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# Diabetes Mellitus Impact on Periodontal Status in Children and Adolescents

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## 1. Introduction

Diabetes mellitus is a systemic disease with several major complications affecting both the quality and length of life. One of these complications is periodontal disease. Periodontal disease (periodontitis) is much more than a localized oral infection, recent data indicating that periodontitis may cause changes in systemic physiology. The interrelationships between periodontitis and diabetes provide an example of systemic disease predisposing to oral infection, and once that infection is established, the oral infection exacerbates systemic disease. The relationship between periodontitis and diabetes has been extensively investigated over the last years, but despite of the numerous scientific studies on the influence of periodontal treatments on glycemic control, there is limited knowledge on the impact of glycemic control upon periodontal status. Moreover, the impact of periodontal treatment on sugar metabolic control in diabetics has not been fully elucidated, the present chapter intending an outlining of the features that governs the interrelationship diabetes mellitus – periodontal disease, a discussion of the present scientific evidences, mainly focusing on clinic-biological research in juvenile groups of population.

## 2. Diabetes mellitus

Diabetes mellitus represents a metabolic disease usually characterized by the classic triad of polyuria, polydipsia and polyphagia, resulted from homeostasis disruption due to impaired glucose metabolism.

### 2.1 Classification

There are two basic types of diabetes mellitus described: insulin-dependent diabetes mellitus (IDDM- type 1) and noninsulin-dependent diabetes mellitus (NIDDM-type 2). The prevalence of type 1 diabetes mellitus exhibits a wide range, with an ever increasing rate within Europe (Neubert et al., 2011). This classification does not designate exclusively the need for exogenous insulin, sometimes the hormone being also required by type-2 diabetic patients. Type-1 diabetes is produced by the destruction of insulin-producing cells, whereas type-2 results from the combination of an increase in cell resistance to endogenous insulin with a defective secretion of this substance. Diabetes mellitus consist of an even more

alarming public health problem, the prevalence of the metabolic disorder recording significant regional and ethnic variations, and a risk factor for several conditions.

### 2.2 Etiology and pathogenesis

The main pivotal mechanisms related to the etiology and pathogenesis of the diabetic complications include: 1) increased oxidative stress with excessive production of reactive oxygen and nitrogen species (Robertson & Harmon, 2006) and decreased antioxidants (Simmons, 2006); 2) the polyol pathway, resulting in toxic complications induced by sorbitol and 3) production of advanced glycosylation end products (AGEs) associated to impaired lipid metabolism. This last theory proposes that glucose binds, by non enzymatic reaction, to proteins such as hemoglobin, collagen, or albumin, determining certain complications triggered by the AGEs-released mediators. Diabetes complications, long time exclusively assigned to hyperglycemia can be equally determined by lipid metabolism impairment, characterized by serum LDL (low density lipoprotein), TG (triglycerides) and FA (fatty acids) level augmentation. Lipid imbalances may be related to monocytes function disorder, monocytes being able to elicit suppression of growth factors production, therefore expressing an inflammatory phenotype (rather than a proliferative one), consecutive stimulation by the pathogenic bacteria endotoxin (lipopolysaccharide). Moreover most of the evidences from the literature prove that higher levels of serum triglycerides induce stimulation of monocytes production of pro inflammatory interleukins on one hand, and of chemotactic and phagocytic abilities of neutrophils on the other hand (Iacopino, 2001).

## 3. Periodontal disease

Among the others cavities of the body, the oral cavity represents a distinctive ecosystem endowed with critical important biological functions, the fluids that bathes the mentioned ecosystem possessing an impressive number of components. Among the inflammatory disorders, periodontal disease-PD represents gram-negative anaerobic infections that involve tooth supporting tissues, the structures that form the periodontium (gingiva, alveolar ligament, root cementum, and alveolar bone). These alterations have mainly episodic evolution affecting first the gingiva and followed by possible secondary alteration of the surrounding connective tissue.

### 3.1 Classification

The most widely used classification was the American Association of Periodontology classification that distinguishes six categories: gingival disease, chronic periodontitis, aggressive periodontitis, periodontitis as manifestation of systemic disease, necrotizing periodontal disease, and periodontal abscess (Armitage, 1999). Actually, being no well-defined clinical criteria for the diagnosis, periodontal disease cannot be classified according only to the etiology, the designation periodontal disease including both reversible, soft form of inflammation, gingivitis, and irreversible, more extensive processes, periodontitis, tightly associated not only to the connective tissue of the tooth support destruction, but also accompanied by apical migration of the whole apparatus. It is one of the most widespread diseases in the world, the clinical importance of periodontal disease deriving partly from its very high prevalence, both in developed and developing countries. The main representative clinical manifestation of periodontal disease is the appearance of periodontal pockets, real

favorable niche for microbiological colonization, relative facile to be revealed by clinical investigation with the periodontal probe and paraclinical X-ray imaging.

### 3.2 Etiology and pathogenesis

It is well known the fact that, although necessary in initiating the state of disease, bacteria represent insufficient criteria to determine its progression in the absence of an associated immune response. Also, despite the fact that the response of the host and environmental factors are important in manifesting the state of disease, nor gingivitis, neither periodontitis can onset in the absence of bacterial triggered mechanisms (Noda et al., 2007). The inflammatory reaction in the context of periodontal disease, initiated by the accumulation of bacterial plaque, starts in early childhood and reflects the special significance of the bacterial impact on the host, in a systemic context. At most children, the inflammatory process of the gum remains superficial – at the clinic stage of gingivitis, but there are cases where the balance between the bacterial aggression and the host response is impaired, leading to destructive processes which induce attachment loss, and even lost of the teeth. Moreover, Armitage (2000) includes in his classification the pre pubertal periodontitis, juvenile periodontitis and the fast progressive forms of manifestation of periodontal disease at children and teenagers, in the aggressive periodontitis class, because of the fast progression and severe impairment of periodontal tissues. This is why, tracking down the disorder as early as possible, is essential for an early establishment of a specific therapy, but especially for preventing the installing and evolution toward more severe forms of disease. On the other hand, the inconsistency between the aggression of periodontal destruction at child and teenager and the reduced quantity of biofilm (in some forms of tooth decay), determined some scientists to claim that the bacterial challenge represents an essential condition, although not sufficient in developing periodontitis, the decisive factor being actually, host susceptibility (Tabholz et al., 2010). Today, it is well known that both genetic and contracted factors are determinants of periodontitis presence, progression and severity in adults, Pihlstrom attributing to genetic causes almost half of the risk in developing a periodontal disease during life (and probable to be revealed even during childhood).

### 3.3 Gingival crevicular fluid as a diagnostic fluid

Present in the gingival sulcus, gingival crevicular fluid (GCF) has been studied since 1955 for its diagnostic potential. Since several decades, gingival fluid reentered into the specialists' attention, its components being analyzed as non-aggressive means of host reaction examination at the periodontal level and early diagnosis of the periodontal breakdown. The gingival crevicular fluid has numerous advantages versus blood and saliva, in particular because of the ability of designation and collection of convenient samples from specific sites containing components derived both from host (in the form of plasma, cellular components, tissue of connection) and bacterial plaque. GCF can thus be considered a true "battle field" (the center of interaction host-microorganism) between the external aggressors (especially of bacterial plaque) and internal aggressors (host derived). Besides, the trend of current understanding of the periodontal pathology suggests that destruction of the periodontal tissue is modulated by host response (Van Dyke, 2009), that release products representing real periodontal destructive markers, suitable for monitoring both, within plasma and gingival fluid. The correct determination of such sensitive markers of destructive periodontium imposes itself as a need for settling the management of the disease

on more rational and less empirical bases. Gingival crevicular fluid (GCF) reflects the complexity of the host-bacteria interaction and offers information, referring not only the equilibrium between the infected germs and the host, but also specific dates concerning involved pathogenic mechanisms (Champagne et al., 2003). Therefore, GCF that reflects both, these influences at the systemic and host level on one side, and the local modulation of these responses following specific bacteria interactions on the other side, appears as a representative biologic sample for searching these indicators and predictors of the bidirectional interplay diabetes-periodontal disease.

## 4. Study approach

The anatomic and functional particularities of the marginal periodontium in child and adolescent, the variety of clinical expression of disease, and also the heterogeneity of etiology and the complexity of the pathogenic mechanisms, make the periodontal disease in child and adolescent to keep being a subject with many unknowns, interesting both the researchers and also practitioners. The lack of concordance between the aggression of periodontal destructions in child and adolescent and the amount of bacterial plaque in some forms of periodontal disease determined a series of researchers to state that bacteria, although absolutely necessary for developing the periodontal disease, are insufficient for developing periodontitis, thus susceptibility of the host being also involved (Kinane et al., 2007). Therefore, the prevalence, onset, progression and especially pathology of periodontal diseases can be modified by numerous endogenous factors. Soluble chemical mediators (prostaglandins, cytokines) or enzymes, sharing significant expression on the oral fluids level, are important in evaluation of the metabolic response within the active stage of the disease. The dental plaque-mediated inflammatory reaction onset within the periodontal breakdown takes place in early stages of childhood, and reflects the important signification of bacterial impact upon the host tissues within systemic context. In most of the children, the gingival inflammation remains superficial, but sometimes further destruction occurs, with loss of periodontal attachment. Over the last years, there has been an emerging interest in the bidirectional relationship diabetes mellitus and oral health. Postulated as a disruption in homeostasis of glucose metabolism, type 1-diabetes is often associated to periodontal disease, inflammation representing the common pathophysiologic feature.

### 4.1 Objectives

Starting from the alarming 2008 World Health Organization reports concerning the continuously increasing incidence of insulin dependent diabetes mellitus in the juvenile population, we focused much of our attention on the binomial relationship between IDDM and periodontal disease within this age group of individuals, considering both the potential of investigation and prevention of this malady and its complications within the juvenile population. The main preoccupations of the present research targeted the following aspects: a. study of the periodontal pathology in child and adolescent, through determination of the role and diagnostic value of certain cytokines determination, within the complex program of identification, evaluation and treatment of the patients with periodontal disease and unaffected general state (control group) and systemically affected individuals; b. analysis of impact on periodontal breakdown pathogenesis of the interleukins IL-1β, IL-2, IL-10 and interferon gamma (IFN-γ), and their expression as potential indicators or predictors of diagnostic and evolution of periodontal disease in systemic context.

### 4.2 Materials and methods

#### 4.2.1 Subject population

The evaluation was carried out on 84 subjects, age 6 – 18 years, divided into two groups, both with several degree of periodontal alteration: 42 non-diabetic subjects who did not suffer from any systemic disease (control group), and 42 IDDM subjects. The subjects were evaluated and divided into subgroups, according to the prepubertal (6-10years old), pubertal (11-14 years) or juvenile age (15-18 years old), and metabolic control of the disease. The diabetic group enrolled in this study comprised half well-controlled (glycosylated hemoglobin levels ≤7%) and half poorly controlled (glycosylated hemoglobin levels >7%). All subjects were submitted blood collection, GCF sampling and clinical periodontal index evaluation. Data on blood glucose, lipid profile and glycosylated hemoglobin (HbA1c) were collected from the medical records. Considering the bivalent nature of the relationship between DM and PD, the evaluation of the gingival fluid comprised records of several immune-chemical inflammatory mediators: interleukin 1β – Il-1β, IL-2, IL-10 and IFN-γ, in parallel with serum mediator determinations. Total amounts and concentrations of serum and gingival crevicular cytokines were analyzed by enzyme-linked immunosorbent assay and flow cytometry. Diabetic patients were recruited from the Metabolic and nutrition diseases department of the University Children Hospital "Sf. Maria" Iasi, and selected based on the following criteria: aged between 6 and 18 years old, diagnosed with type 1 DM. Patients were excluded if they had non-type 1 diabetes, any inflammatory diseases, liver or renal impairment (depending of the blood creatinine levels), a periodontal treatment in the last 6 months prior to the assessment, any severe pathology of the teeth or were receiving medication that could influence the studied parameters (corticosteroids, antibiotics). The age matched control group was selected among the non-diabetic individuals that followed regular treatment in the dental unit of the Pediatric Dental Clinic. Informed consent was obtained in all cases, the local ethics committee approving the protocol deemed to conform to the guidelines issued in the Helsinki Declaration.

#### 4.2.2 Clinical study design

Periodontal status was assessed by clinical evaluations of plaque index (PI), papillary bleeding index (PBI) and clinical attachment loss (CAL), and correlation with the degree of metabolic control (levels of glycemia and glycosylated hemoglobin). The mentioned periodontal parameters were evaluated in a randomized half mouth examination on six sites of each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) by a calibrated examiner. The level of oral hygiene was estimated with a plaque index – Quigley Heine index (based on the score from 0 to 5) (Silness & Löe, 1964). The scores of the plaque index were calculated according to the formula: per person = sum of individual scores/number of teeth present for each person, subsequently, the group scores being subtracted. Other clinical records consisted of papillary bleeding index evaluation, based on gentle probing and clinical attachment loss determinations of the total teeth in the mouth by periodontal probe exploration. PBI score (Saxter and Muhleman) was recorded based on four different grades of bleeding intensities subsequent to careful probing. Subsequent of completed probing, the bleeding intensity was scored in four grades: grade 0 = no bleeding; grade 1 = appearance of a single bleeding point; grade 2 = a fine line of blood or several bleeding points become visible at the gingival margin; grade 3 = blood filled interdental triangle; grade 4 = profuse bleeding consecutive probing. The bleeding value was given by the sum of the recorded scores and PBI by dividing the bleeding value to the total number of

examined papilla. CAL, the distance from the cementoenamel junction to the base of the periodontal pocket, a measure of the amount of alveolar bone lost due to periodontal disease, was measured to the nearest millimeter using a North Carolina periodontal probe. Measurements of 1–2 mm were considered to be slight, 3–4 mm moderate, and ≥5 mm severe (Costa et al., 2007).

#### 4.2.3 Gingival crevicular fluid and serum sampling

Collection and analysis of GCF represent noninvasive methods for the evaluation of host response in periodontal disease. Gingival crevicular fluid samples were obtained from the mesiobuccal site of every tooth (excluding third molars) from two randomly selected contralateral quadrants. Consecutive plaque evaluation and following isolation of the site with cotton rolls, supragingival plaque was removed, and the tooth air dried. GCF sample was collected on periopaper strips (Periopaper®, Amityville, NY) gently inserted 1–2 mm subgingivally, into the periodontal pocket. Gingival fluid volume was assessed using an electronic device, Periotron 8000® (Oraflow Inc., Plainview, NY). Collected samples were immediately placed into sterilized plastic tubes on ice, shipped to the laboratory and stored at −80°C till the day of determination. GCF samples were always collected prior to clinical measurements and samples contaminated with blood were discarded. Using the venipuncture technique, approximately 5 ml of venous blood was also drawn from the antecubital vein, using the vacutainer system (Becton Dickinson, NJ, USA), and analyzed for the lipid and carbohydrate metabolic profile. The degree of metabolic control was evaluated considering the glycosylated hemoglobin values (HbA1c), measured by high performance liquid chromatography (HPLC). Good metabolic control was taken to be represented by HbA1c ≤ 7%, while poor control was defined as HbA1c > 7%, (American Diabetes Association), normal values being considered for HbA1c < 6%.

#### 4.2.4 Measurement and quantification of cytokines using multiplex cytometric bead array

Serum and local gingival fluid cytokine levels were determined using the high sensitivity human CBA cytokine multiplex (Cytometric Bead Array®, BD Pharmingen, San Diego) for flow cytometry. Prior to assay, GCF samples were eluted into 50 µl of the assay buffer by vortexing for 30 minutes and further 10 minutes centrifugation at 8,000 rpm. Flow cytometry is an investigation method that allows various cells sorting according to size, granularity, and specific markers expression. Cytometric investigation of cytokines has substantial advantages compared to ELISA immunoassay method, allowing simultaneous detection of multiple cytokines, fast and with very small sample volumes (50µl). CBA kit contains microspheres coated on the outside with anti-cytokine monoclonal antibodies. Each type of microsphere has a characteristic fluorescence level detectable on third channel (FL 3) of the cytometer. Detection of cytokine amount is performed through the second category of anti-analyte antibody, marked with a fluorescent protein, phycoerythrin, whose fluorescence is detectable on channel FL 2. The FACS Caliber (BD Biosciences, San Jose, CA, USA) monitors the spectral properties of the beads to distinguish the different antigens, simultaneously measuring the amount of fluorescence associated with phycoerythrin and reported as median fluorescence intensity. The concentrations of the assessed cytokines were estimated using a standard curve obtained following the manufacturer's instructions, by testing standard samples included in the kit and expression as pg/ml.

#### 4.2.5 Data analysis and statistics

Periodontal parameters of subjects according to the age group were described by means of standard deviation and analyzed by analysis of variance (ANOVA).

Clinical data were collected from 6 sites per tooth for visible plaque, papillary bleeding index, and CAL. The levels of each inflammatory mediator were measured for up to 14 GCF samples per subject and expressed as pg/ml. Interactions between variables were studied using Pearson's correlation. The Mann–Whitney *U* test was used to compare values between groups. Paired non-parametric (Wilcoxon) *t* tests established significance for cytokine level within gingival fluid and serum from the same individual, while $p<0.05$ established significance.

### 4.3 Results

The association between DM and periodontal disease has been debated over decades, with conflicting conclusions. Most of the recent studies tend to support a higher prevalence and severity of periodontitis in diabetic adult patients, less literature data being available in what concerns insulin dependent diabetes upregulation of periodontal breakdown in children and adolescents. It has been shown that diabetes strongly influences the production of inflammatory mediators, cytokines and chemokines (Joo & Lee, 2007) resulting in abnormal immune inflammatory reaction and tissue injury in patients with periodontitis. Periodontal disease represents a group of alterations with episodic evolution that affects gingiva and could secondly alter the surrounding connective tissue.

The main goal of the present study was to examine the interplay between the local and systemic cytokine profile, and immune-inflammatory mediated clinical response, in systemically healthy and insulin dependent diabetes mellitus young subjects. In order to achieve this goal we employed flow cytometric techniques to characterize the levels of some pro and anti inflammatory cytokines both in serum and gingival fluid. In addition, the study tempted to reflect the clinical changes in the oral health within children and adolescents with type 1 diabetes mellitus, to assess the rate of gingival inflammation accompanying the systemic disorder and to contribute to the incidence data on periodontal disease for groups of patients where factors attributable to aging are not confounding variables.

The investigation was carried out on 84 subjects age 6-18 years, divided into two main groups: The first group (diabetic group) consisted of 42 subjects with type 1 diabetes mellitus diagnosed with marginal chronic periodontitis (n=6), aggressive periodontitis (n=6) and gingivitis (n=30). The diabetic group was subdivided according to the level of metabolic control, into well control diabetes, glycosylated hemoglobin levels ≤7% (n=22), and poor control diabetes with glycosylated hemoglobin levels > 7% (n=20). In the second group (non diabetes group=ND), there were 42 age-matched subjects who did not suffer from any systemic disease, most of them experiencing the mildest form of periodontal breakdown, gingivitis (n=36), followed by marginal chronic periodontitis (n=4) and aggressive periodontitis (n=2).

#### 4.3.1 Statistics on periodontal breakdown in the two groups

After setting up lots of study, analysis of parameters related to distribution, diagnosis and age reveals the highest prevalence of gingivitis, the mildest form of plaque-induced inflammatory disease (85,7%), followed by breakdown of the superficial periodontal support (chronic superficial marginal periodontitis) (9,5%) and aggressive periodontitis

(4.8% ) in the non diabetes group. Considering the diabetes group, there were different incidences of periodontal disease in the two subgroups: children and adolescents with good metabolic control (n=22) displayed generalized bacterial gingivitis in a proportion of 81% (n=18) and 19% aggressive periodontitis (n=4), while in the poorly controlled diabetes group, besides bacterial gingivitis (60%, n=12), 30% were diagnosed with chronic superficial marginal periodontitis (n=6) and 10% with aggressive periodontitis (n=2). Thus, despite of some previous results that founded no significant correlation between gingival condition and glycosylated hemoglobin levels (De Pomereau et al., 1992), our data suggest that at young ages, there is a higher incidence and severity of periodontal breakdown in poorly controlled diabetes, where the incidence rate increases after puberty and continuously increases by age, with an overall elevation in resorption of the bone and epithelial attachment, and predisposition to infection.

### 4.3.2 Oral hygiene levels

The level of oral hygiene was assessed for all the patients by plaque index evaluation (Quigley Heine index), based on the score from 0 to 5. Our results highlighted high incidence of values in the 2-3 range for the non diabetes group compared with the distribution of values in other groups. In the analysis presented in figure 1, statistical indicators display a high average of plaque index in poorly controlled diabetic patients (3.293 ± 1.06) compared to mean values calculated for the non diabetes group (2.995 ± 0.58) and individuals with well controlled diabetes (2.881 ± 0.857), respectively. Standard deviation registers the minimum value for the nondiabetics (SD = 0.58) while for the group with poorly controlled diabetes, standard deviation reaches a maximum value (SD=1.06).

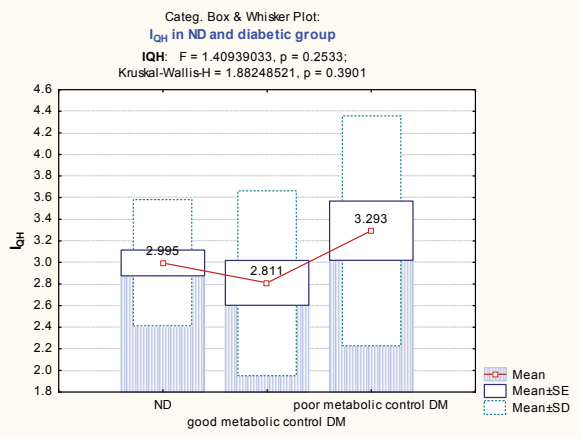


Fig. 1. Mean values of Quigley Heine Index in nondiabetics (ND), good control- and poor control DM groups.

ANOVA test used to compare by analysis of variance the mean plaque index values corresponding to studied groups, highlights the significant difference between mean values corresponding to the groups and subgroups ($p = 0.013$, $p<0.05$). Significant difference exist also between the values corresponding to the ND and poorly controlled DM individuals, the significance level (p) corresponding to 95% confidence interval being 0.0451. Moreover, a

statistically significant difference is record between the plaque index values within the two diabetes subgroups: good control versus poor control DM, p=0.003 (p<<0.05, CI=95%).

| QH Index / Group | Mean | Std. Dev | Min | Max | Q25 | Q50 | Q75 |
|---|---|---|---|---|---|---|---|
| **Pre pubertal age: 6-10 years** | | | | | | | |
| ND | 3.176 | 0.500 | 2.500 | 4.133 | 2.830 | 3.058 | 3.50 |
| Good control DM | 3.132 | 0.736 | 2.000 | 4.000 | 2.660 | 3.500 | 3.50 |
| Poor control DM | 2.830 | 0.626 | 2.000 | 3.660 | 2.330 | 2.830 | 3.33 |
| **Pubertal age: 11-14 years** | | | | | | | |
| ND | 2.734 | 0.445 | 2.330 | 3.660 | 2.330 | 2.660 | 3.00 |
| Good control DM | 3.125 | 0.888 | 1.833 | 4.133 | 2.660 | 3.000 | 4.00 |
| Poor control DM | 3.076 | 0.884 | 1.833 | 4.133 | 2.330 | 3.080 | 4.00 |
| **Juvenile age: 15-18 years** | | | | | | | |
| Martor | 3.036 | 0.668 | 2.166 | 4.000 | 2.500 | 3.000 | 3.66 |
| Good control DM | 2.356 | 0.679 | 1.500 | 3.500 | 1.833 | 2.000 | 3.00 |
| Poor control DM | 3.925 | 1.207 | 1.833 | 5.000 | 3.660 | 4.133 | 5.00 |

Table 1. Statistical indicators of Quigley Heine Index for studied groups, according to age.

Quigley Heine Index can be properly evaluated in the studied groups taking into account the patient's age. The maximum standard deviation was found in the group of patients aged 15-18 years (juvenile period), significant differences being registered in this group between average values of nondiabetics and diabetics (table 1). Maximum values (QHI= 5) were recorded for the juvenile group (15-18 years old), in patients with poorly controlled IDDM. Considering the age intervals, comparative statistic studies of the oral hygiene parameter highlight the most significant differences among the juvenile age individuals from all studied groups (p<<0.05).

### 4.3.3 Periodontal status

**Papillary Bleeding Index (PBI)**

Bleeding index shows differences values in the two populations. Thus, the non diabetes group stands 0.5 minimum and 2.66 as maximum values, lower than those for patients with poorly controlled diabetes (PBI min = 1, max = 4.66). Large variations recorded among the bleeding index values in the group with poor metabolic control are also highlighted by the large standard deviation (SD = 0.97).

As displayed in figure 2, the average PBI in poorly controlled diabetes is 2,964, almost two times higher than in the non diabetic group (PBI = 1.56) and 1.75 times higher than in the well balanced diabetic disease group (PBI = 1.69), p<<0.05. Statistic analysis reveal no significant differences between PBI values of the systemically unaffected population and

diabetic subgroup with good metabolic control (p = 0.58), while significant differences are registered between the two subgroups of diabetics (p = 0.000018, p<<0.05).
Statistic analyses on PBI correlated to age stages highlights minimum values in the ND group within juvenile age ($PBI_{min}$=0.50) and maximum values ($PBI_{max}$=4.66) recorded in the prepubertal age group of patients with poorly controlled diabetes (table 2).

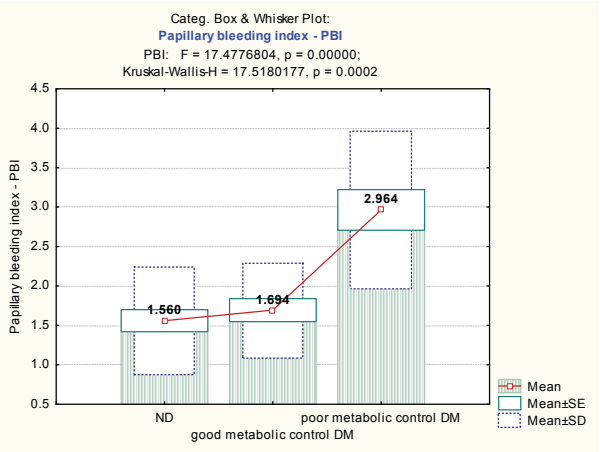


Fig. 2. Mean papillary bleeding index (PBI) in the non diabetes group, poorly controlled and good metabolic controlled diabetic children and adolescents.

| PBI / Group | Mean | Std. Dev | Min | Max | Q25 | Q50 | Q75 |
|---|---|---|---|---|---|---|---|
| **Pre pubertal age: 6-10 years** | | | | | | | |
| ND | 1.914 | 0.472 | 1.330 | 2.660 | 1.580 | 1.830 | 2.250 |
| Good control DM | 2.130 | 0.899 | 1.330 | 3.660 | 1.660 | 2.000 | 2.600 |
| Poor control DM | 3.039 | 1.187 | 2.000 | 4.660 | 2.165 | 2.748 | 3.913 |
| **Pubertal age: 11-14 years** | | | | | | | |
| ND | 1.235 | 0.593 | 0.660 | 2.330 | 0.660 | 1.330 | 1.500 |
| Good control DM | 2.650 | 0.850 | 1.330 | 3.660 | 2.600 | 2.660 | 3.000 |
| Poor control DM | 2.775 | 0.777 | 2.000 | 3.660 | 2.000 | 2.665 | 3.660 |
| **Juvenile age: 15-18 years** | | | | | | | |
| ND | 1.499 | 0.819 | 0.500 | 2.660 | 0.833 | 1.500 | 2.330 |
| Good control DM | 2.747 | 0.457 | 2.000 | 3.300 | 2.330 | 3.000 | 3.000 |
| Poor control DM | 3.132 | 1.260 | 1.000 | 4.000 | 3.000 | 3.660 | 4.000 |

Table 2. Statistics on papillary bleeding index (PBI) for the studied groups, according to age.

Statistic significant differences were recorded for the mean PBI values for all groups of patients divided per age groups ($p<0.5$). For prepubertal stage (6-10 years) mean PBI did not registered significant differences between ND and good metabolic control patients ($p>0.5$), while for pubertal stage significant differences were recorded across all studied groups (ND, good control and poor control DM). Considering the juvenile period (15-18 years), average PBI was higher in poor controlled diabetes compared to mean values of well metabolically balanced diabetics and of ND, the difference being statistically significant ($p<0.5$).

**Clinical attachment loss**

The highest incidence of increased clinical attachment loss along with the most elevated mean value were recorded in poorly controlled diabetics (CAL = 1.053 mm, figure 3).
Significant differences were recorded between the two subgroups of DM children and adolescents and between the groups of non diabetes and good metabolic control DM, respectively ($p=0.002$).
For a description of the lots included in the study based on loss of attachment, table 3 presents statistical indicators that define the characteristics of the groups in terms of this clinical indicator. For pre pubertal stage no real attachment loss was registered in all groups of patients enrolled in the study. The pubertal phase recorded a slight increase in the CAL levels, with maximum values up to 2mm, and 0.5mm as average. Quartile analysis (Q75) indicates that 75% of the children belonging to this age group presented mean CAL levels below 1 mm. Individuals aging between 15-18 years old recorded different values, with minimum CAL=0mm and peak CAL=4 mm, statistic analysis highlighting mean values below 2.3 mm for 75% of nondiabetics, while 75% of poor controlled SM subjects of this age group presented CAL up to 3.5 mm. Moreover, standard deviation was also higher for this age population compared to the others.

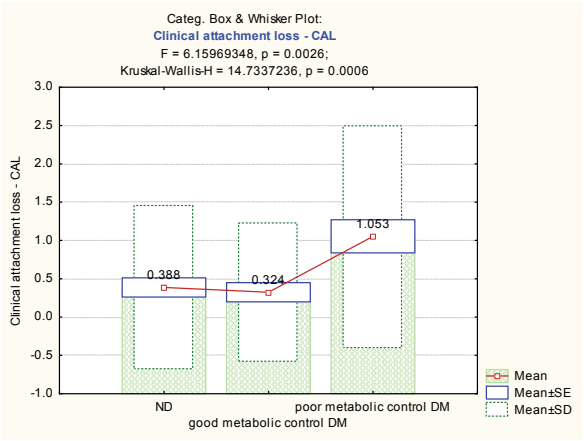


Fig. 3. Mean clinical attachment loss in the studied groups.

### 4.3.4 Analysis of serum and GCF inflammatory mediators

The inflammatory mediator profile in human whole blood and gingival fluid was characterized in more detail. Whole blood and crevicular fluid were collected from all subjects enrolled in the study, according to the previous mentioned protocol, and analyzed

for IL-1β, IL-2, IL-10, and IFN-γ production. The degree of local and systemic inflammatory response was assessed by multiplex flow cytometry blood and gingival fluid cytokines level determinations. A significant interindividual variability in the amounts of inflammatory mediators secreted during the association of the periodontal breakdown with systemic alteration was observed for all the mediators tested.

| CAL(mm) / Group | Mean | Std. Dev. | Min | Max | Q25 | Q50 | Q75 |
|---|---|---|---|---|---|---|---|
| **Pre pubertal age: 6-10 years** | | | | | | | |
| ND | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0 |
| Good control DM | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0 |
| Poor control DM | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0 |
| **Pubertal age: 11-14 years** | | | | | | | |
| ND | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0 |
| Good control DM | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0 |
| Poor control DM | 0.500 | 0.786 | 0.000 | 2.000 | 0.000 | 0.000 | 1.0 |
| **Juvenile age: 15-18 years** | | | | | | | |
| ND | 1.033 | 1.545 | 0.000 | 4.000 | 0.000 | 0.000 | 2.3 |
| Good control DM | 0.786 | 1.280 | 0.000 | 3.000 | 0.000 | 0.000 | 2.5 |
| Poor control DM | 2.560 | 1.447 | 0.000 | 4.000 | 2.300 | 3.000 | 3.5 |

Table 3. Statistic indicators of clinical attachment loss (CAL-mm) for studied groups, according to age.

As shown in figure 4, diabetes mellitus elicited a significant increase ($p<0.05$) in local secretion of IL-1β.

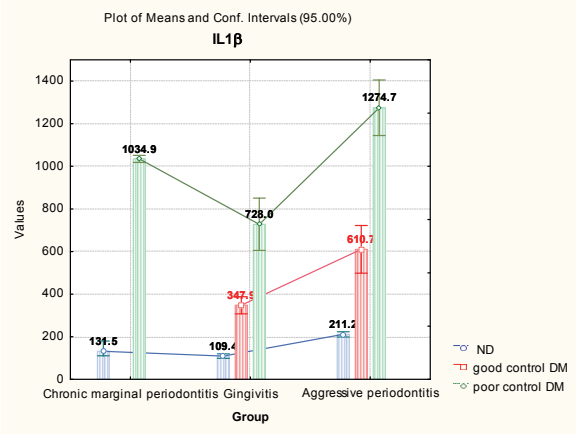


Fig. 4. Levels of gingival fluid IL-1β in the studied groups.

The lowest mean local interleukin 1β value was recorded in the systemically healthy population, the diabetic status associating a considerable increase in gingival fluid interleukin levels. In addition, systemically healthy patients with gingivitis recorded the lowest gingival fluid IL-1β level, a significant elevation of this mediator being associated to IDDM children and teenagers. Moreover, IL-1β, IL-2 and IFN-γ analysis according to the values of $HbA_{1C}$, revealed the existence of a statistic significant positive correlation betwen the measured parameters (Pearson test, r=0.73; 0.65 and 0.71 respectively), thus reflecting important elevations of cytokine levels induced by impaired metabolic balance of the diabetic young population.

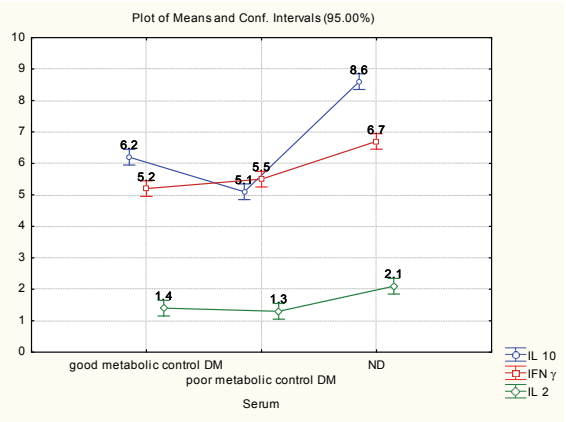


Fig. 5. Serum levels of IL-2, IL-10 and IFN-γ in the studied groups.

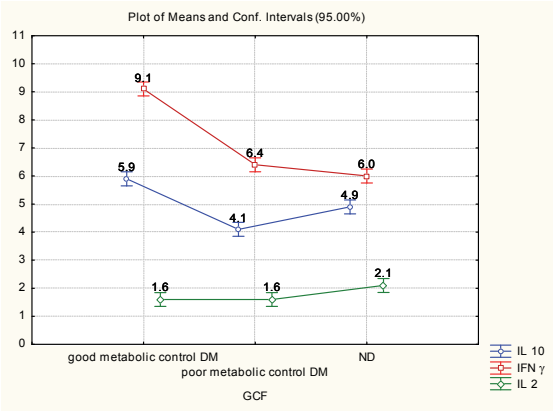


Fig. 6. GCF levels of IL-2, IL-10 and IFN-γ in the studied groups.

Serum IFN-γ in diabetic children recorded moderate values compared to that of ND, and significantly higher levels in gingival fluid (figure 5). While IL-10 gingival fluid secretion was enhanced in some diabetic subjects with good control of the metabolic disorder, the most common elevated levels were present in the serum of systemically unaffected group

(figure 5 and 6). IL-10 registered a decreased average level of blood and GCF secretion in the diabetic population, with significantly differences between the two systemically affected subgroups. Considering IL-2 level, there is a very low secretion in diabetic patients with periodontal breakdown, both in blood and gingival fluid, probably determined by a local production of a blocking factor that induces this specific profile.

### 4.4 Discussions

Analysis of clinical parameters related to distribution, diagnosis and age highlights significant differences in the prevalence of severe periodontal breakdown between the two principal studied groups, with an 14.3% overall prevalence of chronic marginal periodontitis and aggressive periodontitis within systemically healthy individuals versus 28.5% in IDDM group. Moreover, the same proportion was maintained when considering the two diabetes subgroups, almost two times more subjects with poor controlled diabetes experiencing severe periodontal injury (40%) compared to good metabolically balanced age-matched diabetic individuals (19%). Thus, despite that the oral health status data showed gingivitis as the main periodontal alter in both groups, there were significant differences among diabetic subpopulations (81% versus 60% within good and poor controlled diabetics, respectively). This was followed by a 3.1 fold increased incidence of chronic superficial periodontitis within the diabetics (30%) compared to non-diabetic group (9.5%), and almost twice more prevalent aggressive periodontitis in IDDM children and teenagers (19% *vs.* 10%). Summarizing the results based on clinical diagnosis of periodontal injury related to age interval, the highest prevalence of gingivitis is specific for the prepubertal age, followed by an increase incidence of marginal superficial chronic periodontitis in pubertal stage and forms of aggressive periodontitis during juvenile age, among all studied groups. Considering the two main population groups, gingivitis is the main periodontal alter among systemically healthy subjects, the associated systemic disease eliciting an increase in the incidence of more severe periodontal breakdown.

Periodontal homeostasis breakdown along systemic alteration of type 1 DM was also assessed through evaluation of clinical parameters (PI, PBI, CAL) correlated with age, duration and metabolic control of diabetes mellitus (HbA1c values). Statistically significant differences were recorded both, between the mean PI values corresponding to the non diabetes group and poorly controlled DM individuals, and between the two diabetes subgroups ($p<<0.05$, CI=95%). Moreover, taking into consideration the age intervals, comparative statistic studies of the oral hygiene parameter highlight the most significant differences among the juvenile age individuals, for all studied groups ($p<<0.05$).

Papillary bleeding index in diabetic children and teenagers have significantly higher values, directly correlated to the age of systemic disease ($r=0.64$). Considering the patient's age, the most important statistical difference is registered along pubertal period, pointing out a significant difference between the mean PBI values in ND patients (PBI=1.23) versus good controlled diabetic group (PBI=2.65) ($p=0.007$, $p<<0.05$). Furthermore, mean BPI significantly differs in patients with poorly controlled diabetes than the average values in patients with good controlled diabetes and ND ($p<<0.05$), this pattern of overall changes in inflammatory periodontal parameter's levels persisting across all age groups (prepubertal, pubertal, juvenile). These results can be explained by increased activity of collagenases and vascular changes within diabetes that increase gums bleeding and thickening of the small vessels basal membrane of the gingiva.

Distribution of CAL values indicated the most elevated (between 1.5 - 4 mm) and highest mean level (CAL = 1.053 mm), in the group of subjects with poor controlled diabetes, about 2.7 and 3.25 times higher than that of ND (CAL = 0.388 mm) and good metabolic controlled IDDM (CAL = 0.324 mm), respectively. Reffering to age, the highest mean loss of attachment characterized the 15-18 years old poorly controlled IDDM subjects (CAL=2.56mm), 2.4 more elevated than in ND (CAL=1.03 mm) and 3.2 times higher than in good metabolically controlled diabetics (CAL=0.79mm) (table 3). In prepubertal stage, almost no one can question the loss of attachment (explained both by anatomic and physiologic characteristics of this phase and the very short period in which teeth are maintained on the arch). In addition, the disease's evolution is insufficient to elicit real periodontal breakdown of chronic marginal periodontitis type, most commonly, loss of attachment being rather related to diabetes time course.

Furthermore, HbA1c values correlated with clinical parameters of oral status indicated that poorly controlled diabetes (HbA1c >7%) is associated with elevated bleeding index. Comparison of the parameters that indicate the degree of periodontal disruption (PBI and CAL) with age of onset of systemic disease reveals that age of diabetes and its metabolic control can be important determinant indicators to evaluate DM as a risk factor for periodontal breakdown within children and adolescents.

Determination of gingival crevicular fluid with parallel serum levels of soluble inflammatory mediators is highly relevant for studying children and teenager periodontitis within systemic context, since this consistent oral fluid, which bathes the periodontal pocket, derives from gingival capillary beds and contains resident and emigrating inflammatory cells. Systemically healthy patients with the mild form of periodontal disease recorded the lowest IL-1β gingival fluid level, a significant increase of this mediator being associated to IDDM group. Moreover, in ND patients there was a dose–response relationship between the severity of periodontitis and gingival crevicular fluid IL-1β levels (two times higher mean values in systemically unaffected subjects with aggressive periodontitis), which suggested that periodontal disease may play a major role in elevating levels of this cytokine. Our results reveal an overall pattern of most prominent variability among poorly controlled diabetic children and teenagers, regardless of periodontal breakdown degree (gingivitis or aggressive periodontitis). Data from the literature are somehow conflicting, certain results on adult population mentioning the lack of correlation between production of IL-1β related cytokine, and $HbA_{1c}$ levels in patients with type 2 diabetes and periodontitis (Engebretson et al., 2007). Our results recorded significant positive correlation (Pearson test, r = 0.73) between IL-1β and glycosylated hemoglobin levels in diabetic young individuals, translated also into increased interleukin levels directly related to the reduction in the degree of metabolic control of the systemic disorder. IFN-γ is an inflammatory cytokine associated with inflammation, tissue destruction, bone resorption and specific elevated production of collagenases, serum and local determinations of this important regulator of immune inflammatory response revealing different levels in diabetic individuals, higher when associated to a good metabolic control and more specific periodontal breakdown. Moderate IFN-γ serum levels were recorded in diabetic population compared to ND, the high expression of gingival fluid cytokines in severe periodontal alteration of these patients being probably a marker of continuous Th1 response against microbiologic challenge, especially bacterial pathogens colonized in gingival tissue. This can be explained by alterations in the oral microenvironment caused by much higher amounts of glucose and urea in gingival

fluid from DM individuals (data recorded by laboratory analysis of gingival fluid), that create a favorable environment for bacterial changes, with alteration of host immune response to periodontal pathogens, and suggests that Th1 mediated cytokine response may play a destructive role in the periodontium. The present results indicate that microbiological overlapping involves considerable efforts of the body, resulting in significant elevation of IFN-γ, but not of IL-2 which was very low both in blood and GCF diabetic individuals, suggesting the possible existence of a local factor that blocks the lymphocyte and macrophage secretion of this T lymphocytes factor of proliferation, mainly in diabetic patients with periodontal deterioration. The reasons for moderate IL-2 secretion are probably complex and may involve transcriptional or translational repression.
IL-10 registered decreased secretion in the diabetic population, both gingival fluid and serum values recording significantly higher differences between the two systemically affected subgroups. It is thus possible that reduction in IL-10 secretion within juvenile diabetic population could play an important role in switch of the oral tissue differentiation toward periodontal injuries.

### 4.5 Conclusion

Our study showed that DM modulates GCF expression of Il-1β, IL-10 and IFN-γ in patients with impaired periodontal territories. Very probably this is the result of immune system cells sensitization by endogenous ligands and bacterial products through various receptors, some of them recognized as important mediators of immune responses in inflammatory diseases. Applied statistic tests showed that the values of all studied clinical parameters referring to periodontal status in diabetic children and adolescents (plaque index, bleeding index, loss of attachment) are much higher than those of systemically healthy group. Thus, the present study clearly reinforce that children and adolescents are susceptible to destructive forms of periodontal disease, especially when the etiologic external factors (microbial flora) are associated with host-related systemic impairment, such as insulin dependent diabetes. In summary, our data support the notion that systemic alteration of IDDM type is associated with distinct patterns of GCF cytokine expression. Poor controlled young diabetic subjects were characterized by local higher IL-1β and decreased IL-10 and IFN-γ amounts, compared to systemically healthy subjects, suggesting that an imbalance between pro- and anti-inflammatory cytokines is associated with the possible switch of the biofilm-modulated periodontal status toward more specific breakdown. IL-1β, IL-10 and IFN-γ might be involved in controlling the inflammatory process at periodontally healthy and diseased sites, the present manuscript indicating that the interactions appeared to be different in subjects that were systemically healthy when compared with IDDM subjects. Moreover, the metabolic equilibrium of the systemic disease is significantly related to the gram negative species mediated cytokine translocation from the periodontal space into the circulation. Further studies of candidate biomarkers and of inflammatory shifts will be necessary to confirm these observations.

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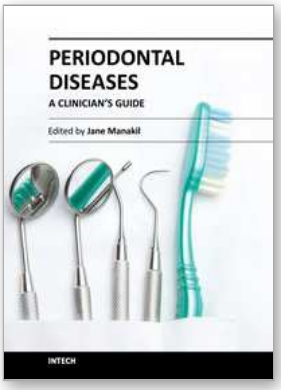


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"Periodontal diseases" is a web-based resource intended to reach the contemporary practitioners as well as educators and students in the field of periodontology. It is fully searchable and designed to enhance the learning experience. Within the book a description is presented of the current concepts presenting the complex interactions of microbial fingerprint, multiple genotypes, and host modulations. In addition, an overview is given of the clinical outcome of the disease's progression, as influenced by the epigenetic factors. Emerging concepts on periodontitis as a risk factor for various systemic diseases and as a bilateral modulating factor have been elucidated in detail as well.



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