University of Medicine and Pharmacy Craiova

Faculty of Medicine

STUDY REGARDING THE EFFICIENCY OF SYSTEMIC ANTIBIOTHERAPY IN PATIENTS WITH DIABETES MELLITUS AND CHRONIC PERIODONTITIS

Ph.D. THESIS ABSTRACT

Scientific coordinator:

Prof. Univ. Dr. Florica Popescu

PhD student:

Gheorghița Lelia Mihaela

CRAIOVA 2014

Table of contents

Key words: diabetes mellitus, chronic periodontitis, glicated hemoglobin, systemic antibiotherapy, non-surgical periodontal treatment.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder, characterized by hyperglycemia, affecting the metabolism of carbohydrates, proteins and lipids. It can be induced by lack of insulin secretion, lack of insulin action or both [Wild S, 2004]. According to the American Diabetes Association, diabetes mellitus reunites a group of heterogen metabolic disorders with a common characteristic that is hyperglycemia [American Diabetes Association, 2011]. Diabetes mellitus and periodontitis are two chronic diseases with a high prevalence among the entire population. A series of studies underline the bidirectional relationship between them [Preshaw PM, 2012; O'Dowd LK, 2010;Choi YE, 2011;Nagasawa T, 2010;Ohlrich EJ, 2010; Lalla E, 2007]. A series of biological mechanisms have been researched in order to explain the interactions between diabetes mellitus and periodontal disease. These mechanisms are similar to those explaining all the other complications of diabetes, therefore periodontal disease was considered the 6th complication of diabetes mellitus [Ribeiro FV, 2011;Santos VR, 2010].

Continuous research and protocols in modern medicine are very important in the treatment of periodontal disease in diabetic patients because it is highly important to maintain oral health in these cases on one hand, and to improve their metabolic control, on the other hand.

THE STAGE OF KNOWLEDGE

Chapter 1 presents the definition and the classiffication of periodontal diseases, the etiology and pathogeny of chronic periodontitis, the pathogenic mechanisms in periodontal disease and the risk factors that are responsable for the progression and evolution of this chronic disease. All these factors are very important in the treatment protocols, as well as in the outcome of periodontal treatment [Dumitriu HT, 2009; Eke et all, 2010; Nesse et all, 2009].

Chapter 2 refferes to the bidirectional relationship between diabetes mellitus and periodontal disease. We would like to underline that diabetes mellitus is an important risk factor for the development and progression of periodontitis, as well as the impact of periodontal disease on glycemic status of diabetic patients [Lamster IB,2008; Mealy BL, 2008; Martinez AB, 2011; Neelima S.R, 2011].

Chapter 3 describes the mechanisms of action of antibiotics used in the treatment of chronic periodontitis, classiffication of antibiotics considering their mechanisms of action and chemical structure, bacterial rezistance mechanisms and the particularities of some antibiotics administered in the treatment of periodontal disease [Thomas J. Pallasch, 2003; Herrera D, 2008; Khocht A. et al, 2012; Rafael Poveda Roda, 2007; Geisla Mary Silva Soares, 2012].

PERSONAL RESEARCH

Chapter 4 describes **The aim and the objectives** of our research regarding the efficiency of systemic antibiotherapy in chronic periodontitis in diabetic patients.

The purpose of our study was to evaluate the degree of periodontal disease in diabetic patients and to investigate the effect of non-surgical periodontal treatment associated with systemic antibiotherapy on periodontal disease and glycemic status of diabetic patients. The histological and immunohistochemical study underline the histopathological changes in gingival epithelium and chorion in diabetic patients with chronic periodontitis.

Objectives. *The first objective of our study* was to identify the patients with diabetes mellitus and chronic periodontitis, assuming that diabetes mellitus is a major risk factor in the development and progression of chronic periodontitis. We anticipated that in diabetic patients we will discover a high prevalence of periodontal lesions, from gingivitis to aggressive periodontitis.

The second objective was to establish the inter-relationship between diabetes mellitus and periodontal disease by correlating the data from the clinical study, the histological and immunohistochemical study with the levels of glycated hemoglobin and

blood sugar levels determined for each patient. We anticipated that in diabetic patients with a long evolution of diabetes and poor metabolic control we will discover a high number of cases with advanced periodontitis.

The third objective was to evaluate the efficiency of non-surgical periodontal treatment associated with systemic antibiotherapy on periodontal disease and glycemic status of diabetic patients. By treating the periodontal lesions we aim to obtain a good control of dental plaque, thus reducing the inflammation on oral tissues. We anticipated that, when treating diabetic patients using non-surgical periodontal treatment associated with systemic antibiotherapy we will obtain a significant improvement of periodontal status and glycemic status, with lower HbA1c levels when measured at 3 months and 6 months, but of course in patients that manage to maintain a good oral hygiene.

Chapter 5 describes the **Clinical study regarding the efficiency of systemic antibiotherapy in chronic periodontitis in diabetic patients.** In order to accomplish all our objectives, this chapter contains the clinical characteristics and periodontal parameters of the patients included in our study and the statistical analysis of periodontal parameters, initial values and the re-call registered values in one month, 3 months and 6 months after periodontal treatment.

Patients included included in our study were selected from a number of 115 diabetic patients from the Diabetes and Metabolism Disorders Clinic of the Emergency County Hospital in Craiova and the Periodontology Department of the University of Medicine and Pharmacy, Craiova. Our research study obtained the Ethics Commitee Aproval and each patients included in our study has been informed about the purpose of the study and has given his consent in order to participate in our research study and follow-up evaluation.

In all cases a detailed case history was compiled, including age, sex, diseases antecedents, type of diabetes, duration of diabetes, current medication, other associated systemic disorders. We selected a number of 63 diabetic patients, 11 patients with type 1 diabetes and 52 with type 2 diabetes. In all cases a clinical examination of the oral cavity was carried out and we evaluated the condition of the oral

mucosa, gingival bleeding, other irritative factors, O'Leary plaque index, papillary bleeding index (PBI), loss of gingival attachment and pocket depth. As complementary tests, all patients were sujected to a panoramic X-ray study and to determine the glycosylated hemoglobin levels (HbA1c).

In all cases non-surgical periodontal treatment was carried out: tartar removal, scaling and root planing in combination with ultrasound and local antiseptic irrigant (0,12% clorhexidine digluconate) for a total of 1-2 sessions, depending on the number of affected quadrans, within 10 days.

All teeth showing excessive mobilty or caries induced destruction not amenable to conservative dental management were extracted, and other irritative factors such as old non-adapted crowns and bridges were removed. Starting with the first visit, oral hygiene instructions were explained, including the use of manual toothbrush and inter-dental brushes and all patients were adviced regarding the importance of good oral hygiene in order to obtain and maintain a reduced level of periodontal inflammation. In smokers we underlined the importance of quitting or at least reducing the number of cigarrettes in order to obtain and maintain a good oral health.

After the mechanical therapy the patients were divided into 2 groups and adjunctive medication was prescribed as follows: the first group of 33 patients received Azithromycin 500 mg tablets, 3 tablets to administer once a day, for 3 days; the second group of 30 patients received Amoxicillin and Metronidazole in association, for 7 days (amoxicillin 500 mg/administered every 8 hours and metronidazole 250 mg/ administered every 8 hours).

One month after the treatment the first follow-up visit was scheduled and then new follow-up visits at 3 and 6 months after the treatment for clinical evaluation and determination of HbA1c.

Statistical analysis was performed using Microsoft Excel programme (Microsoft Corp., Redmond, WA, USA) with XLSTAT for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA). The data were then analysed regarding the relationship between clinical and paraclinical parameters with Microsoft Excel. The secondary analysis - the descriptive analysis,

mean and standard deviation was performed using Excel Programme, Pivot Tables, Functions-Statistical, Chart and Data Analysis. To assess if the variables were distributed normally we used Kolmogorov-Smirnov, Shapiro-Wilks şi Anderson-Darling tests statistical complexe test (Mann-Whitney-Wilcoxon and Kruskal-Wallis etc) XLSTAT module or SPSS programme.

Results

After statistical analysis we obtained a significant improvement of periodontal status and glycemic status of diabetic patients after non-surgical periodontal treatment associated with systemic antibiotherapy.

After analysing the patients included in our study group we noticed that 58,73% of the patients suffered from diabetes for more than 10 years, with a mean value of HbA1c of 9,2%, which proves a poor metabolic control.

When considering the plaque and tartar index (O'Leary) we obtained high initial values. The mean value of the plaque index was 53%, with a relative maximum frequency of 49,2% for index values between 60-74%. Tartar index had also high levels, with a mean value of 49%, all these demonstrating a poor oral hygiene of the patients included in our study.

When considering the papillary bleeding index , initially we determined a mean frequency of 52,4% for values between 1-1,2, with a relative frequency of 36,5% for values between 1,61-2,2 and a relative frequency of 11,1% for values between 2,81-3.

When considering periodontal attachment loss and probing depth in periodontal pockets, the initial mean value of gingival recession was 2,87 mm, 73% of the patients presented levels of 2 mm or higher of attachment loss. 50,79% of the patients included in our study presented periodontal pockets on the first clinical examination, 62,5% of them presenting 4-6 mm probing depth, 25% of them presenting periodontal pockets deeper than 6 mm and 12,5% presenting probing depth lower than 4 mm.

We noticed a significant difference ($p=0,0016<0,05$) between HbA1c levels in patients with short evolution of diabetes (<10 years) compared with those with long

evolution of diabetes (>10 years), long evolution diabetic patients presenting significant higher initial levels of HbA1c.

To compare the efficiency of two types of antibiotics, we compared the differences between those two using the initial values and follow-up determination for each parameter, using the Kruskal-Wallis test. To verify if there is a significant difference between the initial and follow-up values we used the Kruskal-Wallis because the date did not present a gaussian distribution, therefore we could not use the ANOVA test.

When considering the HbA1c values there was a statistical significant difference ($p=0,043<0,05$) between the inital values and the glycosilated hemoglobin levels determined in 3, respectively 6 months after the non-surgical periodontal treatment (Fig. 1).

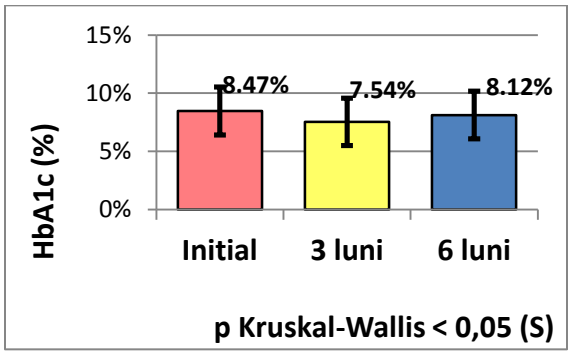

Fig. 1 HbA1c values before and after treatment

Using the Dunn method to compare the pairs of analyzed data, we determined that a statistically significant difference exists between the initial values of HbA1c and those determined after 3 months after perio-treatment. There was no significant difference between HbA1c levels measured at baseline and those determined 6 months after perio-treatment, respectively between the HbA1c values measured at 3 and 6 months after periodontal treatment.

We did not determine any significant difference between the reducing rate of HbA1c values in the two groups of patients, all patients presented a similar reduction of HbA1c levels, regardless of the type of antibiotic administered. All the patients included

in our research presented an improvement of glycemic status after non-surgical periodontal treatment with systemic antibiotherapy, with lower levels of HbA1c determined at 3 months and 6 months re-calls, although HbA1c levels determined at 6 months re-call were slightly higher, with a tendency towards the initial levels, regardless ofn the evolution of diabetes, the initial values of HbA1c or the type of the antibiotic.

After non-surgical periodontal treatment associated with systemic antibiotherapy we noticed the improvement of all clinical periodontal parameters, when measured after one month, 3 months and 6 months after periodontal treatment. Significantly better results and maintained for a longer period of time were obtained in the Azythromicin group compared to Amoxicillin+Metronidazole group.

Chapter V.5 is dedicated to **Discussions** regarding the results of our research, by analysing and comparing our data with those found in the literature. The patients included in our study did not represent a randomised romanian population group, but, nevertheless, we can gather important information regarding the oro-dental health of diabetic patients, the oral hygiene and metabolic status of diabetics. We cannot sustain the hypothesis that long evolution of diabetes mellitus is the direct cause of severe periodontal lesions, but, we can sustain that the progression of chronic periodontal disease was more rapid towards distructive and agressive formes in the presence of heavy dental plaque and tartar in poorly metabolic controlled diabetic patients.

The most significant **Conclusions** of the clinical study are:

1. Our study group consisted of 63 diabetic patients, 11 type 1 diabetics and 52 type 2 diabetics, ages between 32-84 years old with periodontal disease.
2. Diabetic patients with long evolution of diabetes, over 10 years, with poor glycemic control and poor oral hygiene presented aggressive forms of periodontal disease.
3. The evolution of periodontal disease was much more rapid and severe in poorly controlled diabetics with poor oral hygiene.

4. All clinical periodontal parameters evaluated in one month, 3 months and 6 months after periodontal treatment were significantly improved, especially in the azythromicin group.

5. After non-surgical periodontal treatment associated with systemic antibiotherapy a decrease in HbA1c levels is obseved, thus indicating improved blood glucose control in diabetic patients. The improvement of HbA1c levels was obtained regardless of the duration of the diabetic disease and also regardless of the degree of periodontal involvement at baseline. The improvement of HbA1c levels was obtained with similar results in both group of patients, regardless of the systemic antibiotherapy administered, with best results when measured at 3 months after the treatment.

Chapter 6 describes the **Histological and Immunohistochemical study** of periodontal lesions in diabetic patients. The study was performed on human tissues gathered from a total of 52 diabetic patients with chronic periodontitis. The biological material collected after tooth extractions or gingivectomy was represented by periodontal biopsy fragments.

Results. The histological study analysed the alterations in gingival epithelium and chorion using a comparative analysis according to the period of evolution of diabetes mellitus. The immunohistochemical study analysed the alterations in basal membranes, the inflammatory infiltrate in gingival chorion, emphasizing lymphocyte cells, macrophages and also the expression of matrix metalloproteinases (MMP 9).

In diabetic patients with an evolution shorter than 10 years analysing gingival epithelium we noticed different degrees of acanthosis with the elongation of the interpapillary epithelium ridges. In patients with diabetes for over 10 years the alterations in gingival epithelium were characterized more by atrophy and tendency to rectilinear epithelium-chorion limit. In diabetic patients with an evolution shorter than 10 years the blood vessels in gingival chorion frequently presented vascular ectasia with hypertrophic endothelial cells, tendency of microthrombosis and in some cases, recent microthrombi located in the capillaries of superficial chorion. In good metabolic controlled diabetic patients, especially in deep gingival chorion we noticed areas presenting granulation tissue like, with neoformation of blood vessels, turgescent

endothelium associated with inflammatory infiltrate usually consisting of lymphocytes and plasmocytes. In patients with diabetes for over 10 years we found mostly thickening of the blood vessels due to the thickening of the basal membranes. This thickening of the basal membranes is strongly related to the period of evolution of diabetes mellitus, therefore gingival bipsies may be used in the diagnosis of the evolution stages of diabetes mellitus. In diabetic patients with an evolution shorter than 10 years collagen fibers presented a reduced number, with disruptions in collagen bundles due to the inflammatory cells and edema. In patients with diabetes for over 10 years we noticed collagen sclerosis with an increased number of fibrocytes, thick bundles of collagen with perivascular disposition. In these collagen sclerosis areas we noticed hyalinisation zones with altered architecture of the collagen fibers. The interstitial collagen sclerosis process was associated with epithelium atrophy, with rectilinear epithelium-chorion limit, reduced inflammatory infiltrate and vascular sclerosis with lumen reduction in blood vessels.

In our study, as well as other studies published before, we noticed that diabetes mellitus causes the degeneration of interpapillary ridges, increases inflammatory cell migration and collagen accumulation in the gingival tissues.

Immunohistochemistry for the collagen IV showed disruptions of the basement membrane that separates the scuamous epithelium from the subjacent chorion, both in patients with diabetes shorter than 10 years and in patients with diabetes longer than 10 years, but in the latter cases the disruptions in basal membrane were more frequent even in the areas where there was no inflammatory infiltrate. In patients with diabetes for over 10 years we noticed thickening of the blood vessels walls due to accumulation of collagen IV, with lumen reduction in blood vessels. The increased quantity of collagen IV appears due to high accumulation of advanced glicosylation endproducts (AGE) in diabetics gingival tissues. The mechanism responsible for the vascular lesions at this level associated with diabetes mellitus may be oxidative stress.

High levels of MMP in periodontal tissues are responsible for the imbalance between the synthesis and the degradation of collagen, followed by alterations in periodontal tissues. In our immunohistological study we noticed that all patients with

diabetes longer than 10 years had a positive immunoreactivity in MMP 9 in all layers of the gingival epithelium, whereas, in the group of patients with diabetes shorter than 10 years only 58,33% of the patients presented this positive immunoreactivity, the rest of the patients presented a positive immunoreactivity only in the basal two thirds of the gingival epithelium. Considering that the highest positive immunoreactivity was noticed in patients with predominant PMN cells in the inflammatory infiltrate, associated with abscesses, we can confirm the theory that MMP 9 is produced mostly by PMN cells.

In the immunohistochemical study of the inflammatory infiltrate we used antibodies for the detection of T lymphocytes (anti-CD 3 and anti-CD45RO), B lymphocytes (anti-CD20cy) and macrophages (anti-CD68). In patients with diabetes shorter than 10 years we noticed mostly inflammatory infiltrate with predominant T lymphocytes (score 3 in 7 cases); in patients with diabetes longer than 10 years the inflammatory infiltrate with T lymphocytes was reduced (score 1 in 12 cases). All the cases that presented inflammatory infiltrate with B lymphocytes where characterized by subepithelial diffuse disposition of the infiltrate. The results of our study underline the presence of T lymphocytes in the gingival inflammatory infiltrate in patients with diabetic periodontal disease, regardless of the evolution of diabetes mellitus. Analysing the immunoreactivity in CD 68 antibody in patients with diabetic periodontal disease we noticed that, regardless of the evolution of diabetes, most of the cases presented a reduced number of macrophage cells (score 1 in most of the cases).

Chapter VI. 4 is dedicated to **Discussions** regarding the results of our research, by analysing and comparing our data with those found in the literature. The results of our histological research were similar to other studies, therefore we can admit there were no pathognomonic alterations in gingival tissues found in diabetic patients with chronic periodontitis. We underline the fact that the influence of AGE on the development and progression of periodontitis in diabetic patients is higher in patients with long evolution of diabetes [Zizzi A, 2013].

The most signifficant **Conclusions** of the histological and immunohistochemical study are:

1. Gingival epithelium hypertrophy is frequent in diabetic patients and most of the time is associated with altered epithelial permeability and the presence of intraepithelial inflammatory cells.
2. Gingival epithelium atrophy and parakerathosis is mostly characterising diabetic patients with a longer evolution than 10 years of diabetic disease.
3. The alterations in blood vessels in diabetic patients are found in superficial chorion and are represented by endothelium turgescent cells, vascular ectasia in diabetes shorter than 10 years, lumen reduction in blood vessels and thickening of the walls in diabetes longer than 10 years.
4. The collagen in gingival chorion is deeply altered in diabetic periodontitis regardless of the evolution os diabetes, with alterations in collagen bundles in diabetes shorter than 10 years and collagen sclerosis and hyalinisation areas in diabetes longer than 10 years.
5. The expression of MMP 9 in gingival epithelium presents a higher intensity with the longer evolution of diabetes, explaining the progressive alteration in gingival chorion matrix, while in the inflammatory infiltrate the expression of MMP 9 is maximum in cases of poor metabolic control and poor oral hygiene regardless of the evolution of diabetes.

FINAL CONCLUSIONS

1. The study regarding the inter-relationship diabetes mellitus-periodontal disease, a prospective longitudinal study contains two clinical research studies:

- Clinical and statistical analysis regarding the efficiency of systemic antibiotherapy (Amoxicillin+Metronidazole versus Azythromicin) associated with non-surgical periodontal treatment in diabetic pations with chronic periodontitis.
- Histological and immunohistochemical study in diabetic patients with chronic periodontitis.

Sixty three diabetic patienths with periodontal disease, 11 type 1 diabetic patients and 52 type 2 diabetic patients were included in our study, age betwee 32 and 78 years.

Patients were included in a research programme of health education in order to improve their glycemic and periodontal status.

2. Periodontal disease is considered the 6th complication of diabetes, both chronic diseases having a two-way relationship and influencing each other. Therapeutic control of periodontal disease may improve metabolic status of diabetic patients.

3. Periodontal status is highly influenced by the evolution of diabetes and the metabolic control. Long evolution of diabetes may influence the severity of periodontal lesions, while the aggressive forms of periodontitis have been correlated with a poor metabolic control and poor oral hygiene.

4. The initial high levels of HbA1c were correlated with aggressive forms of periodontitis.

5. All clinical periodontal parameters evaluated in one month, 3 months and 6 months after periodontal treatment were signiffically improved after non-surgical periodontal treatment and systemic antibiotherapy.

6. The efficiency of systemic azythromicin was superior compared with amoxicillin+metronidazole, maintaining better results for a longer period of time.

7. After non-surgical periodontal treatment associated with systemic antibiotherapy a decrease in HbA1c levels is obseved, thus indicating improved blood glucose control in diabetic patients. The improvement of HbA1c levels was obtained regardless of the duration of the diabetic disease and also regardless of the degree of periodontal involvement at baseline. The improvement of HbA1c levels was obtained with similar results in both group of patients, regardless of the systemic antibiotherapy administered, with best results when measured at 3 months after the treatment.

8. The alterations in blood vessels in diabetic patients are found in superficial chorion and are represented by endothelium turgescent cells, vascular ectasia in diabetes shorter than 10 years, lumen reduction in blood vessels and thickening of the walls in diabetes longer than 10 years.

9. The collagen in gingival chorion is deeply altered in diabetic periodontitis regardless of the evolution os diabetes, with alterations in collagen bundles in diabetes shorter than 10 years and collagen sclerosis and hyalinisation areas in diabetes longer than 10 years.

10. The inflammatory infiltrate in diabetic periodontitis was polimorphyc, usually consisiting of lymphocytes, plasmocytes, macrophages and PMN. In aggressive periodontitis with poor metabolic control there is a high prevalence of plasmocytes; in periodontal abscesses there is a high prevalence of PMN.

11. The immune response in diabetic periodontal disease involves different types of cells like macrophages, T lymphocytes, B lymphocytes, plasmocytes, PMN. The immune response of periodontal tissues in diabetic patients is generally characterized by the presence of chronic inflammatory infiltrate with T lymphocytes CD 3 and CD 45RO positive, followed by B lymphocytes CD 20 positive and macrophages CD 68 positive.

12. Our research theme is very important and still very actual although still needs further investigations in order to understand and improve the level of therapeutic support for diabetic patients. Dental practitioners should receive a more expanded formation in diabetes and learn to work in association with dental hygienists, dietitians and other health professionals in order to give patients a more complete interdisciplinary attention.

13. Both the results of periodontal treatment and the complications in diabetic patients depend on the follow-ups and regular visits in the dental office, reinforcement of dental hygiene, periodic scaling, and, not least, the improvement of personal oral hygiene habits of the patients.

14. In chronic periodontal lesions in diabetic patients it is necessary to use local or systemic antibiotics, but the eficiency of these therapeutic protocols must be reinforced by maintaining a very good personal oral hygiene and periodic scaling and root planing in dental offices.

15. Maintaining a good glycemic and metabolic control in diabetic patients represents the second strategic approach in the treatment of periodontal disease, together with the periodontal treatment in the specialized office and a good oral hygiene applied by the patient.

REFERENCES

1. American Diabetes Association. *Diabetes statistics*. Data from the 2011 National Diabetes Fact Sheet released Jan. 26, 2011.
2. Choi YE, McKeown RE, Mayer-Davis EJ, et al, Association between periodontitis and impaired fasting glucose and diabetes, Diabetes Care, 2011;34:381-386
3. Dumitriu HT. *Parodontologie*. Editura Viaţa Medicală Românească, ediţia a V-a, Bucureşti, 2009.
4. Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA (2010). Accuracy of NHANES periodontal examination protocols. *J Dent Res* 89:1208-1213.
5. Herrera D, Alonso B, Leon R, Roldan S, Sanz M, Antimicrobial therapy in periodontiotis: the use of systemic antimicrobials against the subgingival biofilm, J Clin Periodontol, 2008;35(Suppl.8):45-66.
6. Khocht A, T Yaskell, M Janal, BF Turner, TE Rams, A D Haffajee, SS Socransky, Subgingival microbiota in adult Down syndrome periodontitis, Periodontal Res. 2012 Aug ;47 (4):500-7.
7. Lalla E, Cheng B, et al, Diabetes mellitus promotes periodontal destruction in children. J Clin Periodontol, 2007;34:294-298
8. Lamster IB, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus, J Am Dent Assoc 2008; 139:19S-24S.
9. Martinez AB, Perez PM, Bermejo ME et al, Periodontal disease and diabetes-Review of the literature, Med Oral Patol Oral Cir Bucal, 2011 Sep 1;16(6):e722-9.
10. Mealey BL, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. Curr Opin Endocrinol Diabetes Obes 2008;15:135-141.
11. Nagasawa T, Noda M, Katagiri S, et al, Relationship between periodontitis and diabetes- importance of a clinical study to prove the vicious cycle, Inter Med 2010;49:881-885.
12. Neelima S.R, Ramesh M.K, Viren G.C, Nilkanth H.M, A clinical study of the relationship between diabetes mellitus and periodontal disease, J of Indian Society of Periodontology, 2011, vol 15, 388-392

13. Nesse W, Linde A, Abbas F, Spijkervet FK, Dijkstra PU, de Brabander EC*, et al.* (2009). Dose-response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetics. *J Clin Periodontol* 36:295-300.
14. O'Dowd LK, Durham J, McCracken GI, Preshaw PM, Patients' experiences of the impact of periodontal disease. J Clin Periodontol 2010;37:334-339
15. Ohlrich EJ, Cullinan MP, Leichter JW, Diabetes, periodontitis and the subgingival microbiota, J of Oral Microbiology, 2010;2:5818-DOI:10.3402/jom.v2i0.5818
16. Pallasch TJ, Antibiotic resistance, Dent Clin Ann, 2003, 47:623-639
17. Poveda Roda R, Bagan JV, Sanchis Bielsa JM, Carbonell PE, Antibiotic use in dental practice: a review, Med Oral Patol Oral Cir Buccal, 2007, 12:E186-92.
18. Preshaw PM, Alba AL, Herrera D, Jepsen S, et al, Periodontitis and diabetes: a two-way relationship, Diabetologia 2012;55:21-31.
19. Ribeiro FV, de Mendonca AC, Santos VR, Bastos MF, Figueiredo LC, Duarte PM, Cytokines and bone- related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. J Periodontol 2011;82:1187-1196
20. Santos VR, Ribeiro FV, Lima JA, Napimoga MH, Bastos MF, Duarte PM. Cytokine levels in sites of chronic periodontitis of poorly controlled and well-controlled type 2 diabetic subjects. J Clin Periodontol 2010;37:1049-1058
21. Soares Silva GM; Luciene Cristina Figueiredo; Marcelo Faveri; Sheila Cavalca Cortelli; Poliana Mendes Duarte; Magda Feres, Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs, J. Appl. Oral Sci. vol.20 no.3 Bauru May/June 2012, http://dx.doi.org/10.1590/S1678-77572012000300002.
22. Wild S., Roglic G, Green A, King H. Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53