Analysis of oral expression of the diabetes-periodontal disease binomial relationship in a juvenile population

Analiza expresiei orale a relației binomiale diabet - boală parodontală în populația juvenilă

Liliana Foia[1*], Didona Ungureanu[1,3], Vasilica Toma[2], Mihaela Zlei[3], Anca Indrei[4], Danisia Haba[5], Daciana Brănişteanu[6]

1. University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Romania, Dept. of Biochemistry
2. University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Romania, Dept. of Pedodoncy
3. Laboratory of Immunology and Genetics, "St. Spiridon" Hospital, Iaşi
4. University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Romania, Dept. of Anatomy
5. University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Romania, Dept. of Radiology
6. University of Medicine and Pharmacy "Gr. T. Popa" Iaşi, Romania, Dept. of Dermatology

Abstract

INTRODUCTION: Based on the alarming 2008 World Health Organization (WHO) reports concerning the continuously increasing incidence of diabetes mellitus (DM) in the juvenile population we focused our study on the binomial relationship between DM and periodontal disease (PD) within this group of individuals. OBJECTIVE: Related to the clinical stage of periodontal injury we evaluated aspartate aminotransferase (AST) and interleukin 1β (Il-1β) within the gingival crevicular fluid (GCF), as relevant indicators of the PD-related cell destruction and inflammatory processes within juvenile insulin-dependent diabetic (IDDM) subjects. Clinical evaluations consisted of plaque index (PI), papillary bleeding index (PBI) and attachment level (AL) assessment and correlation with the degree of metabolic control. MATERIALS AND METHODS: Measurements of AST (spectrophotometry) and IL-1β (ELISA) were performed within GCF samples from two groups of nondiabetic and diabetic young patients, aged between 6 and 18 years. Glycated hemoglobin (HbA_{1c}) levels were measured by affinity chromatography system. RESULTS AND DISCUSSION: Regardless of the dental pattern (incisive, molar and premolar) AST levels in poorly controlled DM patients displayed higher values compared to well controlled DM patient group, with a considerable increase noted at puberty. Significant differences of the IL-1β levels within the GCF were evidenced between control and IDDM patients, which strengthen the previously reported association between the level of this cytokine and DM in adult population. CONCLUSION: Our study provides an evidence base for associating DM to an increased extent and severity of periodontal destruction even early in life. Therefore, periodontal management of children with DM should be considered in order to diminish the unfavorable effect of such prominent inflammatory process upon metabolic control of DM.

Key words: *diabetes mellitus, periodontal disease, interleukin 1 beta, aspartate aminotransferase.*

[*]**Corresponding author:** Liliana Foia, Email: lilifoia@yahoo.co.uk
Institution adress: University of Medicine and Pharmacy Iaşi, Universității Str. Nr. 16, Iaşi, RO-700115, Romania

Rezumat

Pornind de la rapoartele îngrijorătoare ale Organizației Mondiale a Sănătății din 2008 privind creşterea continuă a incidenței diabetului zaharat (DZ) în populația juvenilă, studiul de față a urmărit evidențierea expresiei orale a relației binomiale DZ – boală parodontală (BP) la această grupă de vârstă. OBIECTIVE: Ca indicatori relevanți ai distrucției parodontale şi procesului inflamator am analizat activitatea aspartat aminotransferazei (AST) şi concentrațiile interleukinei 1β (Il-1β) în lichidul gingival (GCF), la un grup de subiecți cu diabet zaharat insulino-dependent (DID). Corelat cu nivelul controlului metabolic au fost evaluați o serie de indicatori clinici: indicele de placă bacteriană (PI), indicele de sângerare papilară (PBI) şi nivelul de ataşament clinic (AL). MATERIAL ŞI METODĂ: Pacienții cu vârste cuprinse între 6 şi 18 ani au fost împărțiți în două grupe mari: diabetici şi nondiabetici. Au fost analizate valorile AST (spectrofotometric), Il-1β (imunenzimatic) în GCF şi ale hemoglobinei glicozilate (HbA1c – cromatografic). REZULTATE ŞI DISCUȚII: Independent de tiparul dentar (incisiv, premolar, molar) valorile activității AST în GCF au înregistrat nivele crescute la copiii şi adolescenții cu control slab al bolii diabetice, comparativ cu subiecții diabetici dar bine echilibrați metabolic. O creştere considerabilă a valorilor a fost observată în jurul vârstei pubertare. În acord cu studiile anterioare realizate în populația adultă şi care atestau corelații ale Il-1β cu statusul diabetic, valorile acestei citokine au înregistrat deasemeni diferențe semnificative între pacienții non- şi cei diabetici. CONCLUZII: Studiul de față relevă existența unei strânse asocieri între DZ şi severitatea distrucțiilor parodontale încă de la vârste foarte tinere. De aceea, pentru a diminua efectele devastatoare ale acestor procese inflamatorii asupra controlului metabolic al diabeticului, managementul periodontal la copiii şi adolescenții cu DID se impune ca o necesitate.

***Cuvinte cheie**: diabet zaharat, boală parodontală, interleukina 1 beta, aspartat aminotransferaza*

Introduction

Recent evidence[7] accumulates on the interrelationship between diabetes mellitus (DM), a systemic disease with multiple major complications affecting the quality of patients' life and periodontal disease (PD), a group of alterations with episodic evolution having a negative impact not only on the integrity of the gum and its surrounding connective tissue, but also on the systemic physiology.

The bivalent nature of the DM-PD relationship has been defined[13] as the predisposition of DM patients for developing PD, on one hand, and the exacerbation of the systemic disease once the oral infection has been established, on the other.

2008 World Health Organization reports point to the continuously increasing DM incidence among children and teenagers – with 3% every year, and among preschool child – with 5% per year. As clinical associations between DM and PD in children and teenagers have been also reported[4] identifying of new biochemical and immunological markers emerges as an essential requirement for an optimal management of both maladies.

The actual trend in current understanding of the periodontal pathogeny[11] suggests that the periodontal tissue degradation is modulated by the host response that will release different mediators, capable of tissue degradation. A special role in the evaluation of the metabolic response within the active stage of the disease belongs to soluble chemical mediators (prostaglandines, cytokines) or enzymes, sharing significant expression on the gingival crevicular fluid (GCF) level. GCF reflects the complexity of the host-bacteria interaction and offers information referring not only to the equilibrium between the infectious germs and the host, but also specific data concerning involved pathogenic mechanisms[3], providing a valuable biologic sample for searching indicators and predictors of the disease.

Therefore, we focused our attention upon the relevance of measuring interleukin 1β (IL-1β) concentration and AST activity in the

GCF for an early detection and prevention of PD and its complications within the juvenile diabetic population. We evaluated oral (GCF) levels of interleukin-1beta (IL-1β), one of the most important indicators of the inflammatory process[19] and aspartat aminotransferase (AST) as an enzymatic marker of cell destruction[9], in a juvenile insulin-dependent diabetes mellitus population (IDDM).

Material and methods

The study group consisted of a sample of 24 nondiabetic children and teenagers (control), who were reffered to us for common dental treatment, at the University Pediatric Dental Clinic, Iasi, Romania. The second group comprised 32 young subjects (twenty girls and twelve boys) who were hospitalized for their type 1 diabetes mellitus evaluation and treatment, in the University Pediatric Hospital, Iasi. In the second group, the subjects were evaluated and subdivided into two groups based upon their diabetic status: 17 with good metabolic control of the disease (values of glycated hemoglobin - HbA_1c <9%) and 15 displaying poor glycemic control (HbA_1c > 9%).

In agreement with the Helsinki convention, informed consent about GF collection for biological examination and dental follow-up examination was obtained from all persons examined or their tutors.

Clinical examination protocol

Dental examination regarded the overall health of fully erupted permanent teeth (third molars were excluded) and the surrounding tissues. The level of oral hygiene and periodontal tissues inflammation and disorder were estimated by the following:

- Plaque index (PI); according to [16] each site was given a score from 0 (absence of plaque) to 3 (abundant soft matter within gingival pocket, margin and adjacent surfaces). Score 1 defines the existence of a thin film adherent to the free gingival margin and adjacent tooth area; score 2 - moderate accumulation of soft deposits within gingival pocket and gingival margin and/or tooth surface.
- Papillary bleeding index (PBI); separates four different degrees of bleeding, subsequent to careful probing into papillary region[15]: 0- no bleeding; 1 – one single bleeding point, 2 – a fine line of blood or several bleeding points become visible at the gingival margin, 3 – the interdental triangle is filled with blood, 4 – profuse bleeding after probing.
- Attachment level (AL) measures the distance from the cement-enamel junction to the base of the pocket[14].

All clinical indicators were evaluated on Ramfjörd teeth level, at mesio-vestibular sites.

GCF collection

Among the biological fluids, we selected the study of GCF because of its numerous advantages: unlike the blood and saliva, convenient samples from specific sites which contain components derived both from host and bacterial plaque can be used. Given that the collection method affects the amount of obtained gingival fluid[10], we used the less aggressive method, with the introduction in gingival sulci of strips with standardized sizes, after the rigorous control of bacterial plaque, isolation from saliva with cotton rolls and dry of the gingival sulci. According to Brill and Krasse[2], the strips were inserted subgingivally, from vestibular to oral, at mesial facet of the Ramfjord teeth (16, 21, 24, 36, 41, 44 or its neighbors) and left in place for 30 seconds. Temporary teeth were not taken into consideration unless their successors were erupted. The strips were placed in cold phosphate buffer pH = 7.4 (at 4ºC), stirred for 5 minutes using a vortex and then the content was divided into 2 tubes, for IL-1β and AST determinations. The samples were immediately prepared or stored at -70º C in plastic containers resistant to that temperature.

Assay of AST and IL-1β in GCF

For gingival fluid **AST** activity deter-

Table 1. Clinical periodontal characteristics of the study population

Clinical parameter	*Age*	*Control*	*Good controlled IDDM*	*Poor controlled IDDM*
PI	6 – 10 years	3,17 ± 0,5	3,13 ± 0,73	2,83 ± 0,63
	11 – 14 years	2,73 ± 0,44	3,12 ± 0,89	3,08 ± 0,88
	15 – 18 years	3,04 ± 0,67	2,35 ± 0,68 p<0,05	3,92 ± 1,2 p<0,05
PBI	6 – 10 years	1,91 ± 0,47	2,13 ± 0,9	3,04 ± 1,19 p<0,05
	11 – 14 years	1,23 ± 0,59	2,65 ± 0,85 p<0,05	2,78 ± 0,78 p<0,05
	15 – 18 years	1,5 ± 0,82	2,75 ± 0,46	3,13 ± 1,26 p<0,05
AL	6 – 10 years	0	0	0
	11 – 14 years	0	0	0,5
	15 – 18 years	1,03 ± 1,54	0,78 ± 1,28	2,56 ± 1,26 p<0,05

p values were related to control in all situations and were mentioned only when recorded statistic significance – $p < 0.05$.

PI – plaque index, PBI – papillary bleeding index, AL – attachment level.

mination, we used spectrophotometric method, on a Hewlett-Packard spectrophotometer and the INIFINITY® AST test (Sigma), using the manufacturer's protocol. The obtained data were directly expressed by the soft in U/l AST**.**

Gingival fluid **IL-1β** investigation was instrumented by enzyme-linked immunosorbent assay (ELISA) using Human Interleukin-1β - hIL-1β (Biosource, Belgium), in accordance with the manufacturer's protocol.

Statistical evaluation

The GCF levels of AST and IL-1β were expressed as average and compared with the control group. The statistical differences between the interested values corresponding to our studied groups were tested using the t-Student test, One-Way ANOVA completed by Kruskal-Wallis test, for GCF IL-1β, AST, and clinical indicators (PI, BPI and AL) investigation. The differences were considered statistically significant for level of significance (p) lower than 0.05, corresponding to a level of confidence of 95%. Moreover, clinical indicators and AST were separately calculated related to age (prepuberal: 6-10 years, puberal: 11-14 years, juvenile: 15-18 years), on incisive, premolar and molar level respectively.

Results

Table 1 describes clinical periodontal parameters of the studied population, by age subgroup (prepuberal, puberal and juvenile).

In both groups, regardless of the glycemic control, the majority of the examined sites harbored dental plaque. Gingival bleeding was present at significantly higher degree in the diabetic young population, with an evidence elevation in the older age group (3.13 ± 1.26 *vs* 1.5 ± 0.82 in the 15-18 years group compared to 2.78 ± 0.78 *vs* 1.23 ± 0.59 recorded among 11-14 years old patients). Considering the third clinical indicator, no attachment loss was recorded in the prepuberal age, while clearly higher levels were correlated to the lower degree of metabolic control of the diabetes. Thus, various degrees of periodontal attachment level were registered between the studied groups in the 15-18 years subjects (1.03 ± 1.54 in nondiabetics and 2.56 ± 1.26 among poorly controlled diabetic teenagers).

The comparative analysis of the mean activities of AST, based upon dental pattern, among our studied groups, is presented in *Figure 1*.

The graphic shows a 3.33 fold increase of the mean incisive AST activity in the poorly controlled IDDM group compared to control

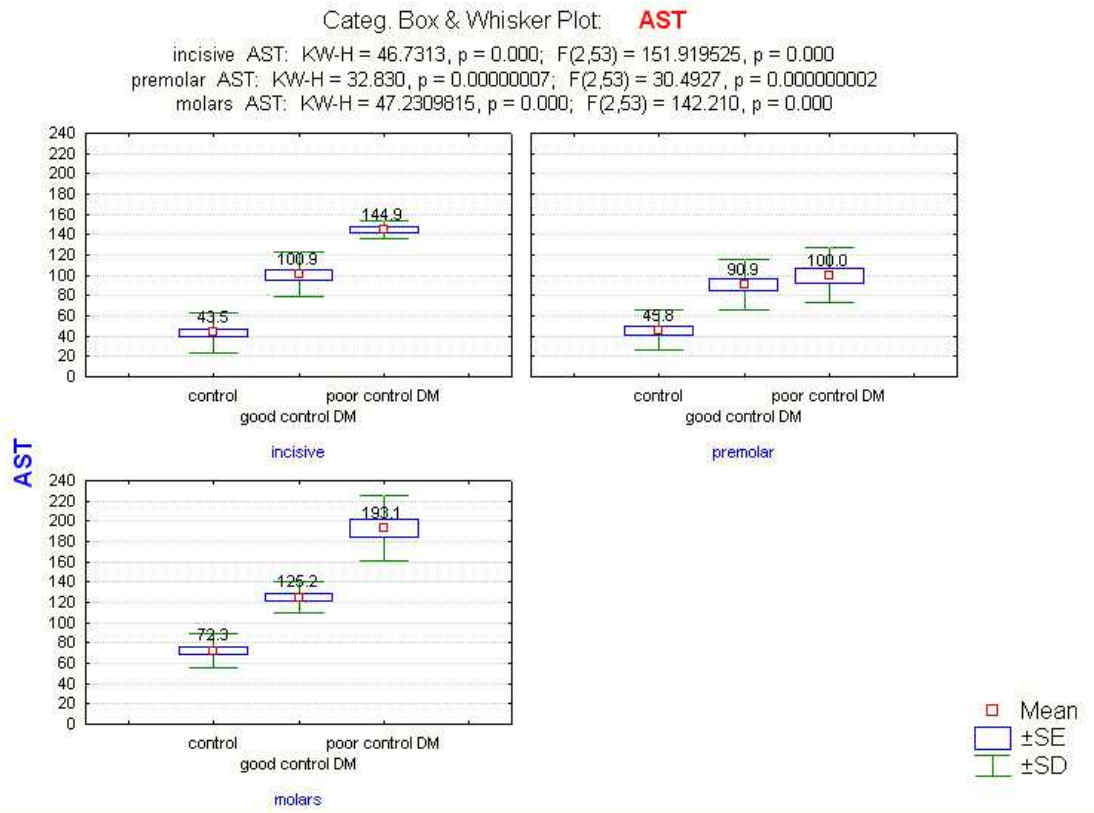

Figure 1. Mean AST activities (U/l) based upon dental pattern in diabetes mellitus patients compared with control

group (144.93 *vs.* 43.5) and of 1.43 compared to well metabolic controlled IDDM subjects.

At the premolar level, children and teenagers with diabetes (regardless of their metabolic control) had significantly more periodontal modifications, as mean enzyme activities registered higher records comparative to controls. Therefore, compared to control, significant increase of 2.18 fold of AST activity among poor controlled IDDM (100 ± 26.7 vs. 45.8 ± 19.76) and less, of 1.98 times among well controlled IDDM (90.94 ± 24.4 *vs.* 45.79 ± 19.76) were recorded (*Figure 1*).

The AST activities around molars recorded higher SD in the group with poorly controlled IDDM compared to the other groups (*Figure 1*), indicating therefore very high variations of the enzyme activities within the mentioned group (min: 143, max: 235). Comparative analysis based on dental site, points out marked AST elevations in the aforementioned subjects around molars.

Among young subjects with poor control of their metabolic disease, the AST activities remained higher for all dental patterns when comparing to well controlled diabetics and control. Thus, poor controlled IDDM recorded AST activities between 100.0 ± 26.7 and 193.1 ± 31.4, approximately 3 fold magnitude than control group (between 43.50 ± 19.15 and 72.25 ± 16.80) and 1.5 times higher than levels of well controlled IDDM group (ranging between 90.94 ± 24.91 to 125.18 ± 15.57) (*Figure 1*). Moreover, based upon age, comparative dental pattern analysis of intracellular enzyme marker within GCF revealed significant differences in and between the studied groups (*Figure 2*). There is a general increase of mean AST

Table 2. IL-1β concentrations in gingival fluid depending on systemic status and periodontal degree alteration

GROUP / IL-1β		Diagnosis	Nr. cases	Mean [ng/ml] IL-1β	Std. Dev	Min	Max
CONTROL		Gingivitis	22	109.36	41.88	0.00	140.00
		Periodontitis	2	211.17	12.02	199.00	230.00
Total			**24**	**117.85**	**49.18**	**0.00**	**230.00**
IDDM	*GOOD METABOLIC CONTROL*	Gingivitis	15	347.89	133.59	0.00	510.00
		Periodontitis	2	610.67	106.57	515.00	785.00
	Total		**17**	**378.80**	**155.41**	**0.00**	**785.00**
	POOR METABOLIC CONTROL	Gingivitis	9	727.96	311.13	0.00	905.00
		Periodontitis	6	1274.67	52.54	1215.00	1314.00
	Total		**15**	**1001.31**	**300.69**	**0.00**	**1314.00**

activity with age, with significant elevation of enzyme activity in puberal and juvenile period.

The comparative analysis of the mean IL-1β levels in GCF was referred for both, diabetic and non-diabetic groups, on the clinical importance of the periodontal breakdown. Thus, most of the individuals displayed a mild form of periodontal alteration (gingivitis), while severe periodontal injury (periodontitis) was diagnosed mainly in the diabetic children with poor metabolic control, and less in nondiabetics and well metabolically controlled diabetics (*Table 2*).

Cytokine concentrations from all subjects were compared between the control and diabetic groups (considering their metabolic status of basic disease), and correlated with the degree of periodontal alteration (gingivitis or periodontitis). Gingivitis (inflammatory process limited to the mucosal epithelial tissue surrounding the cervical portion of the teeth) was assessed in patients displaying signs of inflammation (rubor, dolor, calor, tumor) strictly localized at mentioned area and bleeding tendency. A higher prevalence of periodontitis was correlated with the degree of glucose metabolic imbalance. Following clinical evaluations and dental indicators, criteria for statement of periodontitis were chosen in agreement with the European Workshop in Periodontology[18]: at

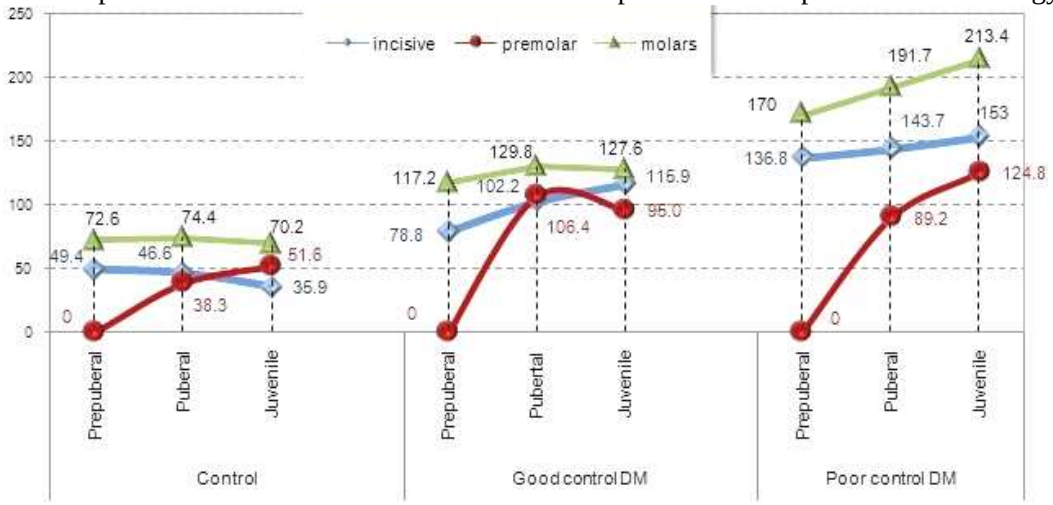

Figure 2. Comparative analysis of gingival fluid AST activities (U/l) based upon dental pattern and age, in diabetes mellitus patients compared with control

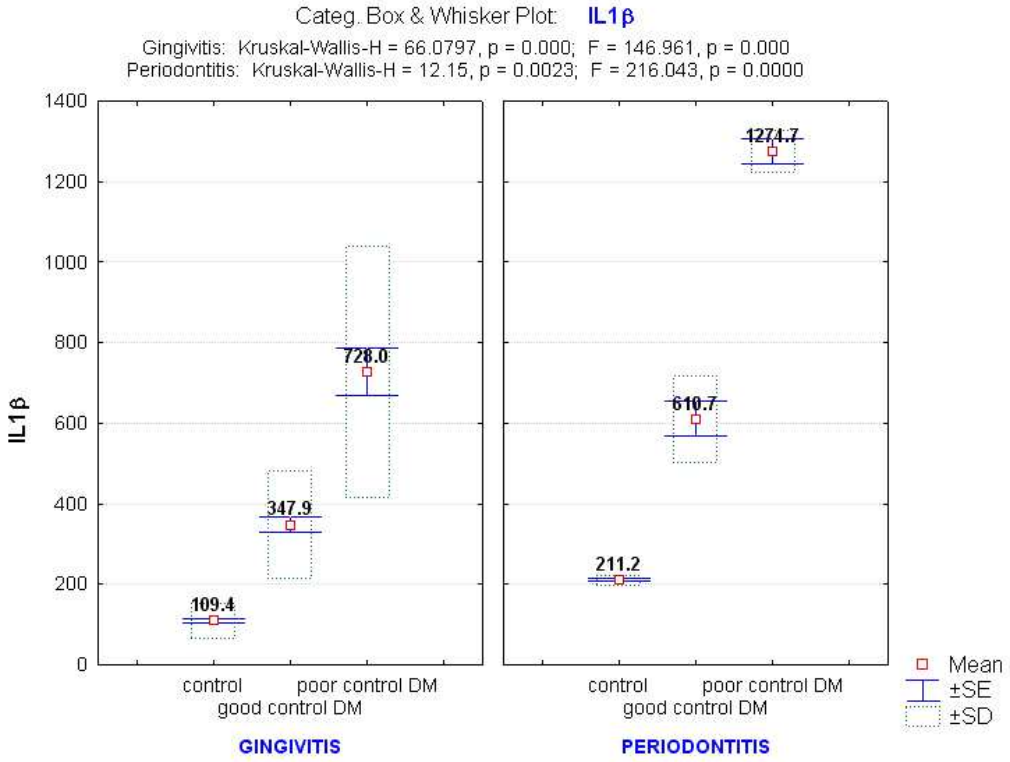

Figure 3. Comparative analysis of gingival fluid IL-1ß concentrations (ng/ml) in normal and diabetes mellitus subjects displaying gingivitis and periodontitis

least two teeth with at least one site with attachment loss >2 mm.

The above data analysis emphasizes that systemically healthy patients with mildest form of periodontal alteration (gingivitis) recorded the lowest gingival fluid IL-1β concentrations (109.36 ng/ml). Furthermore, the values of the gingival cytokine in the aforementioned group increased significantly concomitantly with the severity of the disease, an approximately 2-fold elevation within gingival fluid of periodontitis subjects being recorded (211.16 ng/ml).

But, an important elevation of the GCF IL-1β is associated to IDDM. The concentrations of this chemical mediator were situated on a foreshore from 347.89 ng/ml in well metabolically controlled IDDM subjects displaying gingivitis, to 1274.67 ng/ml in poorly controlled diabetics with periodontitis (*Figure 3*).

Comparative analysis of the mean gingival fluid IL-1β among the young subjects and correlation with the degree of metabolic control of diabetes points out significant data. Thus, related to the GCF cytokine concentration in control, an increase of 3.21 fold in well-controlled IDDM (378.80 *vs.* 117.85 ng/ml) and a dramatic increase, with a 8.5 magnitude in poorly controlled diabetic individuals (1001.31 *vs.* 117.85), was recorded. The level of significance of the Newman-Keuls test used to compare the average values for IL-1β between control and poor/well controlled IDDM showed significant statistical differences between these values ($p < 0.05$).

Discussions

The prevalence of type 1 DM exhibits a wide range, especially in Europe, the children

being extensively and continuously affected. Patients with diabetes have increased incidence and severity of periodontal disease. Poor glycemic and metabolic control has been consistently associated with periodontal disease severity[5]. The main complications of DM are secondary to the development of microangiopathy, PD being also recognized as the sixth complication of diabetes[6].

Few studies, however, have examined local immune-biochemical reflection of periodontal inflammation in children and teenagers with IDDM. The influence of diabetes on the risk of developing PD has been our point of study, by determination of the extent on which diabetes and the level of its metabolic regulation was related to gingival fluid concentration of IL-1β and AST activity.

In this direction, we had to consider the anatomo-functional particularities of marginal periodontium, the variety of clinical expression for its alterations, all these rendering PD in children and teenagers a continuous subject with many unknowns, of interest to both researchers and clinicians. Considering the clinical evaluation of the studied groups, both, gingival bleeding and level of attachment were positive and significantly correlated with metabolic control of the basic disease (*Table 1*). The lack of any attachment loss in prepuberal period might be explained by the anatomic and functional particularities of this stage and also by a probable very short history of diabetic disease up to that age. Our findings are consistent with some and conflicting with other studies, that found also good correlations of gingival bleeding but no correlation between attachment loss alone and diabetic control[8]. Thus, the relationship between diabetic status and specific periodontal parameters is difficult to be conclusively defined.

The diagnostic potential of AST (as cellular component of tissue degradation) and IL-1β (important mediator of periodontal breakdown) in PD monitorization was evaluated through GCF records of enzyme activity and cytokine concentrations, within nondiabetics and IDDM juvenile subjects. The AST activities were compared between the studied groups, based upon the level of metabolic control of diabetes, age and dental type, in order to evaluate potential specific particularities of the assessed parameters upon these variables.

Experimental data from longitudinal studies of chronic PD in adult population pointed out that GCF content of AST might serve as a site-specific marker for ongoing periodontal destruction[1]. It was suggested by some authors[20] that AST is highly correlated with PD sites in adults with chronic PD, whether diabetics or systemically healthy. To our knowledge, there are no reports concerning pattern of AST activity distribution in GCF of children and teenagers with type 1 DM. In the present study, among young subjects with poor control of their metabolic disease, the AST activities remained higher for all dental patterns when comparing to nondiabetics and metabolically controlled DM group.

The comparison of AST activity on dental position indicates the significant difference between AST values at molar level compared to the other two dental types, the aspect being maintained in all groups (diabetics and nondiabetics – *Figure 1*). The linear increase of gingival fluid AST activities in diabetic individuals (more significant in poor- compared to well controlled DM), in all stages of age, irrespective to dental pattern (but with highest levels recorded around molars), is very probable a reflection of a generalized periodontal destruction associated to diabetic disorder. Moreover, the alveolar lysis and periodontal alteration within diabetic young subjects seems to be more important around molars.

Referring to age as a continuous variable (*Figure 2*), our results showed that, there is a general increase of mean gingival fluid AST with age, and diabetics were significantly more affected than controls. Taking into consideration that our analysis is not based on longitudi-

nal data, the mentioned results should be taken with caution. However, it appeared that in both groups, enzyme peaked around puberty, followed by a slow elevation in the 15-18 years in most of the subjects. These findings could probably be attributed to puberty-hormonal related changes around this age. The present investigation comes as a paraclinical confirmation of some recent studies that, assaying by demographic and clinical periodontal parameters the same bivalent relation between PD-DM in children and teenagers[8], highlights the more prevalent PD in children with DM than those without diabetes, bleeding being also more important at puberal period.

IL-1β has been correlated with PD destruction[12], but to our knowledge, less available data is currently displayed upon its correlations in young type 1 diabetic population, as most of the literature is offering information about the interrelation of cytokine mediated periodontal disorder with type 2 DM and adult population[17]. Our study determined the local concentrations of this cytokine in sites of gingivitis and periodontitis of diabetic and nondiabetic young subjects. The local GCF production of IL-1β increased with increasing inflammation. Diabetics recorded marked IL-1β elevation, irrespective of clinical stage of disease and level of metabolic control. As a consequence, the diabetic status is associated with a considerable 5.85 fold increase of gingival fluid IL-1β (*Table 2*). Furthermore, considering the degree of metabolic regulation, different oral cytokine concentrations, with significant elevations in poorly controlled young diabetics compared to control (8.5 fold increase) have been recorded.

More significant IL-1β differences were registered among gingivitis subjects, 6.65 fold increases ($p = 0.000018$) being recorded between children with poor diabetes regulation and control (*Figure 3*). IL-1β displayed higher levels in the GCF from periodontitis than those from gingivitis patients. These results suggest that there is a strong association between severity of PD and IL-1β concentrations in diabetic children and teenagers. Some recent studies[12] upon adult population with no diabetes history have also stated the correlation between GCF levels of IL-1β and PD.

Our experimental and clinical data offers supportive basis for local production and/or release of IL-1β in PD at concentrations sufficient to mediate tissue inflammation and bone resorption. Considering the young IDDM patients and correlating with clinical indicators of periodontal injury, there are evidences that IL-1β and AST may serve as markers of the extent of diabetes-induced periodontal breakdown.

Conclusion

In conclusion, measurements of IL-1β concentration and AST activity in gingival fluid of IDDM children and teenagers, can reflect the degree of inflammation within periodontal tissue. These data are consistent with the hypothesis that hyperglycemia induces a heightened inflammatory response, and propose a mechanism to account for the associating poor metabolic control to periodontal breakdown in diabetic young individuals. Clinical parameters can thus provide reliable means for evaluation of the severity of PD in IDDM children, and immune-biochemical mediators play a pivotal role in the pathogenic mechanism of periodontal destruction.

Revealing an unexpected high level of GCF mediators among the IDDM subjects even in early stages of life, we suggest that IDDM is a significant risk factor for more severe PD among juvenile population. Therefore, good metabolic control should be the standard care in addressing periodontal complications, in young patients with diabetes. The rich content in reaction end-products associated with periodontal inflammation, facile way of collection, with minimum time, risk, capital and material investment, are reasons that claim GCF as a proper medium for PD markers evaluation in dental practice and research.

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