IL 18 as an Important Gingival Inflammatory Biochemical Marker in Children and Adolescents with Insulin-Dependent Diabetes Mellitus

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Interleukin 18 (IL-18) might mediate the gingival inflammation with increased potency in patients with diabetes. To establish possible positive correlations between the degree of gingival inflammation and IL-18 amounts in gingival crevicular fluid (GCF) of children and adolescents with insulin-dependent diabetes. Clinical and GCF IL-18 assessment were performed on 30 children and adolescents with diabetes and 30 healthy subjects. GCF samples were collected from mesial sites of incisors and first permanent molars for all subjects, the level of IL-18 being determined by ELISA. The highest concentrations of IL 18 were recorded in IDD children with severe gingivitis. In all studied groups, the IL 18 values were higher at the level of the incisors. Based on clinical indices, no cases of chronic marginal or aggressive periodontitis were diagnosed. The IL 18 concentration in the GCF increases proportionally with progression toward periodontal inflammation and is strongly associated with the presence of diabetes. The results suggest that the periodontal impairment can start very early in children with diabetes. The inflammation monitored with the IL 18 levels in the GCF was much more obvious and earlier detected than by clinical indices. Moreover, IL 18 can be a prognostic marker of the periodontal inflammation in patients with diabetes where the clinical alterations are not yet evident.

Keywords: interleukin 18, biomarker, gingival crevicular fluid, insulinodependent diabetes mellitus, periodontitis, children, adolescents

Insulindependent diabetes (IDD) and periodontitis (P) are inflammatory conditions with a binomial relationship. Periodontal disease, including gingivitis and periodontitis, is a complex of diseases of the marginal periodontium caused by microbial attack upon tooth support tissues. Gingivitis is inflammation of the gum tissue only, a reversible condition that can further progress to periodontitis, a less controlled system, which emerges through periods of disruption and balance, where breakdown of connective tissue attachment and alveolar bone can eventually lead to tooth unit loss. There are extensive epidemiological studies that claim gingivitis as ubiqvitous in children and teenagers, some indicating the presence of gingivitis among school children (aged 5-14 years) in 84.37%, the prevalence and severity of this condition increasing with age [1]. Moreover, at some point there is a quite common belief that periodontitis detected clinically by level of clinical attachment loss must be preceded by gingivitis [2].

A large number of evidences support the hypothesis that persistent gingivitis is a risk factor for tooth and periodontal attachment loss [3], gingival inflammation and tartar being associated with early periodontal impairment. The factors that forward gingivitis to periodontitis have not yet been entirely deciphered [4].

Diabetus mellitus (DM) is a chronic condition characterized by an altered sugar, protein and fat metabolism, displaying ever increasing incidence in industrialised states [5]. According to the International Diabetes Federation (IDF) recent data, more than 387 million people are suffering from diabetes (prevalence 8.3%) and in 2040 the number will increase probably to 640 million people [6].

Linked to a total or partial destruction of the pancreatic beta cells responsible for insulin production in response to an autoimmune event, type 1 diabetes (IDD) usually occurs in childhood or adolescence, being less common than type 2 as it includes a small proportion - less than 10% of all forms [7].

Since 1993, when was asserted that P is the sixth complication of diabetes [8] (statement that was further reconfirmed in 2011) [9], and 1996, with claiming that DM is the third periodontitis risk factor (after age and smoking), the bivalent relationship P-DM has been extensively promoted by large-scale research. In the early 1997, the report of the expert Committee on the diagnosis and classification of DM reffered P as one of the pathologic conditions often found in adults with diabetes [10].

Multiple studies proved that the prevalence, severity and progression of the P are significantly increased in patients with diabetes. This increase is often correlated with a poor control and other complications of the disease [11,12].

The studies approaching the periodontal disorders in children and adolescents with diabetes are far fewer compared to those investigating the adult population. Periodontal condition in individuals with diabetes was reviewed and reported that, in patients with childhood-onset-diabetes, periodontitis seems to occur around puberty and to progress with age [13]. Recently, it was stated that in diabetes periodontal destruction can start very early in life, and becomes more prominent as children become adolescents [14].

The current trend in periodontal pathogenesis suggests that the impairment of the periodontal tissue caused by the microbial attack is modulated by the inflammatory response of the host, which will release various destructive

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mediators. The concentration of these mediators controls the nature and intensity of the inflammatory response. One class of these mediators, the cytokines, has an important role in the inflammatory cascades of various diseases.

IL-18 is a multifunctional proinflammatory cytokine from the IL 1 family. It was initially identified as an IFNγ inducer [15] and subsequently associated to obesity, atherosclerosis, insulin resistance, glucose intolerance and cardiovascular diseases [16]. It was also reported that high levels of IL -18 could be considered as predictive for type-2 diabetes progress [17].

IL-18 is a multifunctional cytokine with a probable regulatory role in the inflamed gingival tissue, as gingival samples with enlarged sulcular depth exhibit higher expression of the cytokine [18]. Moreover, gingival crevicular fluid (GCF) IL-18 amounts have been found increased at sites of both gingivitis and periodontitis, suggesting an association between the degree of periodontal breakdown and IL-18 local flow [19]. Considering the clinical grounds, some findings pointed out more relevant local IL-18 levels in patients with chronic periodontitis, as compared to those with gingivitis, either at sites with similar pocket depths [20].

However, recent studies reported IL-18 local levels in juvenile type 2 diabetic individuals associating periodontal impairment [17]. The link between IL-18 levels in both diabetes and periodontitis remains questionable, and very probable, if properly explored would direct to an improved understanding of the bidirectional relationship between the two inflammatory conditions.

Thus, in our study we investigated GCF IL-18 levels in children and adolescents with IDD as compared to those of healthy children in terms of general condition, also with all with various degrees of gingival inflammation. Furthermore, statistical correlations have been made between the level of IL-18 in GCF and clinical characteristics of periodontal impairment: plaque index (PI), gingival index (GI), probing depth (PD), bleeding on probing (BOP) and clinical attachment level (CAL) for all subjects included in the study.

The objectives of the present study were to find possible positive correlations between the concentration of IL-18 in GCF and degree of gingival inflammation in IDD children and adolescents, and to explore the probable impact of GCF IL-18 expression as early biomarker for periodontal injury within young IDD subjects.

Experimental part

The study protocol was conducted in agreement with Declaration of Helsinki, being approved by the Ethical Commitee of University of Medicine and Pharmacy Grigore T. Popa - Iasi. Eligible subjects or their parents/tutors were informed of the nature, potential risks and benefits of their participation in the study and signed a consent form.

30 young subjects with type I diabetes (IDD), aged between 7-18 years, were recruited from Diabetes and Metabolic Diseases Department of the University Clinical Hospital St. Mary in Iasi and assigned as active group. Other 30 patients (also aged 7-18 years) with general state unaffected by systemic diseases, but with various degrees of periodontal impairment, constituted the control group and were selected from children that addressed to the Clinic for Paediatric Dentistry for common dental issues. Exclusion criteria included previous orthodontic treatment, current cigarette smoking, periodontal and antibiotic therapies in the previous 6 months, systemic condition other than diabetes and subjects that presented diabetes complications (other than periodontal injury), that could possibly influence cytokine level.

Periodontal assessment

The enrolled individuals underwent a detailed periodontal evaluation using a periodontal probe 3.5/5.5/8.5/11.5 mm and Tweezer kit (Kerr-Total/Metrex research, Hamburg, Germany).

Clinical investigation envisaged the following periodontal variables:

- plaque Index (PI) was assessed according to Silness and Löe method, using an erythrosine plaque detector, through evaluation of the presence or absence of 4 surfaces around each tooth (mesial, distal, buccal and lingual), scoring from 0 (absence of plaque) to 3 (serious plaque accumulation) [21];

- gingival Index (GI, table 1) reflect the qualitative changes in the gingiva, by scoring the marginal and interproximal tissues separately on the basis of 0 (normal gingiva) to 3 (severe inflammation). The original index of Silness and Löe was evaluated by the presence or absence of 4 surfaces around the tooth and giving a recorded from 0 (no inflammation) to 3 (severe inflammation). For PI and GI data, percentages of positive sites were obtained per subject and mean values were further scored for the groups;

- bleeding on probing (BOP) was determined through gentle probing of the hole of the gingival crevice on 4 surfaces (mesial, distal, vestibular, lingual) of each tooth. For bleeding occurring within 10 s a positive finding was registered, recording the whole number of positive sites and expression as percentage of the number of sites examined [22];

- probing Depth (PD) reflect the distance from the gingival margin to the bottom of the groove and was measured following insertion of the probe along the teeth axis on mesial, distal, buccal and lingual sites. For all subjects, the percentage of sites with values over 3 mm have been estimated;

- clinical attachment level (CAL) represents the length between the cementoenamel junction and the bottom of the groove, the percentage of sites with value > 2 mm, indicating a loss of bone support being evaluated for all enrolled subjects.

Based on their periodontal status, each subject of the active and control group was included in the gingival healthy group or gingivitis group according to the following criteria:

A. Gingival healthy group, GI=0, with PD < 3mm and no attachment loss or clinical sign of BOP, no erythema or supuration;

GI = 0	Health Normal gingiva
GI= 1	Minor inflammation- narrow colour change, negligible edema No bleending on probing
GI= 2	Moderate inflammation- redness, shining, edema Bleeding on probing
GI= 3	Severe inflammation- redness, edema, ulceration Move toward spontaneous bleeding

Table 1
GINGIVAL INDEX

B. Gingivitis group, GI≥1, no relevant clinical attachment loss, PD < 3 mm, bleeding on probing and the presence of either sweling or redness. Furthermore, based on GI values, gingivitis group was subdivided into the mild (GI=1), moderate (GI=2) and severe (GI=3) gingivitis subgroups.

Finally, in order to make a relevant analysis of the putative relationship between IL-18, IDD and P, the global 60 young individuals were assigned into 8 batches as follows:
Control:

Gr. 1= healthy (GI= 0), n=10 (subjects), s=80 (number of GCF collected samples);
Gr. 2 = mild gingivitis (GI=1), n=11, s = 88;
Gr. 3 = moderate gingivitis (GI=2), n=8, s=64;
Gr. 4 = severe gingivitis (GI=3), n=1, s=8;
IDD:
Gr. 5 = healthy (GI= 0), n=3, s=24;
Gr. 6 = mild gingivitis (GI=1), n=5, s=40;
Gr. 7 = moderate gingivitis (GI=2), n=16, s=128;
Gr. 8 = severe gingivitis (GI=3), n=6, s=48.

Procedures for site selection and sample collection

GCF samples collected using paper strips have been used to assess the cytokine levels. For all the selected participants, GCF harvesting and subsequent periodontal examination have been done in the dental office, after seating them comfortably, the procedures being clearly described. The sample collection was performed in mesial sites of central incisors (I) and the first permanent molars (M, maxillary and mandibular) of each individual, resulting in a total of 8 strips per patient. Sample collection was achieved before clinical evaluation to avoid any contamination of the strips with blood released during the periodontal evaluation.

The area was isolated with cotton rolls and gently air-dried to remove possible saliva contamination. Gingival fluid was collected through inserting standard paper strips (Periopaper, Oraflow Inc., NY, USA) into the sulcus for 30s. Strips that were contaminated with blood were discarded. The GCF volume was recorded with a calibrated device (Periotron 8000, Proflow Inc., Amityville, NY, USA), the readings being subsequently converted to accurate volumes by reference to a standard curve. Strips were placed into specific vials containing 100 µL phosphate saline buffer and stored at -70°C until analysis for IL-18.

IL-18 assay

The GCF samples were assayed for IL-18 levels using Human IL-18 ELISA Kit (Bender Med Systems GmbH, Viena, Austria) according to the manufacturer's instructions. In brief, the technique is based on anti-IL-18 monoclonal coating antibody that is adsorbed onto microwells. IL-18 present in the sample or standard binds to antibodies adsorbed to the microwells. Subsequent to the add of a biotin conjugated monoclonal anti-IL-18 antibody, it binds to IL-18 captured by the first antibody. Streptavidin-HRP is added and binds to the biotin conjugated anti-IL-18. Finally, a colored product is formed, the intensity being directly proportional to the amount of IL-18 present in the sample. Absorbance of each well is read on ELISA reader using 450 nm as primary wavelength. The concentration of IL-18 in the analyzed samples was estimated using the standard curve.

Statistical analysis

Statistical analysis was performed using a software package. The assess the data distribution normality, the Kolmogorov-Smirnov test was used. The parametric tests were needed to compare the means of IL-18 values in different groups. Statistical analysis was performed using One Way ANOVA completed with Holm-Sidak method, with confidence level of 95% ($p < 0.05$). Finally, the use of Pearson's correlation was involved to observe any existing correlation between the IL-18 GCF amounts and clinical parameters.

Analysis considered the $p<0.05$ values statistically significant. Mean ± SD was used to express results per groups.

Results and discussions

Periodontal assessment

Table 2 shows the distribution of the patients in all groups according to the gingival health status. It was seen that 10 subjects in the control group showed no periodontal altering (GI = 0), significant difference compared to IDD group of children, where only three individuals displayed no periodontal modification (33.33 % *vs* 10 %).

Most children in the two studied groups exhibited different degrees of bacterial gingivitis, as follows:

-mild gingivitis (GI=1) was recorded in 11 subjects of the control group and 5 subjects of the IDD batch (36.66% *vs* 16.66%);

-moderate gingivitis (GI=2) was recorded in 8 subjects of the control group and 16 subjects of the IDD group (26.66% *vs* 53. 33%);

-severe gingivitis (GI=3) presented in only 1 children from the control group and 6 subjects of the IDD group (3.33% *vs* 20%).

Is to be noted that while for most children in the control group gingival inflammation was mild or absent, in the case of IDD children, moderate to severe gingival inflammation were dominating.

No case of chronic or aggressive periodontitis have been diagnosed in the studied groups, as no attachment loss greater than 2 mm (CAL > 2 mm) nor PD > 3 mm were detected.

Clinical indices

The average values for clinical indices in studied groups are found in table 4.

There are no statistically significant differences in PI, PD and CAL values between control groups and the corresponding IDD groups ($p > 0.05$), (5 *vs* 1, 2 *vs* 6, 7 *vs* 3 and 4 *vs* 8).

When comparing control groups and their corresponding IDD subjects, statistically significant differences were revealed for BOP and GI indexes, more relevant for the inflammatory status ($p < 0.05$) (table 3).

Periodontal diagnosis		Control (n=30)	DID (n=30)
Absence of periodontal disease (GI=0)		10 (33.33%)	3 (10%)
Gingivitis (GI≥1)	GI=1	11 (36.66%)	5 (16.66%)
	GI=2	8 (26.66%)	16 (53.33%)
	GI=3	1 (3.33%)	6 (20%)

Table 2
PERIODONTAL DIAGNOSIS (PATIENTS AND PERCENTAGE)

Table 3

CLINICAL INDICES (t-TEST ANALYSIS)

Parameters	Control	IDD	P value
PI (mean±SD)	Gr. 1 (GI=0) Gr. 2 (GI=1) Gr. 3 (GI=2) Gr. 4 (GI=3)	Gr. 5 (GI=0) Gr. 6 (GI=1) Gr. 7 (GI=2) Gr. 8 (GI=3)	Gr 1 vs 5, p=0.431 Gr 2 vs 6, p=0.199 Gr 3 vs 7, p=0.075 Gr 4 vs 8, p=0.802
GI (mean±SD)	Gr. 1 (GI=0) Gr. 2 (GI=1) Gr. 3 (GI=2) Gr. 4 (GI=3)	Gr. 5 (GI=0) Gr. 6 (GI=1) Gr. 7 (GI=2) Gr. 8 (GI=3)	Gr 1 vs 5, p=0.445 Gr 2 vs 6, p=0.218 Gr 3 vs 7, p=0.025* Gr 4 vs 8, p=0.023*
BOP (mean±SD)	Gr. 1 (GI=0) Gr. 2 (GI=1) Gr. 3 (GI=2) Gr. 4 (GI=3)	Gr. 5 (GI=0) Gr. 6 (GI=1) Gr. 7 (GI=2) Gr. 8 (GI=3)	Gr 1 vs 5, p=0.073 Gr 2 vs 6, p=0.195 Gr 3 vs 7, p<0.01* Gr 4 vs 8, p=0.025*
PD < 3mm (mean±SD)	Gr. 1 (GI=0) Gr. 2 (GI=1) Gr. 3 (GI=2) Gr. 4 (GI=3)	Gr. 5 (GI=0) Gr. 6 (GI=1) Gr. 7 (GI=2) Gr. 8 (GI=3)	Gr 1 vs 5, p=0.308 Gr 2 vs 6, p=0.719 Gr 3 vs 7, p=0.151 Gr 4 vs 8, p=0.397
CAL < 2mm (mean±SD)	Gr. 1 (GI=0) Gr. 2 (GI=1) Gr. 3 (GI=2) Gr. 4 (GI=3)	Gr. 5 (GI=0) Gr. 6 (GI=1) Gr. 7 (GI=2) Gr. 8 (GI=3)	Gr 1 vs 5, p=1.000 Gr 2 vs 6, p=1.000 Gr 3 vs 7, p=0.355 Gr 4 vs 8, p=0.183

*p < 0.05, statistically significant

Table 4

DESCRIPTIVE STATISTICS OF STUDIED PATIENTS SHOWING MEAN, STANDARDS DEVIATION, MEAN AND RANGE FOR THE PI, GI, BOP, CAL, PD AND IL-18 LEVELS IN GCF

	Study group	IL-18 (Mean ± SD)(pg/ml)	PI (Mean ± SD)	GI (Mean ± SD)	BOP (Mean ± SD) %	PD (Mean ± SD) (mm)	CAL (Mean ± SD) (mm)
control	Goup 1 GI=0	I_1=8.3±0.2 M_1=3.2±0.1	0.5±0.4	0.3±0.4	0	1.0±0.9	0
	Group 2 GI=1	I_2=17.4±0.9 M_2=12.5±0.3	0.8±0.3	0.6±0.4	0.7±0.2	1.8±0.4	0
	Goup 3 GI=2	I_3=19.5±0.8 M_3=12.8±0.3	1.5±0.3	1.2±0.6	16.2±5.4	2.0±0.7	0.5±0.8
	Goup 4 GI=3	I_4=25.7±1.3 M_4=15.9±0.4	1.8±0.7	2.1±0.9	28.6±4.3	2.4±0.3	0.7±0.9
DID	Group 5 GI=0	I_5=32.6±1.7* M_5=24.2±1.4*	0.7±0.2	0.5±0.3	0.5±0.9	1.6±0.6	0
	Group 6 GI=1	I_6=35.7±2.6* M_6=26.8±3.3*	1.0±0.2	0.9±0.5	0.9±0.4	1.9±0.7	0
	Group 7 GI=2	I_7=44.4±5.3* M_7=33.8±4.1*	1.8±0.4	1.9±0.7	31.9±6.2	2.3±0.3	0.8±0.7
	Group 8 GI=3	I_8=68.9±8.2* M_8=42.5±6.3*	2.0±0.7	2.8±0.2	47.4±5.5	2.5±0.1	1.2±0.3

I = incisor; M = molar; IL-18 = interleukin 18; PI = plaque index; GI = gingival index;
BOP = bleeding on probing; PD = pocket depth; CAL=clinical attachment loss
*statistically significant (p < 0.05) as compared to control groups (1-4)

Interleukin-18

The descriptive data for concentrations of IL-18 in GCF in all the 1-8 subgroups are presented in table 4.

The results indicated the mean IL-18 concentration in GCF was highest in group 8-IDD subjects with severe gingivitis and lowest in group 1-control group and gingivitis-free.

Between these uttermost values, mean IL-18 levels in GCF were found, as descending trend, in IDD children with moderate gingivitis, in IDD subjects with mild gingivitis, in IDD and no gingival inflammation, in control group with severe gingivitis, in control group with moderate gingivitis and in control group with mild gingivitis. These data highlighted the clear conclusion that the effects of diabetes on GCF IL-18 levels is still to be debated. Differences in parameters to define periodontal status, management of various insulin types and others such as the protocol to assess interleukins values should account for these results.

Our results pointed out that the mean values of GCF IL-18 in group 5-IDD without clinical expression of gingivitis (GI=0)- were more elevated than those recorded in the control group regardless of mild, moderate and even severe gingivitis (group 2, group 3 and group 4).

One Way ANOVA completed with Holm-Sidak method pointed out that the differences were statistically signifiant between group 1 *vs* 5 ($p<0.001$), 2 *vs* 6 ($p<0.001$), 3 *vs* 7 ($p<0.001$), 4 *vs* 8 ($p<0.001$), 5 *vs* 2 ($p<0.001$), 5 *vs* 3 ($p<0.001$), 5 *vs* 4 ($p=0.021$), for both incisors and molars.

Pearson's correlation exhibited the positively correlation between the GCF IL-18 level and GI as well as BOP (and not with PI, CAL, PD) in groups 4, 5, 6, 7, 8 (table 5) for both incisors and molars.

Furthermore, IL-18 could serve as a marker of inflammation at the preclinical stage of the periodontal impairment, in the context of juvenile insulin-dependent diabetes. Hence, the concentration of IL-18 in GCF

Table 5

PEARSON'S CORELATION COEFFICIENT TEST COMPARING GCF IL-18 AND OTHER VARIABLES FOR INCISORS (I) AND MOLARS (M)

Groups	IL-18 and PI (I/M)	IL-18 and GI (I/M)	IL-18 and BOP (I/M)	IL-18 and PD (I/M)	IL-18 and CAL (I/M)
Group 1	0.925/0.843	0.924/0.811	0.867/0.735	0.996/0.984	0.841/0.719
Group 2	0.961/0.979	0.969/0.920	0.899/0.969	0.843/0.961	0.827/0.811
Group 3	0.979/0.961	0.920/0.969	0.925/0.979	0.867/0.920	0.985/0.719
Group 4	0.841/0.956	0.956*/0.843*	0.989*/0.827*	0.827/0.735	0.757/0.811
Group 5	0.935/0.949	0.991*/0.996*	0.960*/0.977*	0.953/0.957	0.959/0.976
Group 6	0.773/0.973	0.872*/0.972*	0.891*/0.991*	0.883/0.971	0.851/0.951
Group 7	0.960/0.984	0.953*/0.978*	0.959*/0.983*	0.978/0.953	0.984/1.000
Group 8	0.953/0.978	0.972*/0.827*	0.973*/0.735*	0.953/0.843	0.960/0.867

*statistically significant

increases with the progression of periodontal inflammation, and is extensively associated to the presence of diabetic systemic alteration (mean GCF IL-18 levels being elevated in all diabetic individuals' subgroups, as compared to non-diabetics).

In the context of the periodontal disorders initiated by the accumulation of bacterial plaque, the inflammatory reaction starts early into the childhood and reflects the major significance of bacterial impact on the host, in a systemic context. For most children, the inflammatory process of the gums remains superficial, at the clinical stage of gingivitis. In some cases, however, the balance between the microbial load and the host response is disrupted and leads to a destruction of the support tissues of the teeth, which can sometimes result in the loss of dental units.

Based on the large evidence in the literature which showed an increased incidence and severity of the P in patients with diabetes, several authors investigated the bidirectional relationship between the two disorders [10,11,23].

When reviewing the recent literature regarding the binomial relationship diabetes P, it was concluded that the two disorders are strongly correlated [24]. While it was clearly demonstrated that diabetes increases the risk of P, equally relevant data exist, regarding the impact of P on the glycaemic control of diabetes and the mechanisms involved. There are, however, studies that show the improvement of the glycaemic control of the patient with diabetes, following the treatment of P [25]. On the other hand, regarding the metabolic control of diabetes, revealed by the values of the glycated haemoglobin (HbA1c), the literature reports conflicting data. Some authors support the correlation between the poor metabolic control and the severe P [26], while others state the opposite [14,27].

Following the microbial attack, the body responds by releasing various cytokines. Their type and concentration control the nature and intensity of the inflammatory response. Of these, IL-18, a multifunctional pro-inflammatory cytokine caught our attention. IL-18 belongs to the IL-1 family and many studies found positive correlations between the IL-18 levels in the serum, GCF or the gingival tissue, and the clinical indexes of P, i.e. GI, PD, BOP and PAC [20,28].

There was found an association between the raise of IL 18 concentrations and the increase of the risk to develop type 2 diabetes, by age, body mass index, systolic blood pressure and physical activity. The study was performed on adult subjects after adjustment for classic risk factors [17].

IL-18 is responsible for the initiation and propagation of periodontal destruction. There are studies indicating that IL-18 induces the synthesis of MMP 9 and IL-1β, both with a pro-inflammatory effect in tissue degradation [29-32]. Since these events also occur in chronic periodontal inflammation, it seems worthwhile to evaluate IL-18 levels in normal and diseased periodontium.

The present study points out that IL-18 level in GCF grows with the severity of the gingival inflammation, but is also highly elevated in the systemic context of IDD.

The study is the first one to investigate IL-18 in the GCF of children with IDD. A number of 30 control subjects (children, 7 18 years old), without systemic disorders, and 30 children (7 18 years old), with IDD and various degrees of gingival inflammation, were included.

Our study included eight subgroups, which resulted from subdividing each of the two main groups (control and IDD) based on the GI value, i.e. two healthy subgroups (GI=0), two mild gingivitis subgroups (GI=1), two moderate gingivitis subgroups (GI=2) and two sever gingivitis subgroups (GI=3).

Regarding *the values of the clinical indices*, the results are the following. No significantly higher values were found for PI in the IDD group as compared to the control group. This is in agreement with some authors [33], which also reported that PI was not significantly different between the control group and the IDD one, and in disagreement with others [27, 28], which found that the values of PI in IDD patients (especially those with a poor metabolic control) were significantly higher ($p<0.0001$) as compared to healthy patients.

Our results showed statistically significant differences between the control and IDD groups for GI and BOP, being in agreement with the extensive literature data [27,28,34].

Previous studies in children with diabets have indicated that gingival inflammation is significantly increased as compared to nondiabetic control subjects, even after adjusting the oral hygiene levels. Periodontal distruction is increased in children and adolescents with diabetes and, extremely important, this starts earlier in life then formerly recognized [13].

In a study that included 182 children and adolescents (6 18 years old) with IDD, it was concluded that the pathogenesis of both diabetes and periodontitis are complex and that the existence of diabetes clearly associated periodontitis even in 6-to-11- yo group. This relationship becomes more pronounced after the age of 12 [14].

We would like to mention that, following the PD and CAL evaluation of all the patients in the study, we did not diagnose any chronic marginal periodontitis or aggressive periodontitis, and no PD > 3 mm or PAC > 2 mm values were recorded (possibly because of the group size), these results being in accordance with some other researchers [27, 35-37]. On the other hand, our results differ from the conclusions of other authors [13,38]. It was also reported an increased prevalence of chronic marginal periodontitis in children with IDD [14]. The studied group, however, was much larger, consisting of 182 children and adolescents with IDD.

The IL-18 was assessed in the gingival crevicular fluid, which was collected with paper strips (Periopaper) inserted into the gingival sulcus for 30 s. This method is generally considered to be non invasive, non traumatic and painless for the patients [39]. Other authors evaluated IL-18 from GCF collected with microcapillary pipettes, a method they deemed more accurate [39]. We considered this procedure to be more traumatic for the child patient. Some other authors measured IL-18 in the serum of the patients with P [28]. We assessed IL-18 in the GCF as we considered this an evaluation of the local inflammation in the P, right in the *battlefield* between the external aggressors (the microbiota) and the immune response of the host [40-42].

Our study clearly demonstrated the increase of IL-18 concentration in the GCF proportionally with the progression of the gingival inflammation. The IL -18 values increased with the GI and BOP values, in both the IDD and control groups, which is in agreement with other studies performed on adults without IDD[19,20,35]. In addition, this increase of the IL-18 in the GCF was strongly associated with the presence of diabetes in children. The IL-18 values were 2.7-2.8 times as average higher in the IDD group as compared with the control group, both with severe inflammation. The same is true for corresponding subgroups as well (IDD *vs* control). These results could not be compared with literature data since we could not find any studies regarding the assessment of IL-18 in the GCF of children with IDD. As mentioned above, the IL-18 was evaluated in the serum/GCF of adults with P but without IDD [19,28,43].

While investigating IL-18 in the serum of subjects with and without periodontal impairment [19, 28], it was found that serum IL-18 levels increased with the severity of the periodontal disease. Moreover, the study has demonstrated positive correlation among IL-18 levels in serum and GI, PD and CAL in chronic periodontitis.

Markedly increased levels of IL-18 were found in serum of juvenile idiopathic arthritis (JIA) patients with incipient attachment loss [44]. Part of the authors of the same group reported a higher elastase activity associated with lower IL-18 in GCF from juvenile systemic lupus (JSLE) patients [45], the IL-18 values being in the GCF, 33 ± 38 pg/mL (JSLE) vs. 52 ± 14 pg/mL (control), but higher in the serum, 453 ± 302 pg/mL (JSLE) vs. 315 ± 61 pg/mL (control). The average age of the adolescent patients in the study was 15.6 ± 2.7 years old.

We presented the above data to be able to compare the IL-18 values from our studied groups with published data regarding children and adolescents (which are scarce and conflicting).

Moreover, the statistical differences between the IL-18 volumes from the IDD group and those from the control group are much higher as compared with the differences between the clinical indexes values (GI, BOP). In contrast with some important studies [14], we found no significant differences between the studied groups regarding the values of the IP and CAL clinical indexes. The present study also shows statistical correlations between IL-18 and the clinical indexes (GI, SBI) in some subgroups, in agreement with others [28,39].

Conclusions

Our results suggest that the periodontal impairment can start very early in children with diabetes. The inflammation monitored with the help of IL-18 in the GCF becomes much more obvious and is earlier and more objectively detected, compared with the subjective evaluation through the clinical indexes of periodontitis.

This may suggest that, in periodontitis, the preclinical alterations are triggered (and can be assessed) before the clinical ones, as already mentioned. Determining IL-18 is useful in establishing an early diagnosis of the alterations in the marginal periodontium of children with diabetes.

Moreover, IL-18 can be considered a potent inflammatory biomarker of periodontal disease in patients with diabetes where the clinical alterations have not yet set in, as IL-18 levels are increased, even in the absence of the clinical gingival inflammation (GI = 0), and hence deserves further consideration in the development of methods for prevention and therapy.

Acknowlegements: This study was suported by University of Medicine and Pharmacy Grigore T. Popa Iasi through the Internal Research Grant 29237/20.12.2013. Daniel Petru Cioloca, Irina Elena Nechifor and Catalina Holban are Ph.D. Students at the Grigore T. Popa University of Medicine and Pharmacy of Iasi, 16, University Str., 700115, Romania.

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Manuscript received: 14.04.2016