RESEARCH PAPER

Serum interleukin-1 beta plays an important role in insulin secretion in type II diabetic

Eizadi Mojtaba*, Kohandel Mahdi, kasbparast JR Mehdi, Sarshin Amir

Department of Physical Education and Sport Science, Karaj Branch, Islamic Azad University, Iran

Abstract

The proinflammatory cytokine Interleukin-1beta is increased in presence obesity or its related disorders. The objective of this study was to determine serum IL-1β in relation to some markers indicative of type II diabetic. For this purpose, blood samples were collected from brachial vein after a overnight fast between 8:00 and 9:00 a.m. o'clock in order to measuring serum IL-1β, glucose, insulin and beta cell function in a group of type II diabetic men patients (n = 30) and none-diabetic healthy subjects (n = 36) matched for BMI and age in order 1) to compare serum IL-1b between diabetic and none-diabetic subjects, 2) to determine serum IL-1b in relation to fasting glucose, insulin and beta-cell function in diabetic patients. All anthropometrical indexes were also measured in two groups. Statistical analysis was performed by Independent sample T-test and Spearman rank correlation method. P value of <0.05 was accepted as significant. Insulin resistance and fasting glucose and serum IL-1b were significantly higher and serum insulin and Beta cell function were significantly lower in diabetic patients than none-diabetic subjects ($p < 0.05$). Serum IL-1b was negatively correlated with insulin and beta cell function and positively related with fasting glucose in diabetic patients ($p < 0.05$). Based on this data, it was concluded that IL-1b as a inflammation cytokine play a important role in type II diabetic and insulin secretion of pancreatic beta cell.

*Corresponding Author: Eizadi Mojtaba ✉ izadimojtaba2006@yahoo.com

Introduction

Diabetes is known to high insulin concentration and is a metabolic disease that occurs when pancreatic islets fail to produce sufficient insulin and/or the sensitivity of glucose-metabolizing tissues to insulin decreases (Wang *et al.,* 2010). In other words, he pathophysiological hallmarks of type 2 diabetes mellitus are insulin resistance and beta cell dysfunction (Kahn, 2003). It is reported that mechanisms of islet β cell failure are different in the progression of type 1 and type 2 diabetes (Wang *et al.,* 2010). In individuals without diabetic, insulin secretion of beta cells is linked to peripheral insulin sensitivity through a postulated negative feedback loop that allows the beta cells to compensate for any change in whole-body insulin sensitivity by a proportionate and reciprocal change in insulin secretion (Retnakaran *et al.,* 2005). It is generally accepted that insulin resistance is the primary defect and that pancreatic beta cell dysfunction occurs later that contributes to the progression of diabetes. However, a number of studies conducted in Asian populations have demonstrated the dominant role of beta cell dysfunction in the pathogenesis of type 2 diabetes (Kim *et al.,* 2011; Matsumoto *et al.,* 1997).

Beta cells dysfunction is mainly destructed by autoimmune-mediated apoptosis, leading to the loss of insulin production. Inflammatory cytokines play crucial roles in this process (Kawasaki *et al.,* 2004). Chronic exposure of β cell to circulating inflammation cytokines induces excessive production of reactive oxygen species (ROS) and activation of caspases, which inhibit insulin secretion and promote apoptosis of pancreatic β cells (Andersson *et al.,* 2001). The inflammation and anti-inflammation cytokines as leptin, resistin or adiponectin play important role in beta cells dysfunction and type 2 diabetic prevalence (Wang *et al.,* 2010).Depending on their roles in regulating pancreatic β-cell function, some cytokines are protective, others can be detrimental (Wang *et al.,* 2010). Among them, interleukin 1-beta (IL-1βb) is a proinflammatory cytokine that plays important roles in inflammation. However, the role of this cytokine under physiological conditions Remain to be clearly delineated. A recent study showed that IL-1β plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiological conditions (Matsuki *et al.,* 2003). A number of studies have described a positive association between IL-1β gene polymorphism and obesity, suggesting functional effects on fat mass, fat metabolism and body mass (Manica-Cattani *et al.,* 2010). In the past decades, it had been well established that inflammatory cytokines including IL-1β play a critical role in the pathogenesis of type 1 diabetes (Wang *et al.,* 2010), although its role in type 2 diabetic are still not completely elucidated. It is necessary to determine the role of Il-1b in insulin action and its secretion of pancreatic beta cells. Therefore, the main objective of our work was to study the relation of serum IL-1β to fasting glucose, insulin and beta cell function in type II diabetic patients. We also compared above mentioned variables in these patients with healthy subjects.

Material and methods

The aims of present study were 1) to compare the baseline serum IL-1β, glucose, insulin and beta cell function between type II diabetic and those none diabetic men subjects, 2) to determine serum IL-1β in relation to the other above mentioned variables in diabetic patients. The study protocol was approved by the ethics committee of Islamic Azad University. For this purpose, blood samples were collected from brachial vein after a overnight fast between 8:00 and 9:00 a.m. o'clock in order to measuring serum IL-1β, glucose, insulin and beta cell function in a group of type II diabetic men patients (n = 30) and none-diabetic healthy subjects (n = 36) matched for BMI and age (BMI ≥ 30 kg/m2, aged 37 ± 5 years). Each participant received written and verbal explanations about the nature of the study before signing an informed consent form.

Inclusion or Exclusion criteria

Participants were non-athletes, non-smokers and non-alcoholics. Adolescents with other respiratory, cardiac, rheumatic, musculoskeletal and orthopedic diseases and with associated neurological sequelae were excluded from both groups. In addition, the exclusion criteria were infections, renal diseases, hepatic disorders, use of alcohol, and use of nonselective β blockers and presence of malignancy. Neither the control or diabetic subjects had participated in regular exercise/diet for the preceding 6 months, nor did all subjects have stable body weight. Those patients who were unable to avoid taking hypoglycemic drugs or insulin sensitivity-altering drugs for 12 hours before blood sampling were also barred from participating in the study. Subjects were asked to avoid doing any heavy physical activity for 48 hours before blood sampling.

Anthropometric measurements

Weight and height of the participants were measured by the same person when the participant had thin clothes on and was wearing no shoes by using the standard scales. Body mass index was measured for each individual by division of body weight (kg) by height (m2). Also, Waist to hip circumference ratio (WHO) was calculated through dividing the abdominal circumference by hip circumference. Visceral fat and body fat percentage was determined using body composition monitor (OMRON, Finland).

Assay

Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). Serum IL-1β was determined by ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-1β), using a Biovendor- Laboratorial kit made by Biovendor Company, Czech. The Intra- assay coefficient of variation and sensitivity of the method were 5.1% and 0.3 pg/mL, respectively. Insulin was determined by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 2.6% and 2.88

respectively. Beta cell function (HOMA-BF) = [(20 × fasting insulin (µ/ml] / [Fasting glucose (mmol/l) – 3.5] (Greenman *et al.,* 2004).

Statistical analysis: All values are represented as mean ± SD. Statistical analysis was performed with the SPSS software version 15.0. The Kolmogorov-Smirnov test was applied to determine the variables with normal distribution. An Independent sample T-test was used to compare the serum levels of all variables between obese and none-obese subjects. The bivariate associations between IL-1β concentration with glucose, insulin and beta-cell function were examined with the Spearman rank correlation analysis in obese subjects. A p-value less than 0.05 were considered statistically significant.

Results

Data were expressed as individual values or the mean ± SD. Baseline characteristics such as age, body weight, BMI and body fat percentage did not differ between two groups (p ≥ 0.05). These data showed that fasting glucose concentration (101 ± 12 vs. 225 ± 35 mg/dl, p=0.000) and insulin resistance index (2.10 ± 0.33 vs. 4.58 ± 0.98, p=0.031) were higher in diabetic patients than none-diabetic. The finding of independent sample T-test were also showed lower serum insulin in diabetic patients than those with none diabetic (8.41 ± 2.11 vs. 711 ± 2.11 mg/dl, p = 0.021). Additionally, serum Il-1b concentrations were significantly higher in the type 2 diabetics than in the none-diabetic group (2.01 ± 0.21 vs. 2.81 ± 0.31 ng/ml, p = 0.012). Also, beta cell function in studied patients was lower than none-diabetic subjects (85.66 ± 21.3 vs 22.14 ± 6.11, p=0.000). Statistical analysis by Pearson method showed Baseline serum IL-1b was not correlated with insulin resistance in diabetic patients, while this inflammation cytokine was significantly negative associated with beta cell function (p = 0.016, r = 0.40, Fig 1). These finding support the role of Il-1b in beta cell dysfunction.

In addition, a significant positive association was observed between fasting glucose concentration with serum Il-1b in studied patients (p = 000, r = 0.62). This study has also shown and negatively association between IL-1b and insulin concentration in studied patients. (p = 0.0083, r = 0.44, Fig 2). Serum IL-1b was found to be positively associated with visceral fat in patients (p = 0.004, r = 0.47).

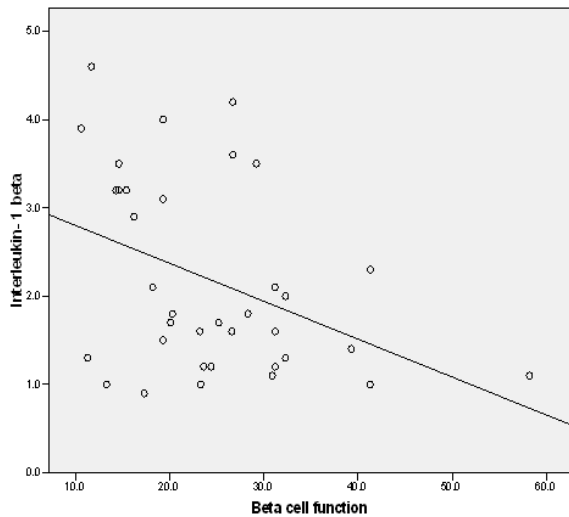**Fig 1.** The correlation pattern between serum Il-1b and Beta cell function in studied patients. This illustration indicates a significant negatively association between serum IL-1b and beta cell function in studied patients.
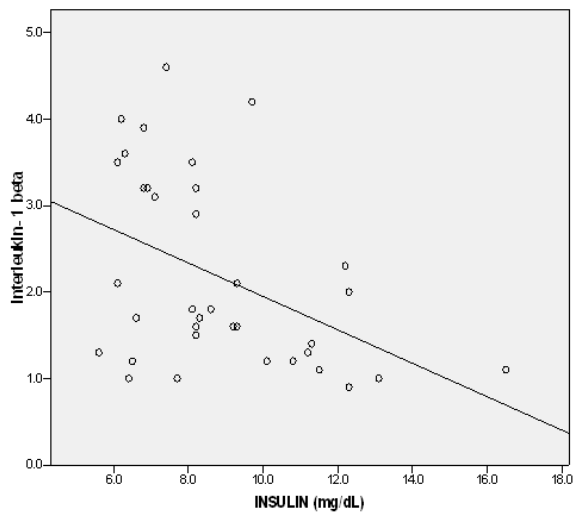

Fig 2. The correlation pattern between serum Il-1b and insulin concentration in studied patients. This illustration indicates a significant negatively association between insulin concentrations with serum Il-1b in studied patients.

Discussion

Our study finding showed higher fasting glucose and insulin resistance in diabetic patients compared to none-diabetic subjects, while serum insulin and beta cell function in these patients were significantly lower than those without diabetes. These finding have been reported repeatedly by previous studies (Maedler *et al.,* 2009). In present study, we also observed that Serum IL-1βconcentrations were significantly higher in the type 2 diabetics than in the healthy subjects. Proinflammatory cytokines secreted by adipose tissue and the other tissues can cause insulin dysfunction in adipose tissue, skeletal muscle and liver by inhibiting insulin signal transduction. Accumulating evidence indicates that the diseases related to metabolic syndrome are characterized by abnormal cytokine production, including elevated circulating IL-1β, increased acute-phase proteins, e.g., CRP (Sauter *et al.,* 2008) and activation of inflammatory signaling pathways (Juge-Aubry *et al.,* 2003). Recent evidence has shown that IL-1β plays a role in various diseases, including autoimmune diseases such as inflammatory bowel diseases and type 1 diabetes, rheumatoid arthritis, as well as in diseases associated with metabolic syndrome such as atherosclerosis, chronic heart failure and type 2 diabetes (Maedler *et al.,* 2009). Macrophage is known as the primary source of IL-1, but epidermal, epithelial, lymphoid and vascular tissues also synthesize IL-1. IL-1β production and secretion have also been reported from pancreatic islets (Maedler *et al.,* 2009).

The major finding of this investigation was negative significant association between serum IL-1β with beta cell function. To support our data, it is reported that increased secretion of IL-1β have been linked not only to various autoimmune and auto-inflammatory diseases, but also to metabolic dysregulation (Dinarello , 2009) and a disturbance in its secretion is associated with type II diabetic and impaired beta cell function (Mandrup-Poulsen *et al.,* 1998; Eizirik, 1998). A number of studies have demonstrated that

low concentration of IL-1β stimulates insulin release and proliferation in rat and human islets (Maedler *et al.,* 2006; Schumann *et al.,* 2007). A recent study states that increased IL-1β levels is accompanied with impaired insulin secretion, decreased cell proliferation and apoptosis of pancreatic beta cells (Osborn *et al.,* 2008). Rather than being directly cytotoxic, IL-1β may drive tissue inflammation that impacts on both beta cell functional mass and insulin sensitivity in type 2 diabetes (Ehses *et al.,* 2009). In addition to impaired insulin secretion, IL-1β was found to induce β -cell death, which was potentiated by TNF-α (Eizirik, 1998). It was found that blocking IL-1β by IL-1β -neutralizing antibodies conferred protection from the cytotoxic effects induced by activated-mononuclear-cell-conditioned medium and this data demonstrated that IL-1β plays an important role in the molecular mechanisms underlying autoimmune β -cell destruction (Bendtzen *et al.,* 1986). Several studies have found an overexpression of MnSOD also protects IL-1β cells from IL-1β or other cytokine induced apoptosis by repressing NF-κB activation and iNOS expression (Azevedo-Martins *et al.,* 2003).

In present study, serum Il-1b also correlated negatively with the serum insulin and positively with glucose concentration in diabetic patients that support the role of insulin secretion of pancreatic beta cells. This proinflammatory cytokine also is known to induce vascular dysfunction and cell death and its inhibition might represent a new strategy to inhibit capillary degeneration in diabetic retinopathy (Vincent *et al.,* 2007). There is evidence that short-time pretreatment of pancreatic β cells with IL-1β, IFN-γ, and TNF-α, alone or in combination, results in significant inhibition of insulin secretion in the absence or presence of stimulatory glucose concentration (Zhang *et al.,* 1995; Kiely *et al.,* 2007). Chronic exposure of IL-1β cell of pancreas to IL-1β activates the expression of inducible nitric oxide synthase and results in excessive production of nitric

oxide (NO), which interferes with electron transfer, inhibits ATP synthesis in mitochondria, and induces the expression of proinflammatory genes (Yang *et al.,* 2010). It is reported that a decrease in cellular ATP content inhibits insulin secretion and results in cell dysfunction (Wang *et al.,* 2010). In a recent study, administration of IL-1β-neutralizing antibody for 13 weeks significantly reduces glycated hemoglobin (0.45%) and improves islet function in HFD-induced diabetic mice (Osborn *et al.,* 2008). it has been demonstrated that pioglitazone also protects human islet IL-1β cells from IL-1β-induced apoptosis by blocking NF-κB activation (Suk *et al.,* 2001). One study reported that the expression of islet-derived proinflammatory cytokines including IL-1β and TNF-α is reduced by IL-1ra treatment with amelioration of islet inflammation (Ehses *et al.,* 2009).

Altogether, this study has two important findings: the first one is that the levels of IL-1β as an inflammatory cytokines in patients with diabetes are far higher than those with non-diabetic counterparts. Secondly the serum levels of this inflammatory cytokine are significantly correlated with beta cell function. Thus the inverse relationship between serum levels of IL-1β and beta cell function somehow supports the role of increased IL-1β in pancreatic beta-cell dysfunction and further corroborates the hypothesis that increased secretion of this cytokine reduces the amount of secretion of insulin from beta cells; because in the present study a significant inverse relationship was observed between levels of IL-1β and insulin and showed that each subject, increased serum levels of IL-1β were associated with decreased insulin concentration. On the other hand, the positive and significant relationship between IL-1β and blood glucose concentration in diabetic patients was another finding of this study which supports the role of this inflammatory cytokine in prevalence of diabetes and the determining parameters of type II diabetes. Finally, although like some previous studies, the findings of this study support the role of IL-1β in

pathophysiology of type II diabetes and beta cell function, the main mechanisms of interaction between them are still unknown and call for further studies in this field.

References

Andersson AK, Flodstr¨om M, Sandler S. 2001. Cytokineinduced inhibition of insulin release from mouse pancreatic β-cells deficient in inducible nitric oxide synthase," Biochemicaland Biophysical Research Communications **281(2),** 396–403.

Azevedo-Martins AK, Lortz S, Lenzen S, Curi R, Eizirik DL, Tiedge M. 2003. Improvement of the mitochondrial antioxidant defense status prevents cytokine-induced nuclear factor-κB activation in insulin-producing cells," Diabetes **52(1),** 93–101.

Bendtzen K, Mandrup-Poulsen T, Nerup J. 1986. Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. Science **232,** 1545 -7.

Dinarello CA. 2009. Immunological and infl ammatory functions of the interleukin-1 family. Annu Rev Immunol **27,** 519 -50.

Ehses JA, Lacraz G, Giroix MH, Schmidlin F, Coulaud J, Kassis N. 2009. IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proc Natl Acad Sci U S A **106(33),** 13998-4003.

Ehses JA, Lacraz G, Giroix MH. 2009. IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat," Proceedings of the National Academy of Sciences of the United States of America **106(33),** 13998–14003.

Eizirik DL. 1988. Interleukin-1 induced impairment in pancreatic islet oxidative metabolism of glucose is potentiated by tumor necrosis factor. Acta Endocrinol (Copenh) **119,** 321 -5.

Greenman Y, Golani N, Gilad S, Yaron M, Limor R, Stern N. 2004. Ghrelin secretion is modulated in a nutrient- and gender-specific manner. Clin Endocrinol (Oxf) **60(3),** 382-8.

Juge-Aubry CE, Somm E, Giusti V. 2003. Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and infl ammation. Diabetes 52, 1104 -10.

Kahn SE. 2003. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia **46,** 3–19.

Kawasaki E, Abiru N, Eguchi K. 2004. Prevention of type 1 diabetes: from the view point of β cell damage," Diabetes Research and Clinical Practice **66(supplement 1),** 27–32.

Kiely A, McClenaghan NH, Flatt PR, Newsholme P. 2007. Pro-inflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic β-cell line. The Journal of Endocrinology **195(1),** 113–123.

Kim DJ, Lee MS, Kim KW, Lee MK. 2011. Insulin secretory dysfunction and insulin resistance in the pathogenesis of Korean type 2 diabetes mellitus. Metabolism 50, 590–593.

Maedler K, Dharmadhikari G, Schumann DM, Størling J. 2009. Interleukin-1 beta targeted therapy for type 2 diabetes. Expert Opin Biol Ther **9(9),**1177-88.

Maedler K, Schumann DM, Sauter N. 2006. Low concentration of interleukin-1 β Induces FLICE-

inhibitory protein-mediated β -cell proliferation in human pancreatic islets. Diabetes **55,** 2713 -22.

Mandrup-Poulsen T, Bendtzen K, Nerup J. 1998. Affi nity-purifi ed human interleukin I is cytotoxic to isolated islets of Langerhans. Diabetologia **29,** 63 -7.

Manica-Cattani MF, Bittencourt L, Rocha MI, Algarve TD, Bodanese LC, Rech R. 2010. Association between interleukin-1 beta polymorphism (+3953) and obesity. Mol Cell Endocrinol **314(1),** 84-9.

Matsuki T, Horai R, Sudo K, Iwakura Y. 2003. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. J Exp Med **198(6),** 877-88.

Matsumoto K, Miyake S, Yano M, Ueki Y, Yamaguchi Y, Akazawa S. 1997. Glucose tolerance, insulin secretion, and insulin sensitivity in nonobese and obese Japanese subjects. Diabetes Care **20,** 1562–1568.

Osborn O, Brownell SE, Sanchez-Alavez M, Salomon D, Gram H, Bartfai T. 2008. Treatment with an Interleukin 1 beta antibody improves glycemic control in diet-induced obesity. Cytokine **44(1),** 141-8.

Retnakaran R, Hanley AJ, Raif N, Hirning CR, Connelly PW, Sermer M. 2005. Adiponectin and beta cell dysfunction in gestational diabetes: pathophysiological implications. Diabetologia **48(5),** 993-1001.

Sauter NS, Schulthess FT, Galasso R. 2008. The antiinfl ammatory cytokine interleukin-1 receptor antagonist protects from high-fat diet-induced hyperglycemia. Endocrinology **149,** 2208 -18.

Schumann DM, Maedler K, Franklin I. 2007. The Fas pathway is involved in β -cell secretory function. Proc Natl Acad Sci USA **104,** 2861 -6.

Suk K, Kim S, Kim YH. 2001. IFN-γ/TNF-α synergism as the final effector in autoimmune diabetes: a key role for STAT1/IFN regulatory factor-1 pathway in pancreatic/β cell death. Journal of Immunology **166(7),** 4481–4489.

Vincent JA, Mohr S. 2007. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. Diabetes **56(1),** 224-30.

Wang C, Guan Y, Yang J. 2010. Cytokines in the Progression of Pancreatic β-Cell Dysfunction. Int J Endocrinol **5,** 15136.

Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. 2010. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells," Nutrition Reviews **68(5),** 270–279.

Zhang S, Kim KH. 1995. TNF-α inhibits glucose-induced insulin secretion in a pancreatic β-cell line (INS-1). FEBS Letters **377(2)**, 237–239.