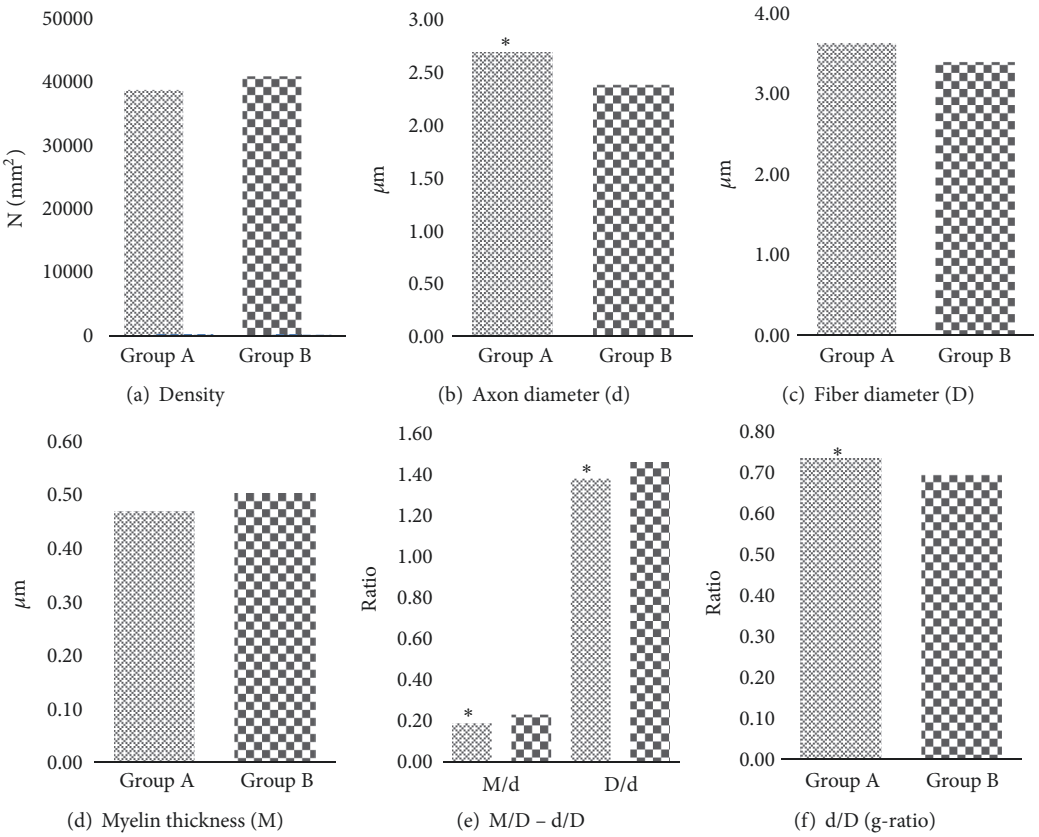

Figure 2: Histograms showing the morphoquantitative analysis of the regenerated myelinated fibers after 7 months from the surgery of Group A (end-to-end suture with a conduit of collagen matrix - OrACELL® - wrapped around the suture into a protective sleeve) and Group B (end-to-end suture only). Values in the graphics are expressed as mean + SD. P>005 (∗ = statistically significant value).

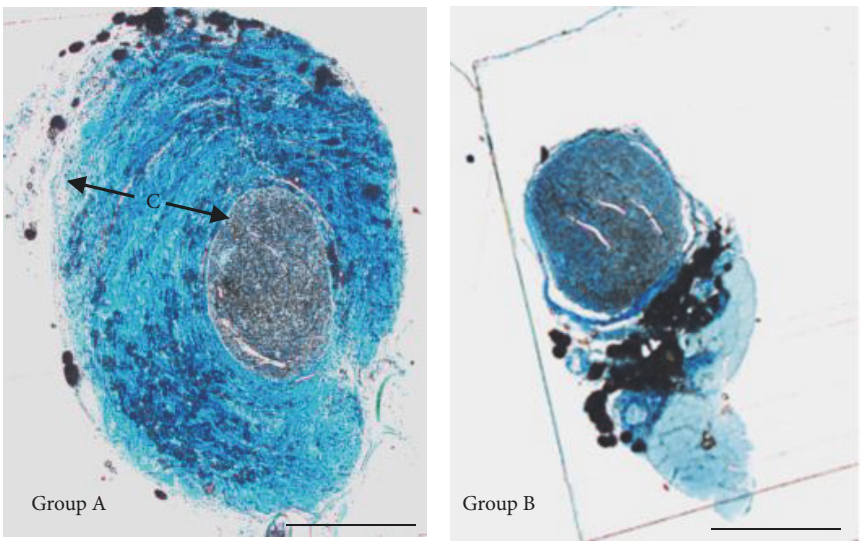

Figure 3: Representative low-magnification (8x) images of toluidine-blue stained semithin transverse sections of regenerated median nerve repaired with end-to-end nerve suture, with (Group A) or without (Group B) a conduit of collagen matrix (OrACELL®) wrapped around the suture into a protective sleeve. Seven months after surgery, the presence of the collagen layer (C) around the suture zone is still visible in Group A. BAR=500μm.

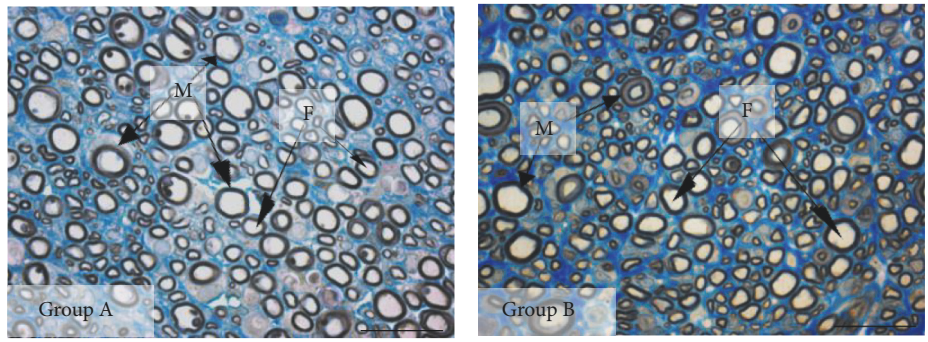

Figure 4: Representative high-resolution (40x) light microscopy images of toluidine-blue stained semithin transverse sections of regenerated median nerve repaired with end-to-end nerve suture, with (Group A) or without (Group B) a conduit of collagen matrix (OrACELL®) wrapped around the suture into a protective sleeve. Seven months after surgery, in both groups, regrowing myelinated fibers (F) with well-organized myelin sheaths (M) are detected. BAR=20μm.

animal. In T_3 the results show a greater ability to exert force in Group A. The difference between the two experimental groups is not statistically significant.

3.1.2. Histology and Quantitative Analysis. The histological observations of the samples seem substantially analogues. The myelinated fibers appear well-organized with regenerated myelin sheaths. The experimental Group A showed a great quantity of connective tissue surrounding the nerve, which is not present in control group. Grafting area in Group B did not show any immunological response or chronic inflammation of nervous and connective tissues (Figures 3 and 4).

The quantitative morphologic analysis in cross-sectional area shows in both groups a similar fiber density (38653±7184 N/mm^2 vs. 40972±4287 N/mm^2). Differences between the groups in axon mean diameter (d) (2,68±0,42 μm vs. 37±0,16 μm) is significant ($p<0,05$)

Fiber diameter and myelin thickness are almost comparable in wrapped nerves than in simple repair (3,62±0,59 μm - 0,47±0,09 μm vs. - 3,37±0,19 μm - 0,50±0,03 μm).

M/d, D/d ratio in the two groups are, respectively: 0,19±0,01 1,38±0,02 and 0,23±0,02 1,46±0,04. The differences are highly significant ($p<0,01$) [Table 2]

In Group A g-ratio (0,73 ±0,01) is higher than the one identified in Group B (0, 69±0,02). The statistical analysis shows differences highly significant ($p<0,01$)

3.2. Discussion. In every district the spontaneous nerve recovery is a physiological possibility subsequent a nerve interruption [27].

Millesi et al. showed that peripheral nerves are considered as gliding structures, made up and surrounded by proper connective tissues themselves capable of gliding [13–17].

When natural healing is not possible the main objective of nerve repair techniques is regenerating sensory, motor, and autonomic axons with limited loss of fibers among the suture line [11].

The wrapping with different materials, whether autologous or heterologous, has been introduced to facilitate nerve repair processes; tubules constitute a regrowth protection and stimulation [18–20, 28].

Some materials were identified as possible wrapping for nerve repair, and collagen has shown the best result for hits biocompatibility and adaptability [18, 20].

The use of collagen conduits could be also considered when complex surgical procedures are planned. Therefore, the wrapping/tubulization of long nerve grafts (whether auto or allografts), such as in cross facial nerve grafting, with collagen or collagen/GAG conduits, could be suggested.

Since collagen has also been successfully used as a conduit for nerve regeneration, for short gaps (no more than 3-4 cm) proximal and distal stump coaptation into a collagen tube, it could also be considered as a valid alternative to autologous vein (filled by free muscle or not) [18].

The animal model used by the authors has been the simulation of an upper limb nerve lesion and surgical therapy. This procedure can be considered a standard in research of nerve regeneration, furthermore, allowing functional assessment [29].

Histological results of our research show how the use of ADM in guided bone regeneration and oral soft tissue correction does not modify the injured nerve healing. This is stressed by the presence of well-organized myelinated fibers in the distal stump of the nerve and no significant difference in fiber density and myelin thickness between the two groups [30, 31].

The morphological evidence of the matrix action is shown by the presence of a connective tissue and collagen cuff around the graft area, as more clearly highlighted in Group A observations. No immunological or inflammatory response vs. the dermal matrix was observed.

A soft, tensionless repair can recreate the gliding apparatus described by Millesi allowing nerves to have their own range of free motion [14–17].

The grasping test progression in time highlights in an initial phase a slow pace in the functional recovery process; this is stressed in T1 by favorable results in the control group in both the number of test animals capable of exert force and the entity of the aforesaid force. In further controls; every test animal shows a grasping ability with progressively

overlapping exerted force values. Seven months after the procedure, the functional analysis shows a superior strength exerted by the rats subjected to the tubulization procedure compared to the group in which the end-to-end anastomosis was not protected, although the data are not statistically significant. Moreover, the conductive ability of the nerves is objectified by the significant increase in fiber diameter since this dimension is proportional to the conduction velocity space constant and the wavelength of the nerve impulse. The same assessment is highlighted by the higher g-ratio identified in Group A compared to the control group that analyses the perineuronal myelin quota and subsequently the jumping conduction potential binds to the action of Schwann cells [32].

Native growth factors present in ADM components as mediators for Schwann cells proliferation and reorganization of molecular and ionic pattern in regenerating nerve sheaths could explicate how these conduits enhance fiber regeneration [23]

Collagen properties in protection against scar through a reduction of TGF beta and other proinflammatory cytokines might play a role in creating a favorable environment for nerve regeneration [23, 30].

The overall assessment of anatomical and functional tests shows the efficacy of our technique. The dermal matrix use shortens the surgical times, avoiding the harvest of conduits from different donor sites. The thick collagen fiber layer observed around the nerve can be an effect of the grow factors contained in the matrix itself. Considering that the dermal matrix use has no adverse effect, we believe that the technique is ready for a clinical application.

4. Conclusions

The collagen membrane tested has never been proposed in conventional tubulisation techniques for nerve repair.

In this animal model study, the use of AMD induced in some aspects a better recovery in neuronal activity.

In the light of the experimental evidence we can conclude that this experimented technique allows, same as others, an efficient nerve trunk repair. The collagen conduit, after clinical trial, can be considered as an alternative to generally used nerve wrap materials to protect nerve repair and its use could be extended to all peripheral nerve surgery. The advantages are the easy availability and handling of the material used and the simplification of the surgical technique since the harvesting of arteries or veins in a different surgical site, in accordance with some nerve wrapping techniques, is avoided.

Data Availability

The data of grasping test results used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

None of has authors has conflict of interests.

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Hindawi
BioMed Research International
Volume 2019, Article ID 5907195, 14 pages
https://doi.org/10.1155/2019/5907195

Review Article

Diabetes: Oral Health Related Quality of Life and Oral Alterations

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Received 6 February 2019; Accepted 27 February 2019; Published 18 March 2019

Guest Editor: Tolga F. Tozum

Background and Objectives. About 5% of the world's population is affected by diabetes; these patients must be further treated during medical and surgical treatments. These patients, due to the glycemic conditions, realize during their life multiorgan changes, in different body districts. Moreover, this condition obliges them to undertake hypoglycemic therapies. Diabetes is a risk factor for many diseases, including those concerning the oral district with immunological implications. *Materials and Methods*. A comprehensive review of the literature was conducted according to PRISMA guidelines accessing the NCBI PubMed database. Authors conducted the search of articles in English language. The results of the last 10 years have been considered, which present useful information regarding the oral conditions. A total of 17 relevant studies were included in the review. The study evaluated only papers with specific inclusion criteria regarding oral health. The works initially taken into consideration were 782; subsequently applying the inclusion and exclusion criteria, there were 42 works. After a careful analysis of the work obtained by two academics who have worked separately, there have been 17 studies. All data from the studies were compared and many of these confirmed alteration in the oral district. *Results*. The studies taken into consideration evaluated different factors, such as OHRQoL, QoL, and oral alterations, involving soft tissue, dental structures, and postrehabilitative complications, as well as immunological alterations. *Conclusions*. We can affirm, in conclusion, that this study has brought to light those that are complications due to diabetic pathology, from different points of view. The psychological and psychosocial alterations, certainly present in these patients, are probably due to local and systemic alterations; this is confirmed by the correlation between oral health and quality of life reported by the patients.

1. Introduction

Diabetes is a term that identifies some diseases characterized by polyuria (abundant production of urine), polydipsia (abundant ingestion of water), and polyphagia (excessive hunger). Commonly the term is used to indicate a chronic disease, which can be included in the group of diseases known as diabetes mellitus, characterized by a high concentration of glucose in the blood, which is in turn caused by a total or partial lack of insulin in the human organism, a hormone that decreases the concentration of glucose in the blood. Diabetes mellitus is a form of diabetes or a group of metabolic disorders united by the fact of persistent instability of the blood glucose level, going from conditions of hyperglycemia, more frequent, at hypoglycemia conditions. Although the term diabetes refers in the common practice to the only condition of diabetes mellitus; that is sweet, there is another pathological condition called diabetes insipidus. The percentage of affected

world population is estimated at around 5%. About 90% of the diabetic population is affected by type 2 DM. WHO estimates that there will be a tremendous increase in the prevalence of DM in the USA, the Middle East, and Southeast Asia, while the increase in Europe will be more modest. In 2030, more than 360 million sick people are expected. There was a higher prevalence in women: (m: f = 1: 1.25). A study of the 15-29 year olds with type 1 diabetes had a higher incidence in males than females, perhaps due to factors such as sex hormones or a different exposure to environmental toxins. Complications of diabetes can be different and spread throughout the body. Among the complications of diabetes mellitus we recognize diabetic macroangiopathies, with atherosclerosis phenomena; diabetic ulcers, carpal tunnel syndrome, glaucoma, diabetic neuropathies, cataracts, oral or dermatological infections, and parodontopathies. We also recognize diabetic microangiopathies, therefore nephropathies, retinopathies, and neuropathies of the peripheral nervous system. This increased susceptibility to develop infections in different districts, including the oral one, is of enormous dental interest. In addition there is a predisposition to the development of periodontopathy; all this makes the patient diabetic, a patient to be treated from a dental point of view [1–3].

2. Material and Methods

2.1. Protocol and Registration. This review was registered on PROSPERO with 120208 ID protocol. PROSPERO includes protocol details for systematic reviews relevant to health and social care, welfare, public health, education, crime, justice, and international development, where there is a health related outcome. Systematic review protocols on PROSPERO can include any type of any study design. Reviews of reviews and reviews of methodological issues that contain at least one outcome of direct patient or clinical relevance are also accepted.

2.2. Focus Question. The following focus question was developed according to the population, intervention, comparison, and outcome (PICO) study design:

What are the oral changes present in diabetic patients?

How much does diabetes affect the patient's oral immunological profile?

2.3. Information Sources. The search strategy incorporated examinations of electronic databases, supplemented by hand searches. A search of four electronic databases, including Ovid MEDLINE, PubMed, EMBASE, and Dentistry and Oral Sciences Source, Human syndrome for relevant studies published in the English language to 2018 was carried out.

A hand search was also performed in other medical journals. The search was limited to English language articles. A hand search of the reference lists in the articles retrieved was carried out to source additional relevant publications and to improve the sensitivity of the search.

2.4. Search. The following key words were used: "Diabetes" AND "Dental" OR "Oral alteration"- "Diabetes" AND "OHIP" AND "Quality of life"- "Diabetes" AND "Dental" OR "Oral" AND "immunologic". The choice of keywords was intended to collect and to record as much relevant data as possible without relying on electronic means alone to refine the search results. The research was also limited to medical journal and only to articles written in English.

2.5. Selection of Studies. Three independent reviewers, of University of Messina singularly analyzed the obtaining papers in order to select inclusion and exclusion criteria as follows. Reviewers compared decisions and resolved differences through comparing the manuscripts. For the stage of reviewing of full-text articles, a complete independent dual revision was performed. The results have been compared at the end of the research. A possible disagreement regarding the inclusion of the studies was discussed among the authors. The first phase of the research consisted of the selection of titles, which allowed us to make a first screening of the manuscript eliminating those not concerning our research. Finally, the full text of all studies was obtained and according to the expected inclusion/exclusion criteria, articles were selected and included in the present review. We obtained a total of 782 results without filters and 256 after the first electronic and manual search with keyword used. We included only 17 full-text English articles on humans.

2.6. Types of Selected Manuscripts. The review included studies on humans published in the English language. Letters and editorials were excluded.

2.7. Types of Studies. The review included all human use studies and literature reviews published on diabetic patients with focus on oral anomalies, Oral Health Impact Profile, and oral immunological profile.

2.8. Inclusion and Exclusion Criteria. The full text of all studies of possible relevance was obtained for assessment against the following inclusion criteria:

(i) Study of patients with diabetic patients.

(ii) Study of patients with dental anomalies and diabetes

(iii) Study of OHIP or quality of life and diabetes

(iv) Study of oral immunological information and diabetes

The applied exclusion criteria for studies were as follows:

(i) Studies involving patients with other specific diseases, immunological disorders, oncological patients, osteoporosis, and genetic diseases

(ii) Not enough information regarding the selected topic, no information about oral status and oral health

(iii) No access to the title and abstract in English language or letters, commentary, PhD thesis and editorials

(iv) Animal studies

(v) Not full-text articles

Table 1: Most common complications of diabetes [2, 3].

CARDIOVASCULAR DISEASE	SENSORY ORGAN DISEASE	KIDNEY DISEASE	OTHER
(i) Macrovascular disease (ii) Coronary artery disease (iii) Peripheral artery disease (iv) Stroke risks (v) Diabetic foot (vi) Diabetic myonecrosis	(i) Retinopathy (ii) Glaucoma (iii) Cataracts (iv) Blindness	(i) Nephropathy (ii) Chronic kidney disease (iii) Urine protein loss (iv) Tissue scarring	(i) Erectile dysfunction (ii) Periodontal disease (iii) Respiratory infections (iv) Restrictive lung disease (v) Lipohypertrophy

2.9. Sequential Search Strategy. After the first literature analysis, all article titles were screened to exclude irrelevant publications, case reports, and the non-English language publications. Then, researches were not selected based on data obtained from screening the abstracts. The final stage of screening involved reading the full texts to confirm each study's eligibility, based on the inclusion and exclusion criteria.

2.10. Data Extraction. The data were independently extracted from studies in the form of variables, according to the aims and themes of the present review, as listed onwards.

2.11. Data Collections. Data were collected from the included articles and arranged in the following fields as seen in Table 1:

"Author (Year)" – Revealed the author and year

"Type of study" - Indicates the type of the study

"Sample" – Number of patients and follow up time

"Parameter Evaluated" – Parameter in the study

"Treatment"- Indicates if any treatment has been performed

"Results" - Info about results

"Statistic"- Statistical analysis

Other information is showed in Table 3.

2.12. Risk of Bias Assessment. This type of work brings together all the studies in the literature in the last eighteen years presenting a review of recent data about dentomusculoskeletal anomalies in MFS. The risk of bias is minimal as the work is intended to be a collection of works carried out about these patients and about only dental anomalies, but we can only consider full text and abstract accessible articles in English language. Regardless of the results of the studies taken into consideration, the evaluation was carried out on the field of action of the analyses carried out by the studies.

2.13. Diabetes. Diabetes mellitus is a chronic disease characterized by an increase in blood glucose concentration. Responsible for this phenomenon is an absolute or relative defect of insulin that allows the body to use glucose for energy processes within cells. When insulin is produced in insufficient quantities from the pancreas or the body's cells do not respond to its presence, glucose levels will be higher than normal in the blood (hyperglycemia) thus favoring the appearance of diabetes mellitus. The diagnosis of diabetes is certain with a blood glucose value of 200 mg/dl, detected at any time of day or two hours after a glucose load. Insulin is a hormone secreted from the islands of Langerhans of the pancreas and essential for the metabolism of sugars. All simple and complex sugars (starches), which are taken with food, are transformed during digestion into glucose, which is the main source of energy for muscles and organs. In order for glucose to enter the cells and be used as "fuel", the presence of insulin is required [3]. The cardiovascular system includes sometimes very serious alterations: the occurrence of an aortic dissection is not uncommon, while a diagnosed patient will be followed throughout the course of the disease and then monitored with an echocardiogram for possible changes in aortic measures. Three subcategories of diabetes mellitus are recognized:

(i) Type 1 diabetes mellitus (also known as juvenile diabetes):

(a) occurs at a young age (within 30 years);

(b) the production of insulin is insufficient; therefore, the therapy consists of the administration of insulin;

(c) glucose is not used by cells and accumulates in the blood (hyperglycaemia);

(d) the high concentration of glucose in the blood prevents the renal tubules from reabsorbing it, with the consequent presence of glucose in the urine;

(e) the renal reabsorption of water and sodium is compromised for osmotic reasons dictated by the high quantity of glucose and ketone bodies in the ultrafiltrate, with consequent production of large quantities of urine (polyuria);

(f) to the polyuria it follows a strong dehydration that, stimulating the center of the thirst, induces the diabetic to drink continuously (polydipsia);

(g) insulin deficiency causes an altered metabolism of fats and proteins, to which is added the inability to store glucose and results in frequent weight loss and increased appetite of the subject (polyphagia);

(h) in the long term serious complications occur, especially in the structural and functional alteration of blood vessels (thickening and hardening of the arterial walls, alterations of the capillaries in the retina and kidneys, suffering of the peripheral nervous system).

Furthermore, type 1 diabetes is further subdivided into the following:

(1) immune-mediated: it is the diabetic immune system that destroys the beta cells, the only ones responsible for the production of insulin and the regulation of glucose levels. This process of destruction is as rapid as the subject is young (children and adolescents, in fact, can develop ketoacidosis very quickly);
(2) idiopathic: the individual does not produce insulin and is subject to ketoacidosis, but there are no autoimmune factors involved.

(ii) Type 2 diabetes mellitus:

(a) occurs in adulthood;
(b) arises due to the inability of cells to use insulin (resistance) correctly and progresses with the gradual loss, by the pancreas, of the ability to produce insulin in adequate quantities;
(c) hyperglycemia occurs when the pancreas is no longer able to meet the organic needs and/or when peripheral insulin receptors are compromised;
(d) administration of insulin as a therapeutic regimen is often not sufficient and/or indicated, while diet control is fundamental;
(e) most people with this type of diabetes are obese;
(f) insulin resistance decreases with weight loss, but returns to rise as soon as weight is regained;
(g) is subject to strong familiarity.
(h) Among the other types of diabetes, we mention the following:

(iii) Gestational diabetes mellitus: type of glucose intolerance that occurs during pregnancy and generally disappears after delivery and returns the patient to normal. It can be managed with or without insulin;

(iv) Secondary diabetes: occurs following other diseases (e.g., genetic defects of beta cells, endocrinopathies, pancreatitis, tumors) or special medical treatments (e.g., corticosteroids);

(v) Diabetes insipidus: pathology characterized by significant polyuria and insatiable thirst due to an alteration of the production, secretion or functioning of the hormone vasopressin (antidiuretic hormone) at the hypothalamus and pituitary level or from its lack of activity at the renal level.

Among the risk factors for the development of diabetes, a distinction can be made that supports factors on which one can act (obesity, lack of physical exercise) to factors that must be taken into account as appropriate (familiarity with diabetes, age, ethnicity, other pathologies, potentially iatrogenic therapeutic treatments). As we said at the beginning, the acute and chronic complications of diabetes are going to significantly affect the life of the person, since it is a chronic and irreversible disease whose chronicization involves other cascade dysfunctions that are added to the events of

(I) acute complications: hypoglycemia, hyperglycemia;

(II) chronic complications: atherosclerosis, retinopathy, nephropathy, neuropathies, diabetic ulcers, increased susceptibility to infections (diabetic foot, phlegmons, cellulitis, necrotizing fasciitis, urinary tract infections, etc.) [1–3].

2.14. Hyperglycemia and Immunological Correlation. The diabetic pathology is closely related to immune factors, both as regards its etiopathogenesis and as regards the complications following the overt pathology. According to the most modern and accredited scientific beliefs, insulin dependent diabetes (type 1) is considered a condition by the rather complex and articulated pathogenesis. However, attention is focused on three aspects of the disease, essentially reducible to genetic, environmental, and immune factors. These three aspects, although generally treated separately, are closely intertwined in contributing to the genesis of the disease. In diabetics some alterations of the immune response are present even when the disease is well established over time and, unlike what has been said so far, can appear both in patients with type 1 and type 2 diabetes. In these patients there is a greater susceptibility to infections, most likely due to a deficient immune response [1–9].

One of the most classic explanations refers to the insufficient use of insulin by the cells of the immune system and the consequent decreased activation and differentiation of the same. Therefore, according to this view, diabetes mellitus, when decompensated, would be included among the secondary immunodeficiency conditions. The result is a vicious circle where acute infections in the diabetic promote the metabolic decompensation which in turn reduces the immune response, as periodontitis. Studies in this direction have shown that in many patients with long-term diabetes there is an increase in circulating immunocomplexes, a decrease in the total number of T lymphocytes, and an imbalance in the lymphocyte subpopulations, with a CD4 / CD8 lymphocyte ratio reduced between 1.5 and 2 due in particular to a decrease in cells with CD4 phenotype (helper). Further clinical confirmation of immunological alteration comes from the evidence that patients with disease duration of more than 5 years show a deficient antibody response after vaccination against hepatitis B. The explanation of this phenomenon would be to be found in the nonrecognition of viral antigenic determinants with consequent reduced specific antibody response, due precisely to the alteration of the ratio and/or function of CD4 and CD8 lymphocytes. In the case of anti-influenza vaccination, the antibody titre was instead normal; this could be explained by the fact that as it is a secondary response and because of the cross reactivity with the different viral strains, a clone of T cells with memory already sensitized towards that particular antigen would be stimulated. This process is not strictly dependent, as in the case of hepatitis B vaccination, from a normal CD4/CD8 lymphocyte ratio and therefore clarifies why in the case of influenza vaccination the specific immune response is normal while it is not after the vaccine administration for hepatitis B; in fact in this last case it is a primary answer. In

perspective, therefore, we can infer that the normalization of immunological parameters may represent a useful attempt to prevent acute infections, whether viral or bacterial, for the long-term diabetic. In this way it is possible to remove the eventuality of a metabolic decompensation that could derive from it or worse than a resulting ketoacidosis with all the consequences that come from this condition. As is well known, diabetes is a morbid condition that can lead to a series of complications with the increase in the duration of the disease, affecting some organs in a characteristic way. In this regard we mention the diabetic retinopathy which, passing through the phase of simple diabetic retinopathy, can reach the most severe proliferative one, leading in some cases to blindness. The kidney is also a target organ of diabetic disease presenting typical lesions such as the peritubular deposit of glycogen and mucopolysaccharides, arteriosclerosis and glomerulosclerosis. The peripheral nervous system can be affected in practically every district and the lesions may belong either to the type of mononeuropathy, such as poly- or multineuropathy. In addition the autonomic nervous system can also be affected; it is widely accepted that the latter is a fundamental factor in determining and/or accelerating diabetic microangiopathy; however, there are some controversial pathogenetic aspects. In fact, clinical experience teaches us how difficult it is sometimes to find signs of diabetic microangiopathy in patients with poor metabolic control or, on the contrary, there are patients who, despite the optimization of metabolic parameters, present a short distance from the diagnosis of the typical lesions. In this context some immunological aspects are inserted which, although over the years have undergone extensive revisions, maintain a certain validity in the essential lines. Proof of this is the detection of immune complex deposits at the level of the renal capillaries and the increase of the same in the circulation, probably related to the alteration of the mechanisms of "clearance" of the immune complexes. The thickening of the basement membrane, typical lesion in diabetic microangiopathy, would be the consequence, among other things, of a complex series of alterations, among which the entrapment of the anti-andean-antibody complexes would play an important part. From this and many other considerations it emerges that the pathogenesis of diabetic microangiopathy is multifactorial, determined not only on the metabolic side, but influenced by the genetic base and individual variability. Precisely because of this important immunological component, immune therapies have been proposed, to counter both the onset of the disease, but also eventually carry out long-term therapy and limit complications [2, 3, 5–12].

2.15. Interdisciplinary Considerations. Periodontal disease (PD) is a chronic inflammatory disease that destroys the gingiva and the tissues surrounding the teeth (periodontal). It is one of the most prevalent chronic infections in adults and affects over 22% of people with diabetes. The two main forms of PD, gingivitis and periodontitis, are the result of bacterial plaque that if destroyed increases the gingival tissue and periodontal. Although it is triggered by bacteria that adhere to the teeth, the individual inflammatory response also plays an important role in the development of periodontal disease: this is why people suffering from chronic diseases such as diabetes, cardiovascular disease, obesity, chronic obstructive pulmonary disease (COPD), and other diseases respirators are much more sensitive and have a greater risk of getting sick. Some studies have documented that periodontitis treatment improves glycemic control, most likely by improving insulin sensitivity. Diabetes and periodontal diseases both have a chronic course but are treatable conditions and should be kept under control with long-term treatment, to ensure better health conditions in general and in the future. In the care of the person with diabetes, the aspect of education and empowerment is of fundamental importance. The diagnosis of diabetes mellitus causes, in fact, the emergence of a situation of fear and insecurity that can negatively affect the correct implementation of the care activities [12–15]. Above all as regards the aspects of containment of anxiety, the intervention of a psychologist is considered necessary both to activate attitudes of coping and to design the training aspects of the patient. Therefore, the themes of psychological interest are identified in the communication phase of the diabetic pathology, in the definition of care interventions and in the phase of support to the patient who is redesigning their existence as a function of this pathology. Diabetes mellitus facilitates the appearance of psychopathological disorders such as depression and anxiety, which in turn affect the management of the disease [13]. The work of the psychologist is an important support in the treatment of the pathology. The practice demonstrated the negative effect that psychosocial dynamics can have on the patient's ability to correctly adhere to therapeutic indications. It is therefore a matter of promoting an attitude of the patient's adherence to therapy and, at the same time, of developing effective coping, making the subject see diabetes as a problem rather than a threat. It follows that good adaptation to the disease depends on the type of individual strategies that the patient puts in place to deal with it. The link between diabetes mellitus and mood disorders is known at least since the 1950s. Symptoms of depression include persistent sadness or inability to experience joy, loss, or increase in appetite, insomnia, apathy, difficulty in concentration, feelings of despair and worthlessness, negative thoughts such as suicidal ideas, irritability, anxiety, nervousness, feelings of guilt. Sometimes depressed people find it difficult to cope with daily programs and activities, and they report significant difficulties in the various fields of life. While depression is very common among the general population, some clinical studies indicate that it is even more common in chronic patients [16–22]. This could be due to numerous factors, including stress resulting from treatment and control of the disease, effects on cognitive function, side effects or complications inherent in drug therapy. There are also both organic and psychological implications in diabetic patients with respect to eating disorders (Table 2) [20–26].

3. Results

3.1. Study Selection. Article review and data extraction were performed according to PRISMA flow diagram (Figure 1).

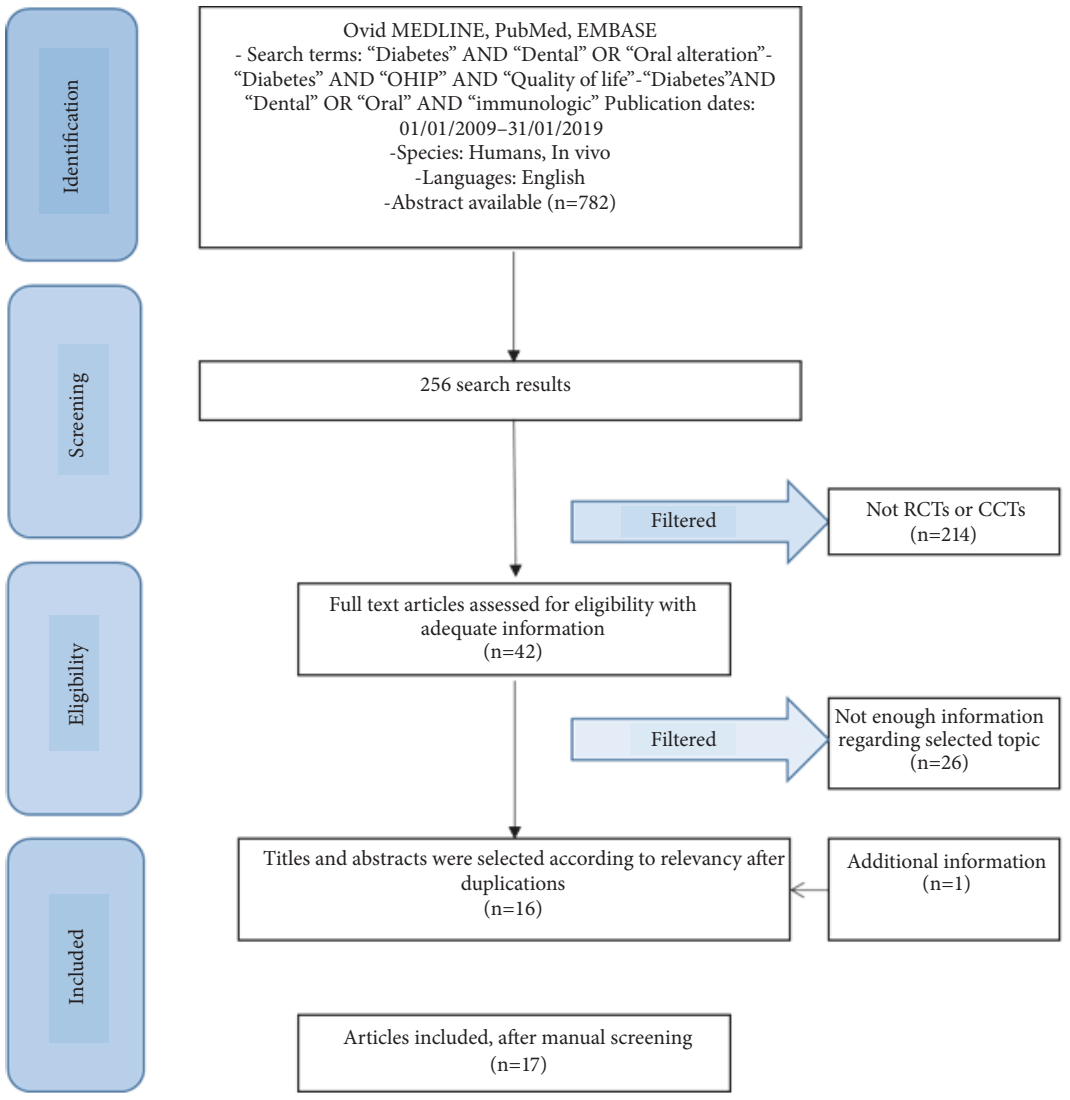

Figure 1: PRISMA flow diagram.

Table 2: Psychological and neurological issues related to diabetes [1–3].

NEURO DISEASE	PSYCHOLOGICAL ISSUE
(i) Neuropathy	(i) Cognitive decline
(ii) Muscle atrophy	(ii) Risk of dementia
(iii) Cognitive deficit	(iii) Alzheimer
(iv) Diabetic encephalopathy	(iv) Depression

The initial electronic and hand search retrieved 256 citations. 240 papers were excluded because were not identified as full text, RCT, or not enough information about this topic. A study was added later to a manual search, as it contributed to our work. At last only 17 studies were included because of topic reasons.

3.2. Study Characteristics. After the study selection a new division related to the kind of bone graft has been performed:

(i) Diabetes and quality of life

(ii) Diabetes and oral alteration

(iii) Diabetes and immunological alteration

3.3. Studies Results. The results were analyzed by the authors independently and were evaluated according to their dental interest field. The review aims to evaluate diabetic patients' symptoms in the medical field, psychology and odontology; all studies have therefore led to satisfactory results. Studies evaluating all types of abnormalities in maxillofacial district are reported. The purpose of this work is to index oral abnormalities and give a rapid diagnostic method proposal for diabetic patients, and for other syndromes early diagnosis.

4. Discussion

As widely discussed in the previous chapters, diabetes is therefore a pathology affecting different areas of the

Table 3: Studies evaluated.

Author (year)	Type of study	Sample	Parameter evaluated	Treatment	Results	Statistic
Cortelli et al. (2017) [27]	RCT, double blinded	206 for 3 months	Oral Health and Quality of Life (OHQoL), pocket depth, plaque and gingival indices, PCR for bacteria evaluation, Periotron®	Gingival treatment	OHRQoL improved over time, confirming that quality of life could be changed by the treatment of oral diseases such as gingivitis	P<0.05
Davis et al. (2018) [28]	Observational cohort study	930 (0, 2, 4, 6 years)	Short Form-12 version (SF-12v2), Audit of Diabetes Dependent QoL 19 (ADDQoL)	Blood glucose lowering therapy	These real-life data show that treatment intensification, including insulin initiation, does not impact adversely on patient well-being in community-based type 2 diabetes	P>0.16
Cinar et al. (2013) [29]	Prospective	186	Community Periodontal Need Index (CPI) HbA1c (glycated hemoglobin percentage)	Health coaching (HC), Health Education (HE)	The present findings imply that HC has a significantly higher impact on better management of diabetes and oral health when compared to formal HE	P<0.05
Tzanetakos et al. (2018) [30]	RCT		quality-adjusted life-years (QALYs)	Insulin Glargine vs. Liraglutide 1.2mg vs exanatide once weekly	ExQW was estimated to be cost effective relative to IG or Lira1.2mg for the treatment of T2DM in adults not adequately controlled on OAD	/
Islam et al. (2014) [31]	RCT	216 for 6 months	HbA1c, quality of life	Short message service (SMS)	Mobile phone SMS services have the potential to communicate with diabetes patients and to build awareness about the disease, improve self-management and avoid complications also in resource-limited setting	/

Table 3: Continued.

Author (year)	Type of study	Sample	Parameter evaluated	Treatment	Results	Statistic
Vora et al. (2015) [32]	RCT	170+165 for 24 weeks	Diabetes Treatment Satisfaction Questionnaire	Glargine/glulisine once daily or insulin aspart/aspart protamine	In long-standing type 2 diabetes with suboptimal glycaemia despite oral therapies and basal insulin, the basal plus regimen was noninferior to biphasic insulin for biomedical outcomes, with a similar overall hypoglycaemia rate but more nocturnal events	
Castro Dos Santos et al. (2016) [33]	RCT	20 at 30, 90, 180 days	Quality of life, public health costs	Antimicrobial photodynamic therapy (aPDT) and ultrasonic periodontal debridement (UPD)	The adjunct application of aPDT to UPD did not present additional benefits for the treatment of chronic periodontitis in type 2 diabetic patients	$P>0.05$
Goodson et al. (2017) [34]	RCT	8173	Salivary glucose concentration, obesity, dental caries, gingivitis		High salivary glucose was associated with dental caries and gingivitis in the study population	/
de Araújo Nobre et al. (2017) [35]	Open cohort study	22009 for 3 years	Periodontitis, dental caries, and peri-implant pathology	Exposure to systemic conditions was prevented	The present study describes an epidemiological approach to the distribution and determinants of the three principal chronical oral diseases	12.2% less periodontitis and 4.3% less dented caries

Table 3: Continued.

Author (year)	Type of study	Sample	Parameter evaluated	Treatment	Results	Statistic
Irani et al. (2015) [36]	RCT	61 + 74, 3 to 6 months	OHRQoL, periodontal status, OHIP-49	Nonsurgical periodontal therapy	T2DM does not impact on overall OHRQoL as measured by OHIP-49	there were significantly higher OHIP-49 scores (indicating poorer OHRQoL) in patients with gingivitis and periodontitis
Peer et al. (2014) [37]	RCT	/	Osteonecrosis of the jaw	Medication	Genetic predisposition for MRONJ, coupled with CYP 450 gene alterations, has been suggested to affect the degradation of medications for DM	/
Fontanari et al. (2014) [38]	review	/	Different implant surfaces on diabetic patients	/	It can be concluded that although the benefits of surface modifications present in individuals with diabetes have biological plausibility, there is little evidence of the benefits of these modifications	No significance
Cairo et al. (2001) [39]	Review	/	Periodontal disease	/	Diabetes mellitus is an important risk factor for periodontitis	/
Al-Zahrani et al. (2011) [40]	Review	/	Halitosis status, HbA1c	/	The results of this study suggest an association between halitosis and increased levels of HbA1c	P=0.03
Domanico et al. (2015) [41]	RCT	68	Reactive oxygen species (ROS)	Antioxidant supplementation	Reduction of ROS levels in patients with NPDR thanks to antioxidant therapy	P<0.001

Table 3: Continued.

Author (year)	Type of study	Sample	Parameter evaluated	Treatment	Results	Statistic
Semba et al. (2014) [42]	RCT	24 for 6 weeks	Peripheral arterial tonometry, serum and urine carboxymethyl-lysine (CML), inflammatory mediators (interleukin-6, C-reactive protein, vascular adhesion molecule-1, and tumor necrosis factor-α receptors I and II), soluble receptor for advanced glycation end products (AGEs), and endogenous secretory receptor for AGEs	High or low AGEs diet	A high- or low-AGE diet had no significant impact on peripheral arterial tonometry or any inflammatory mediators after 6 wk of dietary intervention	\
Orban et al. (2014) [43]		24 months	Peripheral blood immune cell subsets (CD4, CD8-naive, memory and activated subsets, myeloid and plasmacytoid dendritic cells, monocytes, B lymphocytes, CD4(+)CD25(high) regulatory T cells, and invariant NK T cells)	Costimulation modulator	The findings show that the quantification of CM CD4 T cells can provide a surrogate immune marker for C-peptide decline after the diagnosis of type 1 diabetes	/

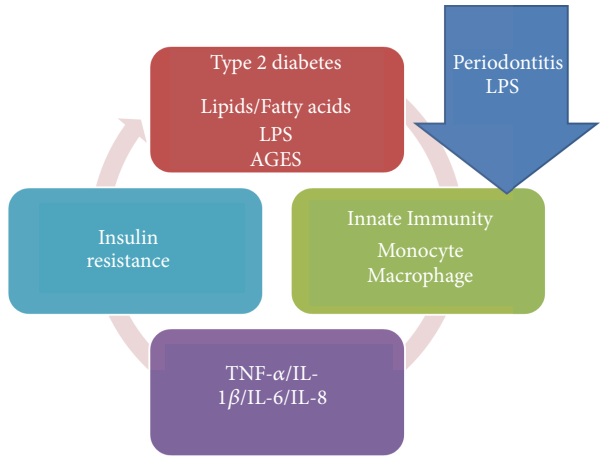

Figure 2: Immunological diabetes aspects and periodontitis.

organism, also having psychological and social implications. In this article, we have considered the alterations caused by diabetes both at the clinical and immunological levels, while evaluating the alterations on the quality of life of the patients. Cortelli et al. in their study evaluate the impact of gingivitis treatment on oral health and quality of life (OHRQoL). This treatment can improve quality of life and emphasize the relevance of periodontal care for individuals' daily life [27]. Another study shows that treatment intensification, because of diabetes, does not affect adversely patient well-being. Insulin use at entry was associated with longer diabetes duration, worse glycaemic control, and a greater risk of chronic complications; it could influence health status and QoL [28]. Health coaching and health education by dentists, physicians, and diabetes educators in order to improve quality of life is significantly reported [29]. Tzanetakos et al. evaluated the differences, from a cost point of view, about diabetes therapy with insulin Glargine, Liraglutide 1.2mg and exanatide once weekly [30]. Islam et al. evaluated the potential to communicate with diabetes patients and to build awareness about the disease using short message service and monitoring HbA1c and quality of life [31]. Through a diabetes treatment satisfaction questionnaire Vora et al. in their study evaluated differences between glargine/glulisine once daily or insulin aspart/aspart protamine [32]. Evaluation about quality of life and public health costs on diabetic patients with the use of antimicrobial photodynamic therapy (aPDT) and ultrasonic periodontal debridement (UPD) did not show additional benefits for the treatment of chronic periodontitis [33]. Goodson et al. evaluated salivary glucose concentration and other oral factors, like dental caries and gingivitis on diabetic patients. High salivary glucose, in this study was associated with a reduction in overall bacterial load and alterations to bacterial frequencies in saliva on 8173 patients [34]. Another cohort study on 22009 patients, with 3-year follow-up, evaluated periodontitis, dental caries, and peri-implant pathology with exposure to systemic condition prevention. This study reported a high prevalence for oral disease with smoking habits [35]. Oral Health Related Quality of Life, periodontal status, and Oral Health Impact Profile-49 were performed in a Irani et al. study. After nonsurgical periodontal therapy, there was a reduction of gingivitis and periodontitis associated with reduced OHRQoL, with improvements on oral soft tissue health [36]. There is an evaluation about osteonecrosis of the jaw and diabetes during medication. There is a genetic predisposition for MRONJ coupled with CYP 450 gene alteration [37]. Different implant surfaces do not influence implant survival rate on diabetic patients [38]. Cairo et al. evaluated periodontal disease in diabetic patients, showing how diabetes mellitus is an important risk factor for periodontitis [39]; this work was considered, although it did not fall as a year, because it brought an important contribution to our work. There is a correlation between halitosis, HbA1c, and diabetes [40]. The evaluation about reactive oxygen species after antioxidant supplementation evaluated a reduction of ROS levels in patients [41]. Semba et al. in their study showed that high and low AGEs diet did not cause alteration in inflammatory mediators like interleukin-6, C-reactive protein, vascular adhesion molecule-1, and tumor necrosis factor-α receptors I and II (Figure 2) [42]. Peripheral blood immune cell subsets after costimulation modulator show that the quantification of CM CD4 T cells can provide a surrogate immune marker for C-peptide decline after diagnosis of diabetes [43]. There are associations between systemic disease and oral alteration like in idiopathic arthritis or other syndromic disease; so these patients need to be attentionated with preventive diagnostic methods (Figure 3) [44–48]. There are no alterations finding about reconstructive technique on these patients [49, 50]. Other studies in literature presented anomalies about peri-implant soft tissue and systemic disease [51]; the approach by the medical staff to these patients is also important, and they can often go against social problems [52].

5. Conclusion

In this study, therefore, we analyzed the disease from a general point of view. We evaluated local and systemic

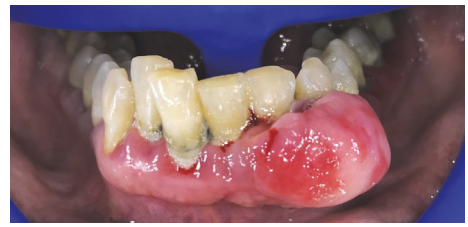

Figure 3: Periodontal disease in diabetic patient, complicated with benign neoformation. This type of lesion, invalidating, also affects the quality of life of the patient, with permission from Dr. L. Fiorillo, 2018.

complications, and we focused on how this disease affects patients' lives. Surely, there is a lowering of the quality of life reported by patients; this is quite clear from the work we have considered. This lowering of quality of life is often related to local or systemic complications of the disease. We must also remember that there is a close connection between this pathology and the onset of pathologies of psychological interest of the patient. Going to oral alterations, there are clear correlated evidences between diabetic patients and periodontal disease; moreover, this pathology affects the healing of oral surgical wounds in a negative way. Immunological and immunological implications are also evident. There are changes in the proinflammatory cytokines, which still affect healing and the health of oral tissues. In conclusion, we can state that these patients should be further treated, compared to healthy patients, going to evaluate their conditions at 360 degrees in case of oral rehabilitation.

Abbreviations

OHRQoL: Oral Health Related Quality of Life
MRONJ: Medication-related osteonecrosis of the jaw
HbA1c: Glycated Haemoglobin
QoL: Quality of Life
ROS: Reactive oxygen species
OHIP: Oral Health Impact Profile.

Conflicts of Interest

Authors declare no conflicts of interest.

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