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INTERLEUKIN-1β EXPRESSION IS ASSOCIATED WITH FASTING BLOOD GLUCOSE LEVELS IN INDIVIDUALS WITH METABOLICALLY DECOMPENSATED TYPE 2 DIABETES, AND AFTER HYPERGLYCEMIA NORMALIZATION TREATMENT

Andrea Elena Iglesias Molli*[1], Fernanda Bergonzi[2], Mónica Spalvieri[3], María Amelia Linari[4], Gloria Edith Cerrone[5] & Gustavo Daniel Frechtel[6]

[1]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina.
[2]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina.
[3]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina.
[4]Unión Obrera Metalúrgica (UOM).Sección de Endocrinología y Nutrición. Vicente López, Argentina.
[5]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina.
Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Microbiología, Inmunología y Biotecnología. Cátedra de Genética. Ciudad Autónoma de Buenos Aires, Argentina.
[6]CONICET-Universidad de Buenos Aires. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Laboratorio de Diabetes y Metabolismo. Ciudad Autónoma de Buenos Aires, Argentina

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Hyperglycemia
Inflammation.

Abstract

Type 2 diabetes (T2D) is characterized by a systemic subclinical inflammation state, which was related with an increase in the expression of pro-inflammatory molecules. Interleukin 1-beta (IL-1β) is one of most important cytokines related to that inflammation state in T2D. The aim of our work was to study the IL-1β serum levels and mRNA expression in mononuclear leucocytes, and to evaluate its relationship with the genotype of the rs16944 (-511C/T) of IL-1β gene, and with biochemical-clinical variables, in 30 recently diagnosed individuals with metabolically decompensated T2D, and after 6 and 12 months of treatment to achieve metabolic compensation. Statistical analyses were performed by multiple linear regression, whit age and gender as covariates. At basal time, the T polymorphic allele of the rs16944 was associated with lower IL-1β mRNA expression (p=0.006); and the higher glycemia was associated with higher IL-1β protein levels (p=0.015). Despite individuals showed a significant decrease in fasting plasma glucose (FPG) and HbA_{1c} after treatment, and we did not found significant changes in IL-1β mRNA expression and protein levels; the greater decreases in FPG and HbA_{1C} were associated with increases in IL-1β circulating levels (p=0.040). These results contribute to the knowledge of the physiopathology of T2D.

Introduction

Diabetes mellitus is a major public health problem, which in recent decades has become a pandemic of uncontrollable proportions [1]. About 90% of the individuals with diabetes mellitus have type 2 diabetes (T2D). In Argentina the prevalence of T2D is close to 10%, similar to that of other Latin American countries and most of the industrialized countries [2,3]. Like other metabolic disorders, T2D is characterized by a systemic subclinical

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inflammation state, secondary to innate immunity hyperactivity [4], which was related with an increase in the expression of pro-inflammatory cytokines.

Interleukin 1-beta (IL-1β) is one of most important pro-inflammatory molecules related to that systemic subclinical inflammation in T2D. It is produced by macrophages deposited in different tissues and by circulating monocytes in response to stimuli such as lipopolysaccharides from the gut microbiota, between others [5]. IL-1β is the prototype of a pro-inflammatory molecule related to pancreatic β-cell function, viability and replication, since pancreatic β cells have been shown to express the IL-1β receptor (IL-1R) [6]. Sustained activation of IL-1R by IL-1β, in the chronic subclinical inflammation situation, causes a progressive decrease in pancreatic β-cell functionality and promotes its death by apoptosis. Modulation of the inflammatory process in the pancreatic islets of individuals with T2D, and in particular the modulation of IL-1β as the mayor intra-islet inflammatory mediator, appears to be a promising therapeutic approach [7].

The deleterious effects of IL-1β in pancreatic β-cells are significantly increased in hyperglycemia states. It was found that the increasing concentrations of glucose causes an increase in the production and release of IL-1β in pancreatic islets [8]. Both persistent hyperglycemia and postprandial hyperglycemia induced the expression of the IL-1β gene in rats, as evidenced by increased levels of IL-1β protein and mRNA in peripheral blood monocytes [9,10]. In a paper published in the Japanese population, circulating levels of IL-1β were found to be significantly correlated with fasting plasma glucose (FPG) levels up to 125 mg/dL, and it was observed that at higher FPG levels there is a greater significant positive correlation [11]. It was also reported that circulating levels of IL-1β were associated with the consumption of foods with high glycemic indexes in men and women with metabolic syndrome (a pre-diabetic state) [12].

T2D is one of the complex, prevalent and heterogeneous diseases, whose etiopathogenesis, not yet completely elucidated, is based on multiple interactions between genetic predisposing factors and environmental triggers [13]. Single nucleotide polymorphisms (SNPs) constitute the genetic basis of prevalent complex diseases, and functional SNPs described in the IL-1β gene have been associated with metabolic diseases [14].

Thus, the low-grade inflammation status of individuals with T2D may be associated with increased expression of IL-1β from activated monocytes in peripheral blood. An altered metabolic microenvironment, in which activated monocytes increased the expression of inflammatory cytokines, may be involved in the development of T2D. In this sense, the influence that micro environmental situations such as hyperglycemia on the expression levels of these pro-inflammatory molecules have not yet been explored in the same individual with T2D. The principal aim of our work was to study the variations in IL-1β mRNA expression in mononuclear leucocytes, and in serum IL-1β levels, in recently diagnosed individuals with metabolically decompensated T2D, and after 6 and 12 months of treatment to achieve metabolic compensation. The secondary aim was to evaluate the relationship between the mRNA expression and serum levels of IL-1β with the genotype of the rs16944 SNP (-511C/T) present in the promoter of IL-1β gene, and with biochemical and clinical variables.

Materials and methods

Population

A prospective controlled study was conducted in individuals with T2D, where the same individual is analyzed pre and post-intervention to a pharmacological treatment and changes in lifestyle. We recruited 30 individuals with recently diagnosed T2D, with hyperglycemia characterized by the glycosylated hemoglobin (HbA_{1c}) levels greater than 8%, which constitute the pre-intervention group (0 months of treatment). Inclusion criteria were: unrelated Caucasian adults; of both sexes; between 30 to 70 years old; native argentine; resident in urban areas of Buenos Aires; without prior personal pathological antecedents except for the possible existence of hypertension, dyslipidemia and/or overweight/obesity. The exclusion criteria were: any pathology except for cardiovascular risk factors; acute myocardial infarction, stroke, pregnancy; psychiatric background; alcoholism; drug addiction; history

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or suspicion of pancreatitis; recent intake of hyperglycaemic agents (<3 months); and instability in body weight (loss >3kg in the last 6 months). These individuals received pharmacological treatment and hygienic-dietary measures, including physical activity and dietary measures, in order to achieve good metabolic control. For each individual, first-line treatment was prescribed to achieve the therapeutic goal. All individuals were reanalyzed after 6 and 12 months of treatment, which constitute the post-intervention groups. Of the 30 individuals recruited, 3 individuals (10%) dropped out the protocol without any particular reason. Therefore, the prospective analysis was performed on 27 individuals of which pre and post-intervention samples were obtained. The study was approved by the Ethics Committees of the Hospital de Clínicas "José de San Martín" of Buenos Aires, and all participants gave their written informed consent.

All participants informed their age and gender. Anthropometric measurements (height, weight and waist circumference), and systolic and diastolic blood pressure (SBP and DBP, respectively) were determined by standardized protocols. Body mass index (BMI) was calculated as weight (kg) / [height (m)]^2. After 12 hours overnight fast, venous blood samples were drawn of every individual. Two aliquots of blood anticoagulated with EDTA K_2 were reserved for mRNA and DNA extraction, and one aliquot of serum was reserved for measurement of IL-1β serum concentrations. All biochemical determinations were done immediately (<6 hours). FPG were determined using standardized procedures, in plasma obtained by the centrifugation of blood anticoagulated with sodium fluoride. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined in serum using standardized procedures. HbA_{1c} was determined by immunoturbidimetric method in an Architect system, with calibrators and controls that correlated with the national standardization program in the USA (NGSP and IFCC). The reference values for HbA_{1c} were 4.40-6.40% for non-diabetic, and 6.00-8.00% for controlled diabetic individuals.

Quantification of serumIL-1β levels

In order to evaluate the IL-1β protein expression, IL-1β concentrations were measured in serum stored at -80°C for short periods (<1 month) by Chemiluminescencemethod, with a sensitivity of 1.5 pg/mL and a measurement range of 1.5-1000 pg/mL. The reference value for serum IL-1β was <5 pg/mL.

Relative quantification of mRNA expression

The aliquot of blood reserved for the extraction of mRNA was processed immediately. The mononuclear cells were separated with Ficoll (FicollPaque Plus, GE Healthcare) and resuspended in guanidiniumthioisocyanate (TRIzol, Invitrogen Corp., Carlsbad, CA) for subsequent extraction of total RNA. Under these conditions, the samples were stored at -80ºC for a maximum period of 3 months. The quality of the extracted RNA was checked on 1% agarose gels. The possible remaining DNA was removed with DNAseI (Amplification Grade DNAseI, Life Technologies). RNA was reverse-transcribed with Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT, Invitrogen Corp., Carlsbad, CA) and DNA hexamers as random primers (Life Technologies), to obtain the first strand of the complementary DNA (cDNA). Gene expression was analyzed by quantitative real time PCR with the StepOne system (Applied Biosystems), using the following primers: IL-1β: forward 5'-ATGATGGCTTATTACAGTGGCAA-3' and reverse 5'-GTCGGAGATTCGTAGCTGGA-3'; TLR4: forward 5'-TAATCCCCTGAGGCATTTAGG-3' and reverse 5'-CCCCATCTTCAATTGTCTGG-3'; and GADPH: forward 5'-TGCACCACCAACTGCTTAGC-3' and reverse 5'-GGCATGGACTGTGGTCATGAG-3'. PCRs were performed in a final volume of 20 µL containing 20 ng RNA, 1X SYBR Green Master Mix (SYBR Select, Applied Biosystems) and 250 nM of each primer. The PCR conditions were: 2 min at 50ºC and 10 min at 95ºC, followed by 40 cycles of 15 sec at 95ºC and 1 min at 60ºC and a melting curve was performed in every assay to evaluate the specificity of the primers with 1 cycle of 15 sec at 95ºC, 1 min at 60ºC, and 95ºC with a temperature ramp of 0.1ºC/sec. Each sample was analyzed in duplicate, with GADPH as the normalizing control gene. The pre-intervention and both post-intervention samples for each individual were amplified in the same assay, and all measurements included the determination of a no-template negative control, in which the cDNA was substituted by water. The $2^{-\Delta\Delta Ct}$ method was employed for relative quantification of gene expression [15]. ΔCt was calculated as the difference between the Ct of the target gene (IL-1β) minus the Ct of the endogenous control gene (GADPH) for each sample. For the pre-intervention group analysis ΔΔCt was calculated as the ΔCt of each pre-intervention

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sample minus the average ΔCt of all pre-intervention samples, and the $2^{-\Delta\Delta Ct}$ was calculated, representing the fold change in mRNA expression of each pre-intervention sample in comparison with the average expression. For the prospective analysis ΔΔCt was calculated as the ΔCt of the post-intervention sample (6 and 12 months) minus the ΔCt of the pre-intervention sample (0 months), and the $2^{-\Delta\Delta Ct}$ calculated represents the fold change in mRNA expression after the intervention. We also calculate the $2^{-\Delta Ct}$ of each sample to evaluate the IL-1β mRNA expression levels normalized by the endogenous control at each time of the study.

Genotyping

We genotyped the rs16944 (-511C/T), located in the promoter of the IL-1β gene, that substitutes the ancestral cytosine (C) with a thymine (T) at nucleotide position -511.

The aliquot of blood reserved for DNA extraction was conserved at -4ºC for short periods of time (<3 months) until its use. DNA was extracted by the cetyltrimethylammonium (CTAB) method [16]. DNA quantification was performed with Qubit 2.0 Fluorometer (Invitrogen Corp., Carlsbad, CA) and samples were prepared at concentrations of 3.3 ng/μL. DNA samples were shipped to LGC Genomics in the UK (http://www.lgcgroup.com) and the genotyping of the SNP was performed by KASP (Kompetitive Allele Specific PCR). KASP technology is a uniplex SNP genotyping platform, developed by LGC Genomics, that utilizes a competitive allele-specific PCR. The KASP assay was carried out with primers with a universal FRET (fluorescent resonance energy transfer) cassette (FAM and HEX), ROX passive reference dye, Taq polymerase, free nucleotides and $MgCl_2$ in an optimized buffer solution. The primers sequences were specific to the SNP to be targeted and were designed by LGC Genomics. They consisted in two competitive allele-specific forward primers and one common reverse primer. Each forward primer incorporates an additional tail sequence that corresponds with one of two universal FRET cassettes.

Statistical evaluation

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). A p value below 0.05 was considered as statistically significant. We considered the logarithms of the $2^{-\Delta\Delta Ct}$ values for the analysis of mRNA expression, and the logarithms of the pg/mL for the analysis of serum IL-1β levels, since those variables showed normal distribution. In the pre-intervention group, we use multiple linear regression, whit age and gender as covariates, to assess the association between IL-1β mRNA expression, serum IL-1β levels, and biochemical and clinical variables. To evaluate the effect of hyperglycemia on IL-1β expression, we created groups with different levels of HbA_{1c} (<10%; 10-11.99%; ≥12%) and FPG (<140 mg/dL; 140-199 mg/dL; ≥200 mg/dL), and compare the IL-1β mRNA expression and serum IL-1β concentrations in each level by multiple linear regression, with age and gender as covariates. Allele and genotype frequencies were calculated by direct counting, and the Hardy-Weinberg equilibrium was assessed by Chi square test. The association between the rs16944 SNP genotype (through dominant, recessive and allelic load models) and IL-1β mRNA expression was also performed by multiple linear regression, whit age and gender as covariates. In the prospective controlled study, the comparison of biochemical and clinical variables, and the levels of gene expression in the individuals in each stage of the study were performed by one-way ANOVA for paired samples and Bonferroni *post-hoc* test. For the analysis of IL-1β mRNA expression we compare the logarithms of the $2^{-\Delta Ct}$ values of the pre and post-intervention stages. The variations in the serum IL-1β levels and in the biochemical and clinical variables were calculated as the value of the post-intervention sample (6 and 12 months) minus the value of the pre-intervention sample (0 months). The fold change in IL-1β mRNA expression and variations in serum protein levels were compared with the variations in biochemical and clinical variables, with the rs16944 SNP genotype, with the type of treatment and with the dose of each antidiabetic drug, by multiple linear regression, whit age and gender as covariates.

Results

Pre-intervention group analysis

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The overall population consisted of 7 women (23.33%) and 23 men (76.67%), with a mean age of 48.17 years (SD=12.37; range: 23-69 years). Table 1 shows the biochemical and clinical characteristics of the population. The biochemical-clinical profile of the pre-intervention group is the expected for individuals with T2D.

The median IL-1β serum concentration at the beginning of the study was 10.40 pg/mL (interquartile range [IQR]: 2.30 - 19.50 pg/mL). 13 individuals (43.33%) showed values within the reference range (<5 pg/mL) and 17 individuals (56.67%) above it (≥5 pg/mL). There was no association between IL-1β mRNA expression and serum protein levels. At the basal time, serum IL-1β level was positively associated with FPG, where the higher intervals of glycemia were associated with the higher IL-1β protein levels (Table 2). IL-1β mRNA expression did not associate with any biochemical-chemical variable analyzed.

The genotypic frequencies of rs16944 in the IL-1β gene were as follows: 8 individuals were CC (26.7%), 15 individuals CT (50.0%) and 7 individuals TT (23.3%). The SNP was in good accordance with the expected genotype distributions calculated by the Hardy–Weinberg law (p=0.995). The study by a dominant model showed a significant association between the presence of the T polymorphic allele (genotypes CT and TT) and the lower IL-1β mRNA expression at the basal time of the study (p=0.006, r=0.831, 95% CI r=0.258/1.405).

Prospective controlled study

The prospective study was carried out in the 27 individuals who finished the protocol. This population consisted in 7 women (25.90%) and 20 men (74.10%), with a mean age of 49.48 years (SD=12.33; range: 23-69 years). The pharmacological treatment of choice in 19 individuals was metformin in doses between 500 and 2550 mg/day; in 5 individuals, it was a combined treatment between metformin and insulin; 1 individual received metformin and glibenclamide; 1 individual received vildagliptin; and 1 individual did not receive pharmacological treatment. In all individuals the treatment included dietary measures and physical activity. Table 1 shows the biochemical and clinical characteristics of the population in the pre and post-intervention stages. The 27 individuals obtained the goal of glycemic control after 6 months of treatment, which was maintained after 12 months, demonstrated by the significant decrease in FPG and HbA_{1c} at post-intervention stages. When analyzing the other biochemical-clinical characteristics, it can be seen a significant increase in HDL-C after 12 months of treatment. The other metabolic variables did not rich significant differences.

We analyzed IL-1β mRNA expression and serum protein levels and we found no significant differences after 6 and 12 months of treatment (Figure 1). The fold change in IL-1β mRNA expression did not associate with any variables analyzed. However, a significant negative association between the variation in serum IL-1β levels after 6 months of treatment and the variation in HbA_{1c} was observed (p=0.021, r=-0.004, 95% CI r=-0.007/-0.001). Also, we found a significant negative association between the variation in serum IL-1β levels after 12 months of treatment and the variation in HbA_{1c} (p=0.004, r=-0.004, 95% CI r=-0.006/-0.001) and in FPG (p=0.040, r=-0.102, 95% CI r=-0.198/-0.005). In all cases, the individuals with the highest glycemia and HbA_{1c} declines were those who showed a higher increased in IL-1β protein levels. The variation in IL-1β serum levels did not associate with any other variables analyzed.

We found no association between the rs16944 genotype and the fold change in IL-1β mRNA expression after 6 and 12 months of treatment.

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Figure:

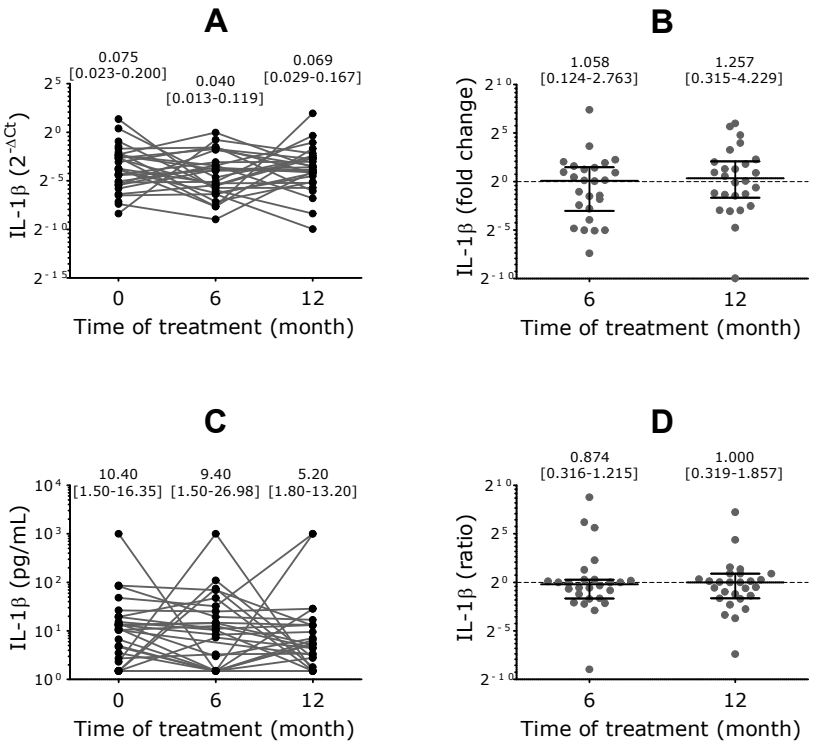

Figure 1: IL-1β mRNA expression and serum levels at each time of the study and variation after 6 and 12 months of treatment. (A) Levels of IL-1β mRNA expression and (B) fold change after treatment. (C) Levels of IL-1β serum protein and (D) ration of variation after treatment calculated as the serum protein level after treatment (at 6 and 12 months) divided by the initial serum protein level (0 months). For each data set the median and interquartile range are informed. Statistical evaluation: One way-ANOVA for paired samples and Bonferroni post-hoc test. For the statistical analysis we considered the logarithms of the $2^{-\Delta Ct}$ values for mRNA expression and the logarithms of the pg/mL for serum protein levels.

Tables:

Table 1: Biochemical and clinical characteristics of the population at each time of the study.

	Pre-intervention group (n = 30)	**Prospective controlled study** (n = 27)					
		Time of intervention (month)			**ANOVA**	**Bonferroni *post-hoc* test**	
		0	**6**	**12**		**0-6**	**0-12**
	M ± SD	**M ± SD**	**M ± SD**	**M ± SD**	**P**	**p**	**p**
Weigth (kg)	95.8 ± 21.5	93.8 ± 21.2	90.8 ± 19.5	90.6 ± 19.8	NS	NS	NS
BMI (kg m^{-2})	34.24 ± 5.57	33.79 ± 5.52	32.88 ± 5.31	32.74 ± 5.66	NS	NS	NS
Waist circumference (cm)	108.1 ± 14.6	106.8 ± 14.8	106.6 ± 14.1	108.0 ± 13.9	NS	NS	NS

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SBP (mmHg)	135 ± 21	134 ± 21	127 ± 12	129 ± 15	NS	NS	NS
DBP (mmHg)	81 ± 14	81 ± 14	75 ± 11	76 ± 11	NS	NS	NS
HbA_{1c} (%)	9.74 ± 1.88	9.69 ± 1.92	6.52 ± 0.99	6.38 ± 1.45	< 0,001	< 0,001	< 0,001
FPG (mg dL^{-1})	200 ± 88	195 ± 87	123 ± 45	121 ± 43	0,005	0,003	0,003
TC (mg dL^{-1})	213 ± 70	212 ± 73	206 ± 50	192 ± 30	NS	NS	NS
HDL-C (mg dL^{-1})	41 ± 8	40 ± 7	42 ± 9	44 ± 9	0,005	NS	0,011
LDL-C (mg dL^{-1})	121 ± 28	119 ± 29	118 ± 34	114 ± 25	NS	NS	NS
TG (mg dL^{-1})	250 ± 357	258 ± 376	214 ± 108	177 ± 101	NS	NS	NS

Statistical evaluation: One way-ANOVA for paired samples and Bonferroni post-hoc test. BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycosylated hemoglobin; FPG: fasting plasma glucose; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; M: mean; SD: standard deviation; NS: not significant.

Table 2: IL-1β mRNA expression and serum protein levels of the individuals at the basal time of the study (pre-intervention group), according to HbA1c (A) and fasting plasma glucose (B) levels.

A	**HbA_{1c}**					
	< 10 % (n = 18)	**10 - 11.99 %** (n = 8)	**≥ 12 %** (n = 4)	**Linear regression**		
	m (IQR)	**m (IQR)**	**m (IQR)**	**p**	**r**	**95% CI r**
IL-1β mRNA expression ($2^{-\Delta\Delta Ct}$)	1.02 (0.28 – 3.44)	0.67 (0.20 – 3.03)	1.36 (0.45 – 2.62)	NS		
IL-1β serum levels (pg mL^{-1})	11.70 (1.50 - 16.35)	6.55 (1.50 - 12.63)	26.45 (2.90 - 762.05)	NS		
B	**FPG**					
	< 140 mg/dl (n = 11)	**140 - 199 mg/dl** (n = 7)	**≥ 200 mg/dl** (n = 12)	**Linear regression**		
	m (IQR)	**m (IQR)**	**m (IQR)**	**p**	**r**	**95% CI r**
IL-1β mRNA expression ($2^{-\Delta\Delta Ct}$)	1.23 (0.23 – 7.62)	0.41 (0.15 – 2.18)	1.32 (0.66 – 2.77)	NS		
IL-1β serum levels(pg mL^{-1})	3.50 (1.50 - 13.50)	6.70 (1.50 - 14.00)	13.25 (10.40 – 74.83)	0.015	0.378	0.078 / 0.678

Statistical evaluation: Multiple linear regression. The dependent variable was considered the logarithms of the 2-ΔΔCt value for IL-1β mRNA expression and the logarithms of pg/mL for serum IL-1β levels, since those variables showed normal distribution. Covariates: age and gender. m: median; IQR: interquartile range; HbA1c: glycosylated hemoglobin; FPG: fasting plasma glucose; r: regression coefficient; 95% CI r: regression coefficient 95% confident interval; NS: not significant.

Discussion

In the present work we describe the mRNA expression and serum protein levels of IL-1β in newly diagnosed individuals with metabolically decompensated T2D and after 6 and 12 months of treatment when they obtained metabolic compensation, and we evaluate the association between the rs16944 genotype with mRNA expression and serum levels of IL-1β and with biochemical and clinical variables.

Our results show that the genotype of rs16944 (-511C/T) present in the promoter of the IL-1β gene has an effect on the transcription of the gene, where the presence of the polymorphic T allele was associated with a lower expression of mRNA. This is in agreement with previous publications were an specific IL-1β haplotype (-3893G, -1464G, -511C and -31T) in the promoter region of IL-1β was described to be associated with increased IL-1β mRNA [17]. Moreover, significantly lower secretion of IL-1β was found to be associated with CTC haplotype -1470G/C, -511T/C, and -31C/T in the promoter of the gene [18]. Polymorphisms in the IL-1β gene promoter region alter the inducing effect of lipopolysaccharide (LPS) on IL-1β gene transcription, leading to susceptibility to inflammatory diseases [19]. Wen et al. suggested that IL-1β promoter haplotypes influence the expression and transcriptional

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activity of the IL-1β gene after LPS exposure, and observed an up regulation in subjects with haplotype GCT (-1470G, -511C, and -31T) [20].

Misaki et al studied healthy and preclinical middle-aged non-overweight and overweight Japanese men and found that plasma IL-1β concentrations significantly associated with glycemia levels up to 125 mg/dL [11]. We were able to replicate these results in individuals with hyperglycemic states (≥ 126 mg/dL), where individuals with higher FPG levels presented higher levels of serum IL-1β. There was also observed that serum pro-inflammatory cytokines, including IL-1β, were significantly higher in individuals with hyperglycemic crises compared with controls, related with an significantly increased oxidative stress [21]. In vitro and in vivo studies had demonstrated that pancreatic beta cells produce IL-1β when exposed to high glucose levels. In vitro exposure of islets from non-diabetic organ donors to high glucose levels resulted in increased production and release of IL-1β, followed by NF-kappaB activation, Fas upregulation, DNA fragmentation, and impaired beta cell function. In addition, it has been observed that beta cells produce IL-1β in pancreatic sections of T2D individuals, but not in non-diabetic individuals [8]. It was also demonstrated in human and murine adipose tissue that high glucose levels induce an increase in mRNA and protein expression of IL-1β, by the activation of caspase-1 and TXNIP. [22]. Pancreatic islets of T2D GK rats presented an increased macrophage infiltration and were demonstrated to have an increased expression of IL-1β, between other pro-inflammatory markers. In these rats, the increased expression of IL-1β was also observed in other tissues, mainly in the liver [23].

It has been described that the regulation of IL-1β expression is very complex, and depends on at least two independent mechanisms, one at the level of transcriptional activation and another at the level of translation efficiency [24], which could justify the lack of association between mRNA and protein levels of IL-1β that we found. Since we associated the glycemia levels with the serum protein levels of IL-1β, but not with mRNA expression, we can suggest that glycemia is one of the factors that exert an effect on the expression of IL-1β at the level of the translation. Furthermore, it must be considered that we measure mRNA in mononuclear cells and there are other sources that may contribute to the circulating protein.

Despite we found a positive association between serum IL-1β levels and glycemia; and despite individuals showed a significant decrease in glycemia after treatment, but did not show significant changes in the serum IL-1β levels; the greater decreases in glycemia after treatment were associated with increases in circulating levels of IL-1β. Endoplasmic reticulum (ER) dysfunction in pancreatic cells is one of the physiopathogenic mechanisms that determine the development of T2D [25,26]. It is prompted by the state of hyperglycemia, among other mechanisms, and resulted in a lower production of insulin and the activation of autophagy signals. Considering that the levels of pathological hyperglycemia may have the same effects on ER of cells that produce IL-1β, the decrease in glucotoxicity would favor the production of proteins such as IL-1β. This would justify the negative association found between the variation in IL-1β serum levels and the variation in FPG and HbA_{1c} after treatment, without being related to changes in IL-1β mRNA expression of mononuclear leukocytes. It must be taken into account that, even though we found a very significant association since the increase in serum IL-1β levels was associated with both FPG and HbA_{1c} after 6 and 12 months of treatment, this association was not enough to generate an increase in IL-1β serum levels after treatment in our population; in which we found a positive association between serum IL-1β levels and glycemia at the beginning of the study, and we did not found significant changes in the serum IL-1β levels after treatment.

Conclusion

Our work has some limitations: (i) it was carried out in a population with different proportion of women and men; (ii) we studied the mRNA expression of mononuclear leukocytes which may not reflect the peripheral circulation protein level for IL-1β; (iii) further studies would be necessary to determine the effect of ER stress in IL-1β production. Nevertheless, we found that IL-1β mRNA expression level was associated with the genotype of the rs16944 SNP present in the promoter of IL-1β gene; and that there is a positive association between the IL-1β

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protein level and the FPG, and a negative association between the variations in IL-1β protein levels and the variations in FPG and HbA_{1c}.

Individuals with T2D constitute a heterogeneous group, for which the treatment objectives must be individualized. In this way, it is important the recognition of new molecular targets for the development of new drugs, guiding the therapeutic strategy in a personalized way (Pharmacogenetics). On the other hand, it will favor the early identification of individuals with high risk, which will allow the development of individualized prevention strategies.

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