Association between serum free IGF-I and IGFBP-3 levels in type-I diabetes patients affected with associated autoimmune diseases or diabetic complications

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ABSTRACT. ***Background.*** **Patients with type 1 diabetes (T1DM) present lower serum free IGF and IGFBP-3 values than healthy people. T1DM patients often present with associated autoimmune diseases such as thyroiditis or coeliac disease, and over time they frequently develop proliferative retinopathy, neuropathy or nephropathy in different combinations.** ***Objective.*** **The aim of this study was to evaluate the effect of two associated autoimmune diseases or three diabetic complications on the serum free IGF-I or IGFBP-3 levels in T1DM patients, who also have a family history of T1DM.** ***Design.*** **246 T1DM patients were enrolled, and then subdivided into groups according to diabetic complications or associated autoimmune diseases. Demographic and clinical data were recorded. Serum free IGF-I and IGFBP-3 levels were determined by IRMA.** ***Results.*** **IGF-I and IGFBP-3 generally present correlated serum values as confirmed in this study. Those patients with autoimmune thyroiditis and coeliac disease presented with significantly lower serum values of IGFBP-3, whereas free IGF-I was significantly lower in patients with the different diabetic complications. Retinopathy presented a slightly significant reduction in serum free IGF-I, while neuropathy and nephropathy showed a more pronounced fall. The number of complications was related to progressively decreasing free IGF-I levels. T1DM family history was associated with lower serum free IGF-I concentrations. These findings were confirmed after correction for age, glycosylated haemoglobin levels, insulin treatment protocol, body mass index, serum creatinine and sex.** ***Conclusion.*** **Despite a direct correlation between serum free IGF-I and IGFBP-3, the correlation between the two molecules in patients with associated autoimmune diseases was lost, possibly due to different mechanisms of metabolic regulation.**

Keywords: type 1 diabetes, insulin-like growth factor

Insulin-like growth factors (IGFs) represent one of the most important molecular complexes (the IGF system) involved in the regulation of cell proliferation, body and organ size, fetus development, apoptosis induction, immune and autoimmune disease responses [1, 2]. Two molecules (IGF-I and IGF-II with their two receptors) and 6 binding proteins (IGFBP-1-6) comprise this system. The binding proteins regulate the bioavailability of the IGF-I molecules. In fact, the active form of IGF-I is the free form (free IGF-I) [1, 3, 4]. IGF-I synthesis is mainly induced by growth hormone (GH) in the liver, and its inhibition is mediated by GH release [2]. However, several cells may produce IGF-I induced through GH activity or other modulators, such as cytokines and growth factors [5, 6].

IGF-I has been largely studied in type 1 diabetes (T1DM) [7], since IGF-I exerts functions similar to those of insulin, which is an IGF-I inducer. Some studies reported that T1DM patients present lower serum IGF-I (both free and total) levels [8, 9]. Interestingly, free IGF-I concentrations are reduced in sera of various diseases with different pathogenic mechanisms, including heart acute infarctions and stroke [10, 11], possibly by modulation of suppressors of cytokine signalling (SOCS) genes [12], as suggested by the inverse correlation between blood SOCS-1-3 products and levels of IGF-I [4, 12, 13].

T1DM is a disease of young individuals that, from its beginning or over time, is often accompanied by autoimmune thyroiditis or gluten-sensitive enteropathy (GSE, coeliac disease), two diseases that may also be present concomitantly in the same patient [14]. Besides these two diseases, other diabetic complications may be found in patients with T1DM, such as retinopathy, nephropathy and neuropathy.

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Previous studies have analyzed the possible involvement of IGF-I in the induction of diabetic retinopathy, although with different interpretations [15-17]. A recent report shows that (in a few patients), the reduction in insulin levels or octreotide treatment improves retinopathy in T1DM patients and this is accompanied by a fall in serum IGF-I levels [15]. It is intriguing that T1DM patients have low IGF-I serum levels, although the development of retinopathy has been associated with higher IGF-I levels [16]. In addition, reductions in IGF-I levels have been noted in serum samples from subjects with chronic renal disease and diabetes mellitus [18], while neurodegenerative diseases have been reported as showing increased serum IGF-I levels [19].

Few observations of reduced serum IGF-I levels have been published regarding children with GSE. In these subjects, a significant correlation between serum IGF-I levels and disease evolution, evaluated by the variations in bone alkaline phosphatase levels, was observed, together with weight reduction, antibody positivity and inflammation of small intestinal mucosa [20, 21]. A possible explanation for a mechanism driving the changes in IGF-I levels is malnutrition [22], known to modulate serum IGF-I values [2]. As far as autoimmune thyroiditis is concerned, the mechanism of IGF-I regulation may depend on the effect of T3 and T4 on IGF-I production. In fact, serum free T3 and free T4 levels have been reported to be correlated with free IGF-I levels, independently from GH [19], and T4 administration is followed by an increase in serum IGF-I [23].

This report aims to study the behaviour of free IGF-I and IGFBP-3 in sera of T1DM patients with or without associated autoimmune (thyroiditis or coeliac) diseases and/or the main complications of T1DM already mentioned.

PATIENTS AND METHODS

Patients

This study involved 201 consecutive, unrelated Caucasian subjects, enrolled between January and September 2004, and belonging to a medium social-economic status. The recruitment was carried out by the Diabetes Care Unit of the Catholic University (where they received regular health care), when presenting with a diagnosis of T1DM according to American Diabetes Association (ADA) guidelines. Enrolment inclusion criteria included: 1) Clinical and laboratory diagnosis of T1DM (WHO and ADA guidelines); 2) all T1DM patients diagnosed were aged under 35 and required permanent therapy in order to optimize metabolic control. They were submitted, on a regular basis, to three injections of insulin at meal times, and one of neutral protamine insulin at bedtime. Exclusion criteria were: 1) non-Caucasian patients; 2) C-peptide serum values over cut-off.

The main characteristics of the subjects enrolled in this study are reported in *table 1*. For each patient, HbA1c mean values of the previous year were obtained by means of HPLC analysis performed on Diamat BioRad (BioRad, Milan Italy). The HbA1c reference range for healthy subjects with normal fasting blood glucose was 3.6-5.0% for individuals < 50 years and 4.0-5.3% for patients > 50 years. In addition, fasting C-peptide values were assayed by means of a commercially available RIA kit (Double Antibody C-peptide kit Behring, EURO/DPC, Gwynedd, UK, detection limit = 0.1 nmol/L). All T1DM patients had fasting serum C-peptide values lower than 0.3 nmol/L, considered as the cut-off between negative and positive subjects.

Three complications were considered: 1) *Neuropathy*: all patients had a diagnosis of peripheral neuropathy with a Neuropathy Disability Score greater than 5 and a pathological conduction velocity. Autonomic neuropathy was diagnosed according to a standardized procedure by Ewing and Clarke, including four cardiovascular autonomic tests [24]; 2) *Retinopathy* was evaluated following the EURODIAB IDDM complication study [25]. The diagnosis evaluation was undertaken by a trained ophthalmologist of the ophthalmology unit of our hospital; 3) *Nephropathy* was defined by albumin excretion rate (AER) (normal AER < 30 mg/24 h; micro-albuminuria 30-300 mg/24 h; macro-albuminuria > 300 mg/24 h). In order to be classified as normal, micro- or macro-albuminuria, AERs had to be elevated on at least two occasions in the absence of a urinary tract infection [26].

Body mass index was calculated as weight (kg)/height (expressed as m^2). Serum creatinine determination was performed using the Jaffè method, suitable for the Olympus AU2700 instrumentation.

The diagnosis of thyroiditis was made following the criteria defined by Glastras *et al*. [13]. All of these patients received T4 replacement (L-thyroxine, 1.8-2.0 mg/kg) and were euthyroid (TSH ranged from 0.1 to 1 mU/L) at the enrolment.

The diagnosis of coeliac disease was arrived at by evaluation of anti-transglutaminase and anti-endomisium anti-

Table 1
Characteristics of 201 T1DM patients

Variables	Overall patients n = 201	Males n = 109	Females n = 92	p
Age	38.7 ± 10.8	38.7 ± 10.6	38.8 ± 11.0	0.96
Age at diagnosis	21.2 ± 10.1	21.6 ± 9.0	20.8 ± 11.2	0.59
Disease duration	17.5 ± 11.0	17.1 ± 10.2	17.9 ± 12.1	0.96
Daily insulin requirement (UI/kg per day)	0.60 ± 0.21	0.63 ± 0.22	0.57 ± 0.21	0.06
HbA1c (%)	7.37 ± 0.83	7.34 ± 0.85	7.41 ± 0.82	0.55
Body Mass Index (kg/m^2)	24.7 ± 3.5	25.6 ± 2.9	23.5 ± 3.9	**0.0002**
Serum creatinine (mg/dL)	0.95 ± 0.40	1.00 ± 0.44	0.91 ± 0.34	0.13

Values expressed as years (Mean ± standard deviation). Statistics: Student's t test between males and females.

bodies, and confirmed by histological analyses. For each subject, age, age-at-diagnosis and disease duration were all tabulated. The information regarding all enrolled patients was obtained by means of a questionnaire filled in by all subjects.
According to WHO guidelines, at family history of T1DM was also considered when a patient had at least one first-degree relative affected with T1DM. The study was in agreement with the Helsinki's statement, and was approved by the Ethics Committee of our hospital. All subjects provided informed consent.

Methods

Sample collection: in order to avoid changes to the free IGF-I levels because of delayed time intervals between centrifugation and freezing, blood samples were immediately centrifuged at 4°C and sera were quickly stored at -80 °C until processed.
Free IGF-I assay: serum free IGF-I was measured in triplicate using the IRMA method (Diagnostic Systems Laboratories, Inc. USA, commercialized in Italy by Pantec S.r.l., Turin – Italy) [26]. CV Intra-assay 6-8% on 10 repetitions, CV inter-assay 7-9% on 10 repetitions (two levels).
This assay determines the amount of free IGF-1, plus a component of IGF-1 defined as "readily dissociable". This component depends on the concomitant presence of various IGF binding proteins.
IGFBP-3 assay: serum IGFBP-3 was measured in triplicate using the IRMA method (Diagnostic Systems Laboratories, Inc. USA, commercialized in Italy by Pantec Turin - Italy) [27]. CV Intra-assay 4-6% on 10 repetitions, CV inter-assay 6-8% on 10 repetitions (two levels).

Statistical analysis

X^2 (2x2 tables) or contingency tables (2x more than 2 tables) were used to analyze qualitative results. Students' t test (two groups) or ANOVA (more than two groups) were used to compare quantitative data. The significance cut-off value was $p = 0.05$. Yates' correction or the two-sided Fisher exact test were performed when necessary. Linear regression coefficient (R) was also used to analyze correlations between variables.
Multivariate analysis: the effects on the relationship between T1DM patient groups and free IGF-I or IGFBP-3 values were analyzed in the multivariate logistic model using the "SPSS enter method" and applying stepwise logistic regression ("SPSS backward and forward LR method", program for SPSS 12.0, Chicago, Illinois (USA)). The following potential confounding factors were evaluated: age, gender, HbA1c levels, body mass index, serum creatinine and daily insulin requirement.

RESULTS

Patients' characteristics

No significant differences for age, age-at-diagnosis and disease duration were found between the male and female T1DM patients *(table 1)*. As expected, age and disease duration were higher in subjects presenting retinopathy, nephropathy and neuropathy. Age-at diagnosis-was lower in patients with complications and in coeliac patients; no difference was noted between the group with thyroiditis as compared to other groups *(table 2)*.
Daily insulin requirement, blood Hb1Ac concentrations, and body mass index and serum creatinine levels were also considered to describe the patient group. Only body mass index presented significant differences between genders.

Effect of gender and diabetic complications on free IGF-I and IGFBP-3 levels

Significant differences of free IGF-I, IGFBP-3 levels *(table 3)* were observed between males and females belonging to groups: 1) T1DM **w**ithout complications or associated autoimmune diseases (T1DM-WCOAAD) and 2) T1DM + retinopathy. Males always presented lower levels than females. The thyroiditis group was characterized by significantly lower levels of IGFBP-3 in males, while free IGF-I levels did not show statistical variations. The values observed for IGFBP-3, both in males and females, were also significantly lower than those calculated in the T1DM- WCOAAD groups ($p = 0.024$ and $p = 0.005$, respectively).

Stratification of patients for diabetic complications and associated autoimmune diseases

In order to distinguish the effects of individual diseases on serum free IGF-I and IGFBP-3 concentrations, the patients presenting autoimmune diseases and/or diabetic complications were stratified into subgroups *(table 4)*, excluding concomitant diseases.

Table 2
Means and SD for age, age-at-diagnosis and disease duration in different groups of T1DM patients presenting or not diabetic complications or autoimmune diseases

T1DM patients	**N**	**Age (years)**		**Age at diagnosis (years)**		**Disease duration (years)**	
Complications		**Mean ± SD**	**p**	**Mean ± SD**	**p**	**Mean ± SD**	**p**
None	110	35.8 ± 10.0.	-	22.5 ± 9.7	-	13.2 ± 8.3	**-**
Retinopathy	59	45.0 ± 11.2	**< 0.0001**	18.4 ± 9.0	**0.007**	26.6 ± 10.3	**< 0.0001**
Neuropathy	34	48.9 ± 9.8	**< 0.0001**	18.2 ± 9.0	**0.022**	30.7 ± 8.1	**< 0.0001**
Nephropathy	20	48.6 ± 9.9	**< 0.0001**	18.6 ± 9.9	**0.008**	30.0 ± 8.8	**< 0.0001**
Thyroiditis	33	39.6 ± 9.6	0.06	24.1 ± 12.7	0.46	15.5 ± 11.1	0.21
Coeliac disease	14	36.7 ± 8.4	0.73	15.8 ± 8.9	**0.015**	20.9 ± 9.6	**0.0017**

All groups were compared with the non-T1DM patients (Student's t test). Total number of patients exceeds the 201 overall subject number since some patients were included in more than one group.

Table 3
Means and SD of free IGF-I (ng/mL) and IGFBP3 (ng/mL) in different subgroups of T1DM patients presenting or not diabetic complications or autoimmune diseases

Pts	No.	No.	Free IGF-I (ng/mL)			IGFBP-3 (ng/mL)		
T1DM +	**M**	**F**	**Males**	**Females**	**p =**	**Males**	**Females**	**p =**
None	69	41	0.63 ± 0.57	1.05 ± 0.76	**0.002**	5221 ± 1239	5967 ± 1315	**0.004**
Retinopathy	25	34	0.34 ± 0.17	0.62 ± 0.45	**0.004**	4699 ± 1172	5525 ± 1085	**0.007**
Neuropathy	15	19	0.38 ± 0.16	0.52 ± 0.39	0.20	4775 ± 1216	5404 ± 1198	0.15
Nephropathy	10	10	0.37 ± 0.19	0.52 ± 0.43	0.66	4922 ± 1319	5575 ± 1258	0.28
Thyroiditis	9	24	0.51 ± 0.39	0.91 ± 0.86	0.19	4240 ± 826	5066 ± 995	**0.03**
GSE	6	8	0.35 ± 0.25	0.58 ± 0.28	0.14	4453 ± 921	5055 ± 1257	0.66

T1DM-positive patients may present more than one diabetic complication or autoimmune disease and therefore the total number in the table above is greater than 201. p was calculated between males and females using Student's t test. GSE = gluten-sensitive enteropathy (coeliac disease).

Table 4
Distribution of T1DM patients in function of associated autoimmune diseases or diabetic complications

Number of T1DM patients (201)	**T1DM + Associated autoimmune diseases (45)**					
	Associated diseases/complications	NO	Thyroiditis	GSE	Thyroiditis + GSE	**Total**
T1DM + complications (**59**)	None	110	22	8	2	**142**
	Retinopathy	17	6	2	0	**25**
	Retinopathy + neuropathy	12	1	1	0	**14**
	Retinopathy + neuropathy + nephropathy	17	2	1	0	**20**
	Total	**156**	**31**	**12**	**2**	**201**

Retinopathy = 59 patients, neuropathy = 34 patients, nephropathy = 20 patients, 33 patients with autoimmune thyroiditis, GSE = 14 patients with GSE. GSE = gluten sensitive enteropathy (coeliac disease).

Only two patients were concomitantly affected with thyroiditis and gluten-sensitive enteropathy (GSE - coeliac disease). In addition, all subjects presenting neuropathy or nephropathy were also affected with retinopathy.

Serum levels of free IGF-I and IGFBP-3 in subjects with thyroiditis or GSE

The comparison between the groups described in *table 5* shows that only IGFBP-3 was significantly different between T1DM patient without associated autoimmune disease and those with thyroiditis or GSE. In particular, subjects with thyroiditis presented significantly lower levels of this molecule ($p = 0.02$) as well as GSE patients ($p = 0.03$) when compared to T1DM patients without diabetic complications. Even if T1DM+GSE patients showed a mean free IGF-I level lower than that of T1DM-WCOAAD patients, the comparison was not significant ($p = 0.096$), possibly due to the small number of these patients.

Serum levels of free IGF-I and IGFBP-3 in subjects with diabetic complications (retinopathy, neuropathy and nephropathy)

Patients with T1DM complications, excluding associated autoimmune diseases, characteristically presented significantly reduced serum free IGF-I values, whereas IGFBP-3 remained statistically unchanged. Interestingly, the free IGF-I concentrations decreased progressively with the number of complications *(table 6)*.

Comparison of serum values between associated autoimmune diseases and diabetic complications

A comparison between the 32 patients with one or both associated autoimmune diseases versus the 46 patients

Table 5
Free IGF-I and IGFBP-3 values (median ± DS) in patients with T1DM or T1DM plus autoimmune diseases (patients with diabetic complications excluded)

Groups	Patient number	Free IGF-I (ng/mL)	IGFBP-3 (ng/mL)
T1DM	110	0.781 ± 0.674	5488 ± 1306
T1DM + thyroiditis	22	0.824 ± 0.854	4783 ± 753
T1DM + GSE	8	0.375 ± 0.219	4448 ± 873
T1DM + GSE + thyroiditis	2	0.600 ± 0.424	4697 ± 124
Total	**142**	P1 = 0.59	**P1 = 0.02**
-	**-**	-	**P2 corrected = 0.038**

P1 calculated by ANOVA; GSE = gluten sensitive enteropathy. P2 corrected after adjusting for age, sex, glycosylated haemoglobin, body mass index, serum creatinine and daily insulin requirement.

Table 6
Free IGF-I and IGFBP3 values (median ± DS) in patients with only T1DM or T1DM plus diabetic complications (patients with autoimmune diseases excluded)

Groups	Patient number	Free IGF-I (ng/mL)	IGFBP-3 (ng/mL)
T1DM	110	0.781 ± 0.674	5488 ± 1308
T1DM + retinopathy	17	0.500 ± 0.310	5425 ± 992
T1DM + retinopathy + neuropathy	12	0.400 ± 0.230	4956 ± 1191
T1DM + retinopathy + neuropathy + nephropathy	17	0.365 ± 0.269	5128 ± 1431
Total	**156**	**P1 = 0.008**	P1 = 0.56
	-	**P2 corrected = 0.01**	-

P1 calculated by ANOVA. P2 corrected after adjusting for age, sex, glycosylated haemoglobin, body mass index, serum creatinine and daily insulin requirement.

with diabetic complications *(table 7)* showed that the differences between the values for all three variables studied were statistically significant.

Analysis of serum free IGF-I and IGFBP-3 for family history of T1DM

Significant reductions in free IGF-I (p = 0.04) were observed when T1DM-WCOAAD were subdivided on the basis of T1DM family history. In fact, 12 individuals with a family history of T1DM presented lower values (free IGF-I = 0.400 ± 0.431 ng/mL, IGFBP-3 = 5688 ± 1693 ng/mL) as compared to 98 subjects without a family history of T1DM (0.828 ± 0.686, 5473 ± 1271, respectively). Age-at-diagnosis was significantly lower in patients with a family history of T1DM (15.5 +/- 8.0 versus 23.3 +/- 9.6 years for those without a family history; p = 0.01).

Correlations

As reported in the literature, serum IGF-I and IGFBP3 presented correlated values. This result is confirmed in this study (R = 0.49, p < 0.001, in 201 subjects and R = 0.57, p < 0.001 in 110 subjects without associated autoimmune diseases or diabetic complications) *(figure 1)*. The same figure shows the correlations observed in T1DM subjects with diabetic complications and with associated autoimmune diseases. Comparisons of these plots show that the correlation is lost in the associated autoimmune diseases group, while it is maintained in the remaining ones, although with different slopes. Furthermore, a significant negative correlation was also found between serum free IGF-I and age (R = -0.24, p < 0.001) and disease duration (R = -0.24, p < 0.001).

Data correction for age, sex, glycosylated haemoglobin, body mass index, serum creatinine and daily insulin requirement

The data correction for all the above mentioned variables, by means of multivariate logistic regression analysis, confirmed the results just listed (p values are reported in *tables 5* and *6*), indicating that IGFBP-3 and free IGF-1 are independent risk factors for associated autoimmune diseases and for diabetic complications respectively.

DISCUSSION

The major amount of serum free IGF-I derives from liver stimulation by GH, thus serum IGF-I determination represents a reliable marker of GH function [2, 7]. In addition, free rather than total circulating IGF-I determines the feedback on GH release in normal subjects [3] and is capable of stimulating its cognate receptor production. Serum concentrations of IGF-I decline as from age 25 to 65 years in both genders. Adult males aged under 65 years present lower serum IGF-I values as opposed to females in the same age group, whereas females over 65 year show lower serum concentrations [2, 4, 10, 28] compared to males.

Many cells may synthesize and release IGF-I, which has several functions both at the local and systemic levels [2]. Besides GH induction, other independent enhancers of the IGF system have been described, including oestrogens in the uterus, FSH in the ovary, PTH in bone tissue and TGF-beta 1. Recently, other molecules have been shown to be involved in the inhibition of the IGF-I system, in particular those defined as suppressor of cytokine

Table 7
Comparison of free IGF-I and IGFBP-3 values between associated autoimmune diseases and diabetic complications

Groups	Patient number	Free IGF-I (ng/mL)	IGFBP-3 (ng/mL)
1) T1DM+ diabetic complications	46	0.424 ± 0.277	5193 ± 1209
2) T1DM + autoimmune disease	32	0.574 ± 0.379	4653 ± 747
3) T1DM + autoimmune disease + diabetic complications	13	0.608 ± 0.410	4823 ± 1429
p (1 *versus* 2) =	-	**0.047**	**0.028**
p (1 *versus* 3) =	-	0.064	0.35

p was calculated by ANOVA. No significant differences between 2) and 3) for the three variables.

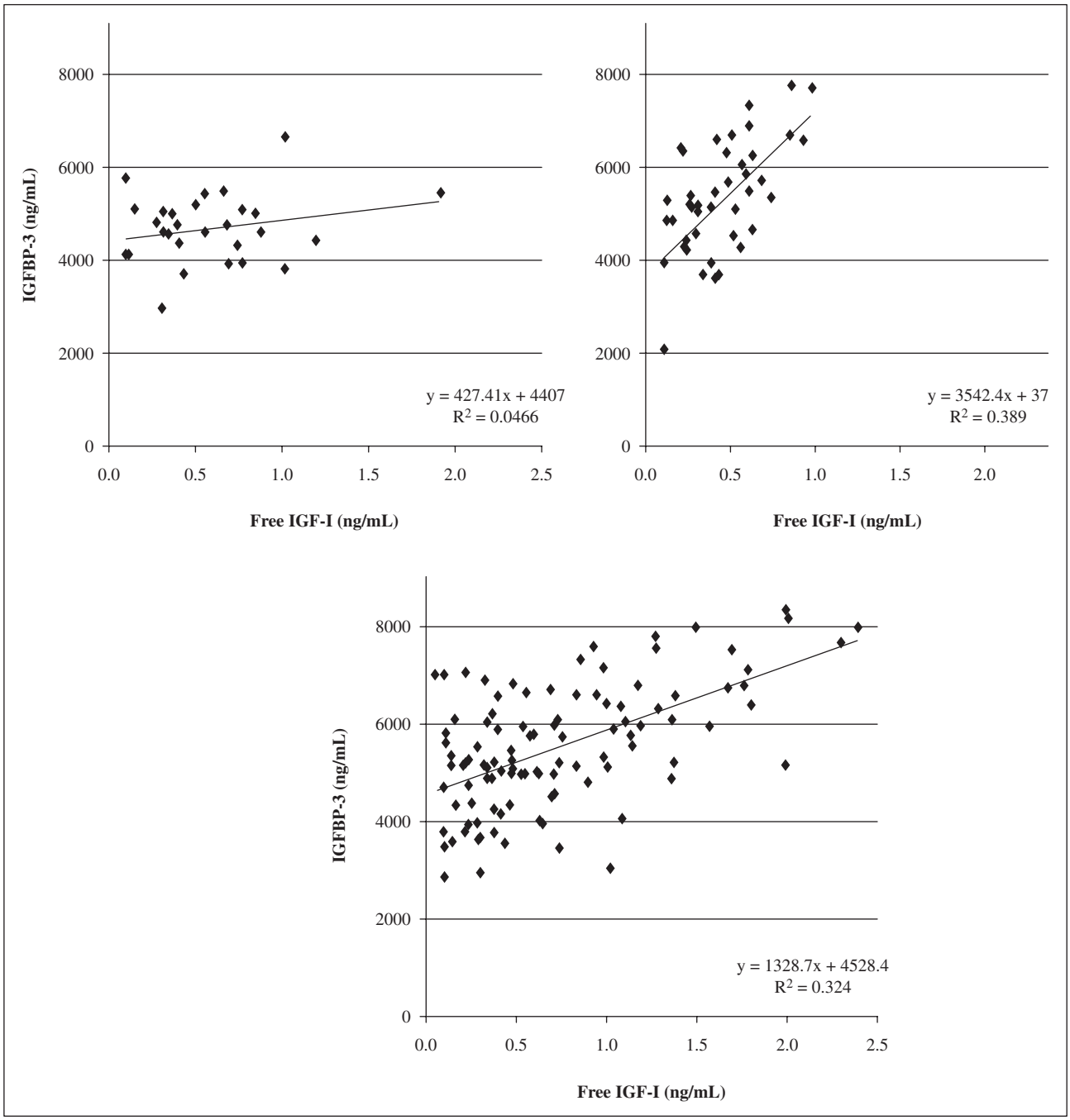

Figure 1

Correlations between serum free IGF and IGFBP-3 in three groups of patients: Top-left) T1DM with associated autoimmune diseases (R = 0.22); Top-right) T1DM patients with diabetic complications (R = 0.62); Bottom-centre) T1DM without diabetic complications and without associated autoimmune diseases (thyroiditis and GSE) (R = 0.57).

signalling1-3 (SOCS), as well as some inflammatory cytokines such as TNF-alpha and IL-1 beta. The latter indirectly acts through the SOCS molecules induction [4]. IGF-I induction by GH determines a concomitant increase of IGFBP-3. However, other mechanisms of regulation of this molecule are separately active, as well as the p53 or TGFβ-1 induction [29, 30]. Moreover, TNF-alpha seems to induce IGFBP-3 synthesis on foetal condrocytes [30], while IGFBP-3 from human adult condrocytes is reported to be enhanced by IL-1 and TNF-alpha [31, 32].

FT4 and FT3 hormones are also known to directly correlate with IGFBP-3 values, independently of GH [23]. Therefore, patients with hyperthyroidism are expected to show higher serum values for IGFBP-3 molecules [20, 23] and, consequently, our patients with thyroiditis presented lower IGFBP-3 concentrations, even after T4 replacement. Finally, the relationship between IGF-I and IGFBP-3 values may be influenced by individual genetic characteristics, high IGF-I intracellular consumption and activity of IGF-I receptor.

Free IGF-I levels are generally decreased in several diseases, including diabetes [33, 34], intestinal inflammation [35], heart failure [10], ischemic stroke [10], stress and physical overtraining [36]. In these conditions, serum IGFBP-3 values are generally correlated with the free IGF-I levels [2, 28, 37].

Previous literature data reported IGF-I and IGFBP-3 levels in some autoimmune diseases associated with T1DM (thyroiditis and coeliac disease) [20, 21, 38-41] as well as in common diabetic complications of T1DM [14, 16, 17, 42-44], such as retinopathy [17, 43, 45], neuropathy [26] and nephropathy [18]. Most of these studies analyzed total IGF-I values more frequently than free IGF-I, although only the latter is considered the really active molecule [34]. A limit of the method used to evaluate the free IGF-1 molecules is that this method also reveals a component of readily dissociable IGF-1. The present study did not evaluate the total serum IGF-1 levels, and therefore a comparison between the concentrations of these two forms of IGF-1 cannot be done.

No studies have analyzed free IGF-I and IGFBP-3 values in T1DM patients, when considering the concomitant pathologies and their possible associations. In this study, a stratification of T1DM subjects was performed, including autoimmune diseases and diabetic complications. Interestingly, the results obtained showed that serum IGFBP-3 values were significantly lower in coeliac disease and thyroiditis patients than in T1DM-WCOAAD controls: a significance that was confirmed after adjusting for age, sex, glycosylated haemoglobin, body mass index, serum creatinine and daily insulin requirement. A similar decline was not found for free IGF-I. As a consequence, the correlation between the two molecules in patients with associated autoimmune diseases was lost when compared to the correlation observed in T1DM with diabetic complications and that of patients without autoimmune diseases or diabetic complications.

Despite the number of patients with diabetic complications being not very high, the statistical analysis clearly showed that the number of complications for each individual induces a progressively decreasing value of free IGF-I. This finding suggests that free IGF-I is a highly sensitive marker of cumulative T1DM complications, independent of age, sex, glycosylated haemoglobin, body mass index, serum creatinine and daily insulin requirement.

A familial history of T1DM also seems to be more closely associated with free IGF-I as opposed to IGFBP-3 values. This finding, also observed for the first time in this study, might suggest a worse clinical situation for familial T1DM, possibly linked to a genetic basis [46, 47]. In fact, more gene activities may be involved in the IGF-I modulation. The most interesting one is represented by the SOCS genes, which might represent one of the general pathophysiological mechanisms regulating IGF-I levels. In particular, SOCS1-3 seems to be very important for the inhibitory effects on IGF-I synthesis, since SOCS molecules are induced by inflammatory cytokines such as those released in autoimmune processes or diabetic complications [47]. Homozygosity for the A-allele of the C-920 $\rightarrow$ A promoter polymorphism of the SOCS-3 gene has been associated with increased, whole-body insulin sensitivity. If confirmed, this association might be involved in the relationships between a family history for T1DM and serum free IGF-I levels [46, 47].

SOCS production is therefore a mechanism able to control the GH-IGF axis, through a first molecular domain capable of inhibiting different cell receptors of these molecules, as well as several cytokines, and a second domain capable of enhancing IGF-I degradation [12, 13].

In conclusion, even if the mechanisms driving the relationships between IGF-I and IGFBP-3 are still to be clarified in different diseases, this paper shows the different behaviours of these two molecules in T1DM complications and T1DM-associated autoimmune disease, underlining, at the same time, the effect of family history in lowering the free IGF-I values.

A real effect of inflammatory processes, such as those active in diabetic complications and associated autoimmune disease, may be speculated, considering that the effective T4 replacement therapy administered in patients affected with thyroiditis did not restored the free IGF-1/IGFBP-3 correlation. At present, the recent information available on the involvement of SOCS genes and cytokine effects may fit with our findings.

Greater exploration of these fields is therefore necessary for a better understanding the phenomena described in this report.

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