MICROBIOLOGICAL ASPECTS IN PERIODONTAL DISEASE AND DIABETES MELLITUS

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Abstract

Background and Aims. Scientists are constantly showing a high interest for the relationship between Periodontal Disease (PD) and Diabetes Mellitus (DM). PD, “the sixth complication” of DM is recognized to be a chronic gram-negative anaerobic infectious disease. This paper is aimed at reviewing and evaluating the correlations between PD and DM from a microbiological point of view. Treatment implications of PD’s management as an important component of DM care is reviewed in the light of microbiological current knowledge. Materials and Methods. Microbiological studies and clinical trials were selected from medical and dental journals, and studied thoroughly. Results. Plaque biofilm and prolonged hyperglycemia increase the risk of PD development in DM. These two features determine inflammatory reactions that end-up in tissue destruction and impaired healing responses. Few pathogens are considered highly prevalent periodontal pathogens, with destructive actions. Studies have shown that metabolic balance or lack of balance determines bacterial variations in diabetic patients with PD. Other results demonstrate the importance of microbial tests (especially PCR techniques) as indicators for healing or disease progression. Conclusions. There aren’t many studies assessing the relationship between PD and DM from microbiological points of view. In light of increasing evidence, larger interventional studies are needed.

key words: periodontal pathogens, polymerase chain reaction, biofilm, glycated hemoglobin, hyperglycemia

Background and Aims.

Society is constantly undergoing a rapid development process associated with both positive and negative outcomes. One of the negative ones is the increased spread of several diseases, such as Diabetes Mellitus (DM) and Periodontal Disease (PD), two of the most prevalent human pathologies which can be frequently found developing concurrently in many individuals. Scientists are consequently
showing a high interest in the bidirectional relationship between these two disorders.

PD is a chronic gram-negative anaerobic infectious disease [1]. This pathology is considered to be the sixth complication of DM [2,3].

The Center for Disease Control and Prevention (CDC) conducted a recent study through which researchers reported that almost half (47.2%) of the American adults over the age of 30 present some form of periodontitis. Higher prevalence (70.1%) of PD is seen in people over 65. Regarding the association with type 2 DM, findings of the United States Third National Health and Nutrition Examinations Survey (NHANES III), revealed that the prevalence of DM among people with PD was around 12.5%. Only 6.3% of the participants having a healthy periodontal status, reported the presence of DM. [4]

The association between chronic inflammation and PD leads to soft tissue and bone structure weakening, ending up in tooth loss, but it has become more apparent that PD has a large impact on the rest of the human body [5].

DM is characterized by an impaired glucose metabolism [6] determined by decreased insulin secretion and/or by reduced insulin sensitivity (insulin resistance- IR) of different tissues, especially the muscular, the fat and the hepatic tissues. Consecutive to the hyperglycemic state, alterations in the proteic, lipidic and hydro-electrolithic metabolism appear, determining a complex imbalance in the energetic metabolism of the human body. As stated, the most common form of diabetes is Type 2 DM (90-95% of all diabetic patients). Scientists think it is associated to a genetic defect of insulin secretion or acquired glucose intolerance. This type of DM includes and is characterized by the association of insulin deficiency with IR [7].

It is also known that the innate immune system brings a big contribution to the development of Type 2 DM. [8]

Type 1 DM includes 5-10% of all diabetic patients. It is caused by a destructive process of pancreatic β cells and exogenous insulin administration is mandatory for patient survival. Type 1 DM can be autoimmune or idiopathic [9].

During the last years, a growing interest has been addressed to the microbiological aspects of the strong relationship involving PD and DM. The present paper is aiming to review and evaluate the correlations between PD and DM from a microbiological perspective. A comprehensive search of microbiological, experimental studies and clinical trials from various medical and dental databases, journals and books has been conducted and identified a large number of reports regarding the DM-PD relationship. These studies covered both type 1 and type 2 DM and helped providing consistent evidence regarding the prevalence, incidence, extent, severity and progression of PD manifestations in DM patients.

The role of metabolic control in DM and PD

DM is recognized to be a major health issue worldwide. Long-time researches proved DM to be associated with tooth deprivation. It has been stated that diabetic individuals present a three times greater risk of developing PD compared to non-diabetic population [5].

Once PD is installed in DM patients, there can be observed: gingival inflammation, higher levels of plaque and calculus and, when present, deeper periodontal pockets [10].

Its complications can lead to high morbidity and premature mortality. In the most recent classifications of PD, DM-associated gingivitis is included as a specific entity. In adults with type 2 DM, it is believed that gingival
inflammation may occur in higher rates compared to non-diabetic patients. Unlike gingivitis, PD is uncommon for example in children younger than 12, even in those with type 1 DM. In adolescence, PD occurs but the attachment loss is usually minimal [10].

Fig. 1 Periodontal status of diabetic patient (S.A) with imbalanced metabolic control (case from personal archive).

Fig. 2 Large amount of plaque and calculus in diabetic patient (S.A) with imbalanced metabolic control (case from personal archive).

Fig. 3 Radiographic evaluation of diabetic patient (S.A) with imbalanced metabolic control and generalized periodontal disease (case from personal archive)

Metabolic control and PD treatment share similar platforms being always in need of a high degree of patient compliance [11]. A large number of clinical studies connect severe
periodontitis with DM and especially with the lack of metabolic control [12,13].

Correlations between glycated hemoglobin (HbA1c) levels and DM chronic complications have been demonstrated through several clinical studies developed on patients with both type 1 and type 2 DM.

Fig. 4a, 4b Aggressive generalized periodontitis in 25 year old type I diabetic patient (Z.R) with imbalanced metabolic control. Duration of diabetes – 6 years. HbA1C value – 14%, and rethinopathy (case from personal archive).

Fig. 5 Radiographic evaluation of diabetic patient (Z.R.) with imbalanced metabolic control, diabetes micro-vascular complications and aggressive generalized periodontal disease (case from personal archive).

The major role that HbA1c fluctuations may have upon long-term complications development has been also highlighted. The variability of HbA1c in contrast to glucose variability may rise the risk of nephropathy and rethinopathy to develop in type 1 diabetic patients. [14]

Glycemic status is very important for the wellbeing of periodontal cells. This statement is sustained by the known fact that periodontal cells are highly susceptible to variation and rapid fluctuation of glucose levels. It has also been concluded that both hyper- and hypoglycemia can directly perturb the biological fluctuations of “periodontal connective tissue via cell-matrix interactions” [15].

Around 600 bacterial species colonize the oral cavity and during some specific conditions, can affect the delicate balance of host bacterial interactions. The result can lead to health or disease.

PD is usually initiated by the sub-gingival microbiota. Researchers accept that connective tissue breakdown mediators are generated by the host’s response to pathogenic infection. [16,17].
Microbiology in periodontitis – implications in DM cases.

65% of systemic diseases are biofilm induced pathologies. The biofilm is the main way of bacterial development in nature and is represented by a complex community of bacteria that adheres to an inert or alive surface. The main cause of persistent infection, the biofilm is a structured community of bacteria arranged into micro-colonies surrounded by a protective polymeric inter-microbial matrix that is being penetrated by various channels with the role of conducting food, enzymes, metabolites and oxygen towards bacteria. It possesses a peripheral aerobic medium and an anaerobic one in the center of the micro-colony. Communication among biofilm microorganisms is made with the help of clinical signals gathered in the Quorum Sensing System. If the inter-bacterial communication is interrupted, infection can be prevented or if installed, it can be treated. The biofilm structure restricts antimicrobial substances to penetrate so its microorganisms are 100/1000 times more resistant to antibiotics and antiseptic substances, in comparison to the planktonic bacteria of the same species.

All biofilm-caused infections have some characteristic clinical aspects: a slow, progressive and difficult to treat evolution. When present, the sub-gingival pathogenic microorganisms, indicate a biofilm alteration from saprophyte to pathogenic; microbial channels represent the consequence of pathogenic biofilm development.

The most studied biofilm is the dental one. Dental plaque represents the biofilm responsible for PD development. The aim of periodontal therapy consists in oral environment changes in order to block multiple periodontal pathogenic bacteria. Simple types of therapies using only antimicrobial substances without the use of mechanical therapy determine an inefficient treatment plan [17].

Other recent researches highlight the differences regarding the composition of the sub-gingival microbiota and the supra-gingival plaque. These are explained mainly by the local blood products availability and second because of a low oxidative-reduction (redox) potential, characteristic for the anaerobic environment [18]. Liliemark and colleagues affirm that in the deeper parts of the periodontal pocket, the microbiota changes and it is dominated by small organisms without a particular orientation [19].

The dental biofilm formation is a rapid process. It takes around 12 weeks for a mature biofilm to develop within a periodontal pocket. It is known that teeth are the primary habitat for periodontal pathogens. Studies state that not long after full-mouth tooth extraction in cases of severe periodontitis, key periodontal pathogens (Aggregatibacter actinomycetemcomitans – Aa and Porphyromonas gingivalis Pg) disappear from their intraoral habitats [20]. More recent studies that used newer microbial techniques proved that these two key pathogens may remain after full-mouth extraction at very low concentrations [21].

The determining factor of PD is the bacterial plaque, primarily composed of microorganisms. One gram of plaque contains approximately $10^{11}$ bacteria [22].

Also, in a periodontal pocket, the quantity of bacteria can range from $10^{3}$ in a healthy crevice to more than $10^{8}$ in a deep pocket as shown by Carranza and collaborators. The current PD etiological concept considers three groups of factors, to determine whether a subject is prone for active periodontitis development:
- A susceptible host;
- Pathogenic species- mandatory present;
- The “beneficial bacteria” - in a small proportion or even absent [23,24].

The susceptibility of the host is only partially hereditary. It can also be influenced by
environmental and behavioral factors. Among others, DM represents an important factor [25,26].

Studies showed that a small group of pathogens is closely associated with disease status, progression and unsuccessful therapy. “Beneficial species” of the host can affect disease progression. One known and documented example is the effect of *Streptococcus sanguis*, which by producing hydrogen peroxide (H2O2), directly or by host enzyme amplification can kill *Aggregatibacter actinomycetemcomitans* [27].

**Which are the known periodontal pathogens and how were they included in this category?**

As history shows us, in the 1870’s, Robert Koch established the classic criteria (Koch’s postulates) by which a microorganism is considered to be a causative agent of human infections. As stated by Quirynen et al. [28] citing Koch’s postulates, a causative agent:
- Must be routinely isolated from diseased individuals;
- Must be grown in the lab, in pure culture;
- Must be recovered from the lesions of a diseased laboratory animal.

Due to problems that may occur in cases of periodontitis, Sigmund Socransky, researcher at Forsyth Dental Center in Boston, proposed criteria that may consider periodontal microorganisms to be potential pathogens [22,24]:
- A potential pathogen must be associated with disease $\Leftrightarrow$ having increased numbers of microorganisms at diseased sites;
- Must be eliminated or at least decreased in sites that show clinical resolution after treatment;
- Must demonstrate host response as an alteration in humoral or cellular host immune response;
- Must be capable of determining disease in experimental animal models;
- Must prove virulence factors that are responsible for enabling microorganisms to cause periodontal tissue destruction.

Based on this criteria, data found in literature support *A. actinomycetemcomitans (Aa)* and *P. gingivalis (Pg)* as having the role of periodontal pathogens [20]. Besides these two, other three microorganisms are considered to be highly prevalent periodontal pathogens: *Tannerella forsythia (Tf)*, *Prevotella intermedia (Pi)* and *Treponema denticola (Td)* [16].

In diabetic patients, high glucose concentrations in the saliva and in the gingival crevicular fluid reduce the salivary flux [29,30]. The accumulation of glucose in the crevicular fluid, observed in periodontal pockets deeper than 4 mm, determines spirochetes and bacteria increase [31,32]. The sub-gingival plaque, associated to bacteria, causes damage in both periodontal tissues and patient’s response ability [12]. Bacteriological researches showed that 70-80% of type 2 DM patients had *Pi*, *Campylobacter rectus (Cr)*, and *Pg* as the most frequent bacterial species. Another demonstrated fact that is particularly damaging in type 2 DM is the interaction between *Pg*, *Td*, *Eikenella corrodens (Ec)* and *Candida albicans* [33].

Studies concluded that in type 1 DM, *Pg* and *Pi* have a major role in the oral epithelium invasion while *Capnocytophaga’s role* is still under serious debate [34].

It has been demonstrated that in patients with DM (controlled or not), the amount of cocci and other bacteria (fusiform and filiform) in periodontal pockets < 4mm, were the same. However, in pockets deeper than 4 mm, the quantity of spirochetes and bacteria was higher in poorly controlled diabetic subjects [35].

Although several investigations and connections have been made, the role of these
microorganisms in PD pathogenesis is still not elucidated. [36].

**PD bacteria investigation techniques**

Sub-gingival microorganisms have been detected for some time now, using several methods: anaerobic culture, enzyme reaction, microscopy, DNA probe, immunohistology and polymerase chain reaction (PCR).

Much of the existing research suggests that the culture method is time-consuming and may lead to false negative or positive results. In exploring and solving these issues, the rapid advancement of molecular biological technologies facilitated PCR methods for bacterial detection. DNA-based methodology for bacteria and viruses detection and identification, has remarkable time-costs saving advantages in comparison to culturing techniques. Using the new techniques, the number of samples examined and the number of microorganisms detected has increased dramatically [37].

Researchers evaluated the role of microbial identification as an aid in treatment planning in patients with aggressive or non-responding periodontitis [38].

Early detection of bacteria associated periodontal disease development provides important information for future diagnosis and treatment. The Polymerase Chain Reaction (PCR), although not an extremely new technique, is considered to be the most powerful tool for the amplification of genes and their RNA transcripts. With the help of this technique, large quantities of DNA are obtained in a simple, automated manner. [39,40].

Scientists developed a new PCR detection method – 16S rRNA or 23S rRNA-based, in order to determine the prevalence of Aa, Tf, Cr, Ec, Pg, Pi, and Td. As stated, PCR amplification of these two bacterial genes (rDNA) is more sensitive and efficient than culturing and biochemical identification of microbial flora. In spite of these acknowledgements, the PCR method still requires the presence of >10^4 bacteria cells for positive detection. This technique can be sensitive enough to detect few DNA strands of the present microorganisms, only if the PCR conditions are optimized enough and if adequate primers are used [41].

Quantitative PCR methods have been developed due to the known importance of quantitative assessment of bacteria. Also, real-time PCR assays are known to overcome limitations regarding diagnostic and prognostic purposes.

A study performed in 2011 at the Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, in collaboration with Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, and conducted by Didilescu and collaborators revealed that although it has originally been designed for periodontal pathogens, the PCR investigation can be successfully used in endodontic samples [42].

Previous studies revealed that the diabetic host could have increased peri-apical lesion sizes or may develop more serious infectious responses to virulent root canal bacteria. Subjects with a history of DM and also peri-apical lesions may develop significantly reduced healing after endodontic treatment compared to that of the non-diabetic population [43].

Although PCR methods are the most appropriate techniques for periodontal pathogens detection, they are still not used as usual diagnostic methods in clinic.

**Benefits of periodontal therapy**

Just like in DM case, PD requires a continuous monitoring and intervention by the dentist or periodontist. The patient plays a crucial role in disease management by
complying with a proper oral hygiene at home. So, it has been proved that patient compliance plays a key role in long term successful treatment of PD. Despite this, only 16% of patients present a 100% satisfactory level of compliance regarding the treatment plan.

Researchers have conducted studies through which they investigated the association between periodontal therapy and metabolic control improvement [43]. While some researchers state that is well known and documented those diabetic patients with periodontitis that undergo periodontal therapy may have beneficial effects on glycemic control, it is not so clear if effective control of PD is entirely associated to a concomitant improvement of glycemic control [44].

Beside the mechanical periodontal therapy, several adjuvant therapies have been developed by researchers, for periodontal infection elimination. They used systemic antibiotics as doxycycline and topical antiseptic substances as Clorhexidine and Povidoneiodine [9].

Metabolic control improvements have been detected in evaluations of different treatment plans combining scaling and root planning and systemic doxycycline therapy in type 1 and type 2 DM patients. Findings of other studies indicate that complex periodontal therapy offers a big improvement in periodontal health but a non-significant one in the metabolic control (small reduction in the HbA1c levels) [45].

These results are more accurate if patients present an advanced PD, with the presence of periodontal pathogens revealed with the help of microbiological exams and if they have had a poor metabolic control before treatment. On the other hand, subjects who have shown little positive clinical effect after periodontal therapy had no change in glycemic control [46].

Another study, this time a placebo-controlled one, was performed on patients with poorly controlled type 2 DM and severe periodontitis that underwent scaling and root planing in combination with the use of systemic doxycycline therapy for a period of 14 days. This treatment was compared to a similar periodontal treatment but combined with systemic placebo [47]. As results, all groups had significant improvements in their periodontal status, but those who underwent systemic doxycycline therapy presented a greater reduction in Pg prevalence. Three months after treatment, patients treated with doxycycline, demonstrated significant improvement in their metabolic control. Placebo-treated subjects didn’t present significant improvement in their glycemic control.

These studies prove that a therapy that combines sub-gingival mechanical debridement with doxycycline may result in metabolic control improvement in poorly controlled diabetic patients with severe periodontitis. In comparison, subjects with moderately well-controlled DM and PD which have been treated only by mechanical therapy may show no significant modifications in the metabolic control, but only improvement in their periodontal status.

Doxycycline was used, in these studies, because DM is associated with greatly elevated production of collagenase [48].

Because of all the controversies, scientists consider that further research is needed to assure clinicians that periodontal treatment improves metabolic control in diabetic patients and to establish the adjunctive benefit of host modulation therapies in diabetic population [48].

Discussions
Several studies and investigations established that the increased risk of developing PD in DM is due to the plaque biofilm and the prolonged hyperglycemic state. Both of these
two features result in a series of inflammatory reactions that, in the end, cause tissue destruction and impaired healing responses. The relationship between DM and PD, in which the negative consequences of DM serve as modifiers of periodontal pathology, was suggested by many researchers but not entirely elucidated [49]. This is why larger studies should be undertaken on diabetic patients with PD.

As stated, the glycemic status is vital for the wellbeing of periodontal cells, knowing that these cells are highly susceptible to variation of glucose levels (hyper- and hypoglycemia) [14]. 

Microbiological investigations revealed the bacterial variations that exist in diabetic patients with PD in accordance to their metabolic balance or lack of balance and the depth of the periodontal pockets. Microbial tests are very important in treatment planning, as a healing or disease progression indicator. This led to important development in molecular biology and the largely use of DNA-based techniques, as the well-known PCR technique [28]. Finally, more researches are being made to improve the quantitative PCR methods and the real-time PCR assays.

A recent meta-analysis showed that the control of PD could represent a very important part of diabetic patient management [50].

**Conclusions**

In summary it has been shown that gram-negative bacteria facilitate the infection pathway in periodontal tissues under certain pathologic conditions as DM. Periodontal cells are susceptible to variation and rapid fluctuation of glucose levels, therefore glycemic status is very important if not vital for these cells. Poor metabolic control and extended duration of a hyperglycemic state represent severe factors for periodontitis and altered host function. On the contrary, well controlled diabetic patients have a response to non-surgical periodontal therapy comparable to that of non-diabetic subjects. Microbiologic testing (PCR) serves as a healing or disease progression indicator.

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