Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Alteration in cytokines secretion with Type 2 Diabetes mellitus

Noha Mohamed Said [1*] and Nahla H. Elshaer [2]

[1]Biochemistry Division, Chemistry Department, Faculty of Science, Zagzig University, Zagazig, Egypt.

[2] Zoology Department, Faculty of Science, Zagzig University, Zagazig, Egypt.

ARTICLE INFO

Type 2 diabetes
Cytokines
Immune system

ABSTRACT

Back ground: The blurred unclear link between the immune system and diabetes has made this issue an exciting research area. Cytokines are excellent evaluating tools for the immune system activity in any disease. They reflect the overall state of the immune system. Therefore, we hypothesized the disruption of the Th1 pathway as a consequence of the type 2 diabetes. We evaluated IL-12 which induces Th1 pathway and IL-2 which mediates Th1 function to prove our hypothesis. Almost, no previous studies gave clear image for the relationship between these two interleukins together and type 2 diabetes. ***Methods:*** Eighty Four diabetic Egyptian patients and 105 of normal control volunteer were chosen to evaluate the level of serum IL-2 and IL-12 in their blood using ELISA technique. To provide a complete image about the state of each subject, we made routine laboratory analysis including fasting blood glucose, fasting insulin, CRP, lipid profile and CBC for each volunteer. ***Results:*** There was a difference in the mean value of both IL-2 and IL-12 serum level between both patients and control. The patients have decreased IL-2 and increased IL-12 than control volunteers. It was interesting to provide a significant correlation between the serum level of IL-2 and IL-12 in patients. ***Conclusion:*** The significant correlation between IL-2 and IL-12 and the mean difference in their serum levels between diabetic patients and control can reflect the importance of these two cytokines in the pathogenesis of type 2 diabetes. Future studies with larger sample can confirm our results.

Corresponding author: Noha M. Said, Fax: +20 552308213, E-mail address: n_saidegy@yahoo.com

Introduction

Cytokines are very important molecules made by numerous immune system cells and carry out a number of utilities in relation to inflammatory and immune reactions. The immune system cytokines are of two types; proinflammatory cytokines that stimulate the immune responses and anti inflammatory cytokines that decrease these responses[1]. Interleukins are important category of the cytokines and had been evaluated in different diseases associated with immune system imbalance [2]**.** Cytokines can be considered as a mirror for the immune system in any disease. Evaluating the level of the cytokines can be used in the prognosis or the following up of the disease progression.

Type 2 diabetes is one of diseases that cause the disturbance of the immune system. This disease is a global threat and has serious complications. The rapid rise of this disease has many social and economic problems that have a terrible impact on the global situation. Unfortunately, more than a third of the world's population is diabetic and they are unaware because they are not interested in initial symptoms of the disease [3]. This disease is accompanied by short and long-term complications on the small and large blood vessels, which is harmful to all body organs, in addition to increase probability of frequent infection [4]**.**

The relationship between type 2 diabetes and immune system has been discussed through several ways and different views. Till now, only the innate immunity disturbance is associated with type 2 diabetes due to inflammation incidence Accompanied by hyperglycemia. The increase in inflammatory cytokines has been reported in many studies but with different percent of elevation. However, the anti-inflammatory cytokines levels in type 2 diabetes are still under debate

Interleukin-2 and interleukin-12 are two cytokines that have different functions in immune system. They were never compared at all during the course of type 2 diabetes incidence.

In the last ten years, research on the role of inflammatory markers on type 2 diabetes progressions has been growing.

However, the mechanism that links the disruption of the serum IL-2 and IL-12 rate to the risk of type 2 diabetes is still unknown. The presence of many environmental stimuli such as infection and chemicals can affect the natural immunity and as a consequence affect both IL-2 and IL-12 serum levels.

IL-2 is a Th1 pro-inflammatory cytokine. It has a great role in T cells activation for production of interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α). More ever, it improves the activity of natural killer cells (NK) [5, 6]**.** Thus, IL-2 is used as a therapy to motivate the immune system responses [7]**.** IL-2 also helps in the growth of regulatory T cells (Tregs), which switch the development and apoptosis of triggered T cells [8]. Furthermore, IL-2 is associated with cell survival, differentiation and the formation of immune memory cells and acts as a negative regulator of immune system stimulation [9]. New studies revealed that IL-2 has a serious role in the differentiation and survival of Tregs and so confirming their importance in the mechanism of the immune response[10]**.** Cytokines efficiently play a role in the pathogenesis of many pathological disorders, such as metabolic, infectious,

autoimmune and inflammatory syndromes [11]. Thus, IL-2 participate in immune functions by contributing to the creation and proliferation of antigen-specific immune responses [12].

IL-12 is a hetero-dimeric pro-inflammatory cytokine. It consists of two subunits p35 and p40 formed by antigen presenting cells in response to PAMPs (pathogen-associated molecular patterns) and DAMPs (danger-associated molecular patterns). It stimulates the switch of the immune response towards Th1 reactions, which makes protection against endogenous microbes [13, 14]. An association between IL-12 and type 1 diabetes was also demonstrated in humans. Glucose-stimulated PBMCs (peripheral blood mononuclear cells) from non-diabetic subjects secreted more IL-12 than non-stimulated cells [15]. Few studies discussed Interleukin-12 role in type 2 diabetes . they suggested the association between IL-12 level and diabetes complications [16–18].

To date, no systematic review has been performed to evaluate the available evidence on the association of neither IL-2 nor IL-12 levels with the risk of type 2 diabetes. The objective of the current study was to estimate the magnitude of the relationships between both IL-2 and IL-12 levels and the risk of type 2 diabetes in Egyptian population. We opted to study whether there will be alterations in inflammatory expression occurred in IL-2 and IL-12 in the context of T2DM.

Methods

Subjects

This work was done in biochemistry and molecular laboratory of Zagaig University. The study is composed of two group. The first group included 84 patients with type 2 diabetes mellitus (mean age of 36.6±12.9 years) chosen from the outpatient endocrinology clinic, Zagazig University Hospitals, during the period from August 2014 to May 2015. The second group included 105 age and sex matched healthy volunteers (mean age of 39.5±11.7) with no history of diabetes in their families.

All patients and healthy volunteers were subjected to: Full history taking, clinical examination, and routine investigations including complete blood count (CBC).

We have taken a written consent from all participants in the study. The diagnosis of type 2 diabetic patients was according to WHO guidelines, 1998 [19]. In addition, an approval of this research was taken from the ethical commite of our university according to the 1964 declaration of Helsinki [20]

Exclusion criteria

The study excluded the patients with type 1 diabetes or chronic liver diseases or renal failure disease or uncontrolled hypertension. We also excluded all the patients who may suffer from chronic inflammatoion, cancer, allergy and and autoimmune diseases. In addition, we didn't take in our study those patients who recieve immune-modulatory drugs. Also, pregnant and breast feeding women were excluded.

Anthropometric assessment

Anthropometric assessment was done for all subjects to evaluate health status of each individual. We took the height and weight for each subject then we calculated body mass index (BMI). In addition, Waist circumference (WC) was taken To measure abdominal obesity for each subject.

Laboratory analysis

Blood samples were taken from patients and healthy volunteers after fasting for 8 h to evaluate concentrations of fasting

glucose and for 12 h for lipid profile. Fasting glucose was measured using the glucose oxidase method, and the fasting serum lipid profile (cholesterol, triglyceride, HDL) was measured by enzymatic colorimetric method. Serum LDL was calculated using the formula of fridwald [21]

Assessment of blood glucose and insulin

Fasting insulin, Fasting blood glucose (FBG), and glycated hemoglobin (HbA1c) were measured using whole blood collected. FBG was detected by a device (COBAS INTEGRA® 400 plus, Roche Diagnostics Ltd, Switzerland). HbA1c level was detected using Siemens DCA analyzer. Fasting insulin level was detected with a human insulin ELISA kit (EMD Millipore, Billerica, USA). We used Fasting insulin and fasting glucose levels to calculate insulin resistant state and β-cell function as shown [22]. Homeostatic model assessment of insulin resistance (HOMA-IR): HOMA-IR = [Fasting insulin (μIU/mL) × Fasting glucose (mmol/L)] / 22.5. Homeostatic model assessment of β-cell function (HOMA-β): HOMA-β = [20 × Fasting insulin (μIU/mL)] / [Fasting glucose (mmol/L) – 3.5].

Assessment of lipid profiles

The colorimetric enzymatic method was done using a device (COBAS INTEGRA® 400 plus, Roche Diagnostics Ltd, Switzerland) to detect triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDL-c) levels. Low density lipoprotein cholesterol (LDL-c) level was calculated using the Friedewald equation as follows: LDL-c = total cholesterol – (HDL-c + TG / 5) [21].

Complete blood count analysis

Whole blood was taken from the donor into EDTA tubes .White Blood Cell (WBCs), Red Blood Cells (RBCs), and platelets with their indices were measured by an autoanalyzer (sysmes XS-500i, German).

CRP detection

Serum CRP was detected using an immunoturbidimetric method applied on an analyzer (COBAS INTEGRA® 400 plus, Roche Diagnostics Ltd, Switzerland). The sensitivity limit of this assay is 0.07 mg/L.

Detection of Serum IL-2and IL-12

Serum samples were collected and stored at -4°C for the measurement of both IL-2 and IL-12 using enzyme-linked immunosorbent assay (ELISA) technique (AviBion Human IL-2 EASIA kit, AviBion Human IL-12 EASIA kit). Samples were brought to room temperature and processed according to company instructions. Colour detection was read at 450 nm. A standard curve was plotted and results were calculated using Excel.

Statistical analysis

All statistical analyses were performed using MedCalc 15.8, GraphPad Prism 5.0 and SPSS 17.0. All data were presented as mean ± standard deviation (SD). Differences between groups were tested using Kruskal-Wallis and Mann-Whitney U tests (for continuous variables and nonparametric analyses for independent samples, respectively). Correlation coefficients (r) were calculated with nonparametric Spearman's correlation analyses. The association between inflammatory markers and T2DM was estimated by odds ratios and 95% confidence intervals (CI), which were obtained from logistic regression. P value <0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curves were generated for the assessment of both IL-2 and IL12 as biomarkers for diabetes. Area under the curve (AUC), sensitivity and specificity were calculated for each factor. The value with the best sensitivity and specificity in AUC analysis (Youden's index) was chosen as the best cut-off. AUCs were compared using the approach described by Hanley and McNeil[23]. The statistical significance was accepted as p value < 0.05

Results

A total of 84 patients of mean age 36.6±12.9 years, and 105 age and sex-matched healthy controls were enrolled in this study. There were no significant differences in age and male/female ratio between the patients and controls (P>0.05). The mean duration of illness for the patients group was 10.5±4.8 years. Neither patients nor controls had a history of clinical findings of impaired hepatic or renal function or any parasitic infection.

Several clinical, biochemical and inflammatory parameters were measured for both patients and control. There was significant difference between patients and control in triglyceride level, white blood cells count, QUICKI and CRP level. More ever, there was high significant difference between patients and control in the level of Homa-IR and Homa-β. It was not a surprise to find very high significant difference between patients and control in the level of Hba1c, fasting glucose and fasting insulin. Regarding IL-2 and IL-12 levels, mean differences was observed in both IL-2 level (169.17 ± 51.47 for patients and 206.7 ± 75.32 for control) and IL-12 (146.11 ± 62.66 for patient and 66.38 ± 25.55 for control). Our results showed that the patients have lower IL-2 level and higher IL-12 level than control but with no significant power **(Table 1).**

Comparative analysis using independent factors

The comparison between diabetic patients in the study according to different independent factors as age, duration of disease, gender, body mass index, diabetes control, Homa-IR, and fasting glucose showed that type 2 diabetes patients can be affected by only duration of disease. There were significant differences in the level of fasting glucose, Hba1c, IL-2, and IL-12 between diabetic patients with different duration of disease (P<0.05). In addition, there was significant difference in the fasting glucose and Hba1c levels between diabetic patients when we used Homa-IR as independent factor (P<0.05). Similarly, there was significant difference in the fasting glucose level when we considered diabetes control as independent factor. Also, there was significant in Hba1c level when we adjusted the comparison to fasting glucose level as independent factor (P<0.05) **(Table 2)**.

Correlation analysis

For the correlation between the significant parameters in this research, we found significant direct proportion between the level of IL-2 and IL-12 (P < 0.05). Both IL-2 and IL-12 were inversely proportional with fasting glucose and Homa-IR but with no significant power (P > 0.05). It worth note that the relation between CRP with IL-2 differs from that with IL-12. The CRP was inversely proportional with IL-2 and directly proportional with IL-12. Unfortunately, these relations lack the statistical power (P > 0.05) **(Table 3).**

Receiver operating curve analysis

Using ROC analysis showed that both IL-2 and IL-12 are not suitable biomarkers for

type 2 diabetes prognosis. (AUC is 0.511 and 0.644) for both IL-2 and IL-12 respectively. The cut off value was (>161, >164.666) for both IL-2 and IL-12 respectively . Also the sensitivity for both the two markers is very small (38% and 33%) for both IL-2 and IL-12 respectively**.** however the specificity was high (80% for IL-2 and 100% for both IL-12 **(Fig 1).**

Discussion

Type 2 diabetes mellitus is the most important prevalent type of diabetes all over the world. And its complications has great social and economic effects [24]. Almost all the previous studies approved the great relationship between type 2 diabetes and innate immunity via inflammation mechanism. However, the alteration of the cytokine secretion due to type 2 diabetes is still under debate.

Our study aimed to assess the effect of type 2 diabetes on the immune system through evaluation of two cytokines; interleukin-2 and interleukin-12. Both of these interleukins have a central role in T cell function and regulation [25, 26]. In addition, they work synergistically in Th1 pathway. IL-12 is Th1 inducing cytokine whereas IL-2 is Th1 cytokine that mediate the function of Th1 cells [27, 28]. Based on that, we suggested that there is association between levels of these two cytokines and that the insufficiency in IL-12 can result in defect in IL-2 secretion.

Type 2 diabetes is not an autoimmune disease but it is inflammatory disease characterized by inflammation incidence due to the hyperglycemia and hyperlipidemia. This inflammation is mediated by several mediators called cytokines which differ in their disturbance level according to their importance and function in inflammation [29].

Generally, the immune system produces two types of the cytokines, pro-inflammatory and anti-inflammatory which act synergistically to regulate the immune system action. It is worth noting that not all the pro-inflammatory cytokines has the same inflammation threshold. IL-1 and TNF-α are the master of inflammation incidence in such an inflammatory disease. Also, IL-4 and IL-10 are the main cytokines responsible for reducing inflammation [30]Some cytokines has a dual pro-inflammatory and anti-inflammatory effect [31]. According to this fact, the levels of cytokines in type 2 diabetes as an inflammatory disease are still under debate due to the variation in the results of the previous studies.

Our results show a decrease in IL-2 level and an increase in IL-12 level in type 2 diabetic patients than control. However, these differences lack statistical power probably because of sample size, life style, age mean and environmental factors. They can be used as predictor and not diagnostic markers for the risk of type 2 diabetes. Our results may be a light for more studies with more samples in other population.

Our results agree with the results of kaye and his group who discussed the relation between IL-2 and diabetes in new England population. They found no significant association between IL-2 and type 2 diabetes. However, they reported the association between IL-2 and type 1 diabetes [32]. This was confirmed by Memon and his team study in Swedish population who concluded to an association between IL-2 and insulin sensitivity index in the Swedish-born population but not in Iraqi- immigrants [33]. The nature of IL-2 has two faces and with a dual function (pro-inflammatory and anti-inflammatory) [31]. This interprets the insignificant decrease in its level in our results. More ever, recent

studies reported IL-2 role in Tregs development from T cells preventing autoimmunity and auto-inflammation by Teffs cells. Regulatory T cells (Tregs) is responsible for regulating effector T cells (Teffs) that respond to self-antigens and lead to autoimmune diseases. An inappropriate Treg/Teff equilibrium can cause most autoimmune diseases such as type 1 diabetes (T1D). To reestablish an appropriate equilibrium, Teffs should be blocked with immune suppressants which are partly effective[34].
Banchereau and his group reported that IL-2 has a great role in controlling inflammation and can be considered as anti-inflammatory as IL-10 and tumor growth factor –beta [35].

Actually, deactivation of IL-2 causes autoimmunity due to its critical role in keeping Treg cells in the periphery [36]. On the other hand, giving low dose of IL-2 to non-obese diabetic mice inhibits the progression of diabetes and can reduce the established disease. IL-2 probably inhibits diabetes by inducing the reactive CD4+Foxp3+ Treg cells that reduce the reactive effector cells. [37].

IL-2 also prevents inflammation by hindering the differentiation of TH17. This is done by interfering with IL-6 signaling events, such as decreasing IL-6 receptor expression and replacing the transcription factor STAT3 with STAT5 on target DNA binding locations in genes essential for TH17 differentiation [38].

More ever, it was proven that there is inversely proportion between serum level of IL-2 and IL-17 that take a part in inflammatory process. Actually, the intake of IL-2 by Treg cells accelerates the differentiation of TH17 cells in vivo and in vitro. Thus, intake of IL-2 can prevent IL-17-dependent inflammatory processes [39].

IL-2 also affects humoral immune response by regulating the progression of follicular helper T cells (TFH cells) that regulate humoral immune response [40]. As shown above for TH17 cells, IL-2 can inhibit THF by indirect way without affecting already differentiated TFH cells. This can be done by interfering with the THF cells production of IL-21 and CXCR5 that help B cells to go through isotype switching and somatic mutations[41].

Thus, by stimulating more Treg cells and reducing the number of TH17 and TFH cells, IL-2 can inhibit the uncontrolled growth of immune responses and reduce inflammation. These outcomes have important helpful implications in treatment strategy.

The regulatory functions of IL-2 were shown in a study done by Koreth and his colleagues 2011. They demonstrated that intake of low dose IL-2 can stop inflammation and improve disease in patients with chronic graft-versus-host disease or hepatitis C virus–related vasculitis [42].

IL-2 contributes to both the induction and the termination of inflammatory immune responses. It has opposing faces during an inflammatory response. It induces T-cell proliferation and help them in achieving the optimal survival and to act as memory cells. On the contrary, IL-2 induces the development and function of regulatory T cells to inhibit the development of inflammatory Th17 cells. Thus, IL-2 has dual and contrasting functions [43].

The decrease in IL-2 level in the results of our patients was supported by the result of Dwyer study that reported that IL-2 can be used as a therapy for type 1 diabetes in some studies but with low doses due to its toxicity [44]. However, no studies discussed the IL-2 as a therapy in case of type 2 diabetes.
On the contrary, Other studies are not in agreement with our results and they found increase in IL-2 in type 2 diabetes in comparison to the control [45]. The Controversy in the results may be due to population ethnicity, life style and environmental factors.

Regarding the level of IL-12, the insignificant increase in in our patients was supported by the results of Momen and his group who reported that there is no association between IL-12 and insulin sensitivity in Swedish population [33]. This was confirmed by Gupta and his team (2017) who demonstrated no significant association between IL-12 and type 2 diabetes [46]. IL-12 is pro-inflammatory and act as activator for natural killer cells. It is a master controller of Th1 differentiation [27]. Its level increase in type 2 diabetes is theoretically logic. However, there is a great variation in its level in the different studies.

Wen and his team showed that the increase in glucose level can increase the mRNA expression of IL-12 by 5.4-fold [47]. Also, Wegner et al., demonstrated elevated IL-12 serum levels in T2DM subjects [16]. In a third study by Ryba-Stanisławowska for type 1 diabetes, they reported IL-12 level increase in type 1 diabetes with decreased Tregs cells due to decreased IL-2 [48].

Besides, it was demonstrated that patients with long-lasting type 1diabetes have high levels of IL-12 in both serum and aqueous humor. This may prove the participation of IL-12 in pathogenesis of diabetic complications [49]. In a study for diabetic nephropathy, Anand and his group reported that IL-12 level increased in diabetic nephropathy [50].

Conversely, IL-12 level in type 2 diabetes was reported to be lower than control in other studies in different populations[51–53]. The controversy may be interpreted with the variation in degree of diabetes, sample size, age environmental factors or population ethnicity. Also, the type of treatment can affect the level of cytokines. More studies with larger sample size and adjusted factors can give more precious results.

Competing interests
The authors declare that they have no competing interests.

Acknowledgements
We appreciate the collaboration of all participants and staff at Zagazig university hospital, Egypt.

References

1. Van der Meide PH, Schellekens H (1996) Cytokines and the immune response. Biotherapy 8:243–249. https://doi.org/10.1007/BF01877210

2. Dembic Z (2015) Cytokines of the Immune System. In: The Cytokines of the Immune System. Elsevier, pp 143–239

3. Wild S, Roglic G, Green A, et al (2004) Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047–1053. https://doi.org/10.2337/diacare.27.5.1047

4. Fowler MJ (2011) Microvascular and Macrovascular Complications of Diabetes. Clinical Diabetes 29:116–122. https://doi.org/10.2337/diaclin.29.3.116

5. Lenardo MJ (1991) lnterleukin-2 programs mouse αβ T lymphocytes for apoptosis. Nature 353:858–861. https://doi.org/10.1038/353858a0

6. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T Cells and Immune Tolerance. Cell 133:775–787. https://doi.org/10.1016/j.cell.2008.05.009

7. Smith AJP, Humphries SE (2009) Cytokine and cytokine receptor gene polymorphisms and their functionality. Cytokine & Growth Factor Reviews 20:43–59. https://doi.org/10.1016/j.cytogfr.2008.11.006

8. D'Souza WN, Lefrancois L (2003) IL-2 Is Not Required for the Initiation of CD8 T Cell Cycling but Sustains Expansion. The Journal of Immunology 171:5727–5735. https://doi.org/10.4049/jimmunol.171.11.5727

9. Gaffen S, Liu K (2004) Overview of interleukin-2 function, production and clinical applications. Cytokine 28:109–123. https://doi.org/10.1016/j.cyto.2004.06.010

10. Malek TR, Bayer AL (2004) Tolerance, not immunity, crucially depends on IL-2. Nature Reviews Immunology 4:665–674. https://doi.org/10.1038/nri1435

11. Lunney JK (1998) Cytokines orchestrating the immune response: -EN- -FR- -ES-. Revue Scientifique et Technique de l'OIE 17:84–94. https://doi.org/10.20506/rst.17.1.1094

12. Raeburn CD, Sheppard F, Barsness KA, et al (2002) Cytokines for surgeons. The American Journal of Surgery 183:268–273. https://doi.org/10.1016/S0002-9610(02)00781-X

13. Watford WT, Moriguchi M, Morinobu A, O'Shea JJ (2003) The biology of IL-12: coordinating innate and adaptive immune responses. Cytokine & Growth Factor Reviews 14:361–368. https://doi.org/10.1016/S1359-6101(03)00043-1

14. Hölscher C (2004) The power of combinatorial immunology: IL-12 and IL-12-related dimeric cytokines in infectious diseases. Medical Microbiology and Immunology 193:1–17. https://doi.org/10.1007/s00430-003-0186-x

15. Adorini L (2001) Interleukin 12 and autoimmune diabetes. Nature Genetics 27:131–132. https://doi.org/10.1038/84732

16. Wegner M, Winiarska H, Bobkiewicz-Kozłowska T, Dworacka M (2008) IL-12 serum levels in patients with type 2 diabetes treated with sulphonylureas. Cytokine 42:312–316. https://doi.org/10.1016/j.cyto.2008.02.014

17. Mishra M, Kumar H, Bajpai S, et al (2011) Level of serum IL-12 and its correlation with endothelial dysfunction, insulin resistance, proinflammatory cytokines and lipid profile in newly diagnosed type 2 diabetes. Diabetes Research and Clinical Practice 94:255–261. https://doi.org/10.1016/j.diabres.2011.07.037

18. Ali M, Mali V, Haddox S, et al (2017) Essential Role of IL-12 in Angiogenesis in Type 2 Diabetes. The American Journal of Pathology 187:2590–2601.

https://doi.org/10.1016/j.ajpath.2017.07.021

19. Alberti KGMM, Zimmet PZ, WHO Consultation (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. Diabetic Medicine 15:539–553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

20. (2014) World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. Journal of the Korean Medical Association 57:899. https://doi.org/10.5124/jkma.2014.57.11.899

21. Jialal I, Inn M, Siegel D, Devaraj S (2017) Underestimation of Low Density Lipoprotein-Cholesterol With the Friedewald Equation Versus a Direct Homogenous Low Density Lipoprotein-Cholesterol Assay. Laboratory Medicine 48:220–224. https://doi.org/10.1093/labmed/lmx023

22. Matthews DR, Hosker JP, Rudenski AS, et al (1985) Homeostasis model assessment: insulin resistance and ?-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419. https://doi.org/10.1007/BF00280883

23. Hanley JA, McNeil BJ (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 148:839–843. https://doi.org/10.1148/radiology.148.3.6878708

24. Connolly V (2000) Diabetes prevalence and socioeconomic status: a population based study showing increased prevalence of type 2 diabetes mellitus in deprived areas. Journal of Epidemiology & Community Health 54:173–177. https://doi.org/10.1136/jech.54.3.173

25. Cote-Sierra J, Foucras G, Guo L, et al (2004) Interleukin 2 plays a central role in Th2 differentiation. Proceedings of the National Academy of Sciences 101:3880–3885. https://doi.org/10.1073/pnas.0400339101

26. Wolf SF, Sieburth D, Sypek J (1994) Interleukin 12: A key modulator of immune function. Stem Cells 12:154–168. https://doi.org/10.1002/stem.5530120203

27. Adorini L, Aloisi F, Galbiati F, et al (1997) Targeting IL-12, the Key Cytokine Driving Th1-Mediated Autoimmune Diseases. In: Adorini L (ed) Chemical Immunology and Allergy. KARGER, Basel, pp 175–197

28. Viallard, Pellegrin, Ranchin, et al (1999) Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). Clinical and Experimental Immunology 115:189–195. https://doi.org/10.1046/j.1365-2249.1999.00766.x

29. Pickup JC (2004) Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes. Diabetes Care 27:813–823. https://doi.org/10.2337/diacare.27.3.813

30. Ostrowski K, Rohde T, Asp S, et al (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. The Journal of Physiology 515:287–291.

https://doi.org/10.1111/j.1469-7793.1999.287ad.x

31. Shachar I, Karin N (2013) The dual roles of inflammatory cytokines and chemokines in the regulation of autoimmune diseases and their clinical implications. Journal of Leukocyte Biology 93:51–61. https://doi.org/10.1189/jlb.0612293

32. Kaye WA, Adri MNS, Soeldner JS, et al (1986) Acquired Defect in Interleukin-2 Production in Patients with Type I Diabetes Mellitus. New England Journal of Medicine 315:920–924. https://doi.org/10.1056/NEJM198610093151502

33. Memon AA, Sundquist J, Wang X, et al (2013) The association between cytokines and insulin sensitivity in Iraqi immigrants and native Swedes. BMJ Open 3:e003473. https://doi.org/10.1136/bmjopen-2013-003473

34. Rosenzwajg M, Churlaud G, Hartemann A, Klatzmann D (2014) Interleukin 2 in the Pathogenesis and Therapy of Type 1 Diabetes. Current Diabetes Reports 14:. https://doi.org/10.1007/s11892-014-0553-6

35. Banchereau J, Pascual V, O'Garra A (2012) From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. Nature Immunology 13:925–931. https://doi.org/10.1038/ni.2406

36. Setoguchi R, Hori S, Takahashi T, Sakaguchi S (2005) Homeostatic maintenance of natural *Foxp3* $^{+}$ CD25 $^{+}$ CD4 $^{+}$ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. The Journal of Experimental Medicine 201:723–735. https://doi.org/10.1084/jem.20041982

37. Grinberg-Bleyer Y, Baeyens A, You S, et al (2010) IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. The Journal of Experimental Medicine 207:1871–1878. https://doi.org/10.1084/jem.20100209

38. Yang X-P, Ghoreschi K, Steward-Tharp SM, et al (2011) Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nature Immunology 12:247–254. https://doi.org/10.1038/ni.1995

39. Chen Y, Haines CJ, Gutcher I, et al (2011) Foxp3+ Regulatory T Cells Promote T Helper 17 Cell Development In Vivo through Regulation of Interleukin-2. Immunity 34:409–421. https://doi.org/10.1016/j.immuni.2011.02.011

40. Crotty S (2011) Follicular Helper CD4 T Cells (T_{FH}). Annual Review of Immunology 29:621–663. https://doi.org/10.1146/annurev-immunol-031210-10140

41. Ballesteros-Tato A, León B, Graf BA, et al (2012) Interleukin-2 Inhibits Germinal Center Formation by Limiting T Follicular Helper Cell Differentiation. Immunity 36:847–856. https://doi.org/10.1016/j.immuni.2012.02.012

42. Koreth J, Matsuoka K, Kim HT, et al (2011) Interleukin-2 and Regulatory T Cells in Graft-versus-Host Disease. New England Journal of Medicine 365:2055–2066. https://doi.org/10.1056/NEJMoa1108188

43. Hoyer KK, Dooms H, Barron L, Abbas AK (2008) Interleukin-2 in the development and control of inflammatory disease. Immunological Reviews 226:19–

28. https://doi.org/10.1111/j.1600-065X.2008.00697.x

44. Dwyer CJ, Ward NC, Pugliese A, Malek TR (2016) Promoting Immune Regulation in Type 1 Diabetes Using Low-Dose Interleukin-2. Current Diabetes Reports 16:. https://doi.org/10.1007/s11892-016-0739-1

45. Penttinen J (1995) High plasma interleukin-2: A potential cause of insulin resistance. Medical Hypotheses 45:614–616. https://doi.org/10.1016/0306-9877(95)90247-3

46. Gupta S, Maratha A, Siednienko J, et al (2017) Analysis of inflammatory cytokine and TLR expression levels in Type 2 Diabetes with complications. Scientific Reports 7:. https://doi.org/10.1038/s41598-017-07230-8

47. Wen Y, Gu J, Li S-L, et al (2006) Elevated Glucose and Diabetes Promote Interleukin-12 Cytokine Gene Expression in Mouse Macrophages. Endocrinology 147:2518–2525. https://doi.org/10.1210/en.2005-0519

48. Ryba-Stanisławowska M, Rybarczyk-Kapturska K, Myśliwiec M, Myśliwska J (2014) Elevated Levels of Serum IL-12 and IL-18 are Associated with Lower Frequencies of CD4+CD25highFOXP3+ Regulatory T cells in Young Patients with Type 1 Diabetes. Inflammation 37:1513–1520. https://doi.org/10.1007/s10753-014-9878-1

49. Gverović Antunica A, Karaman K, Znaor L, et al (2012) IL-12 concentrations in the aqueous humor and serum of diabetic retinopathy patients. Graefe's Archive for Clinical and Experimental Ophthalmology 250:815–821. https://doi.org/10.1007/s00417-011-1905-4

50. Anand G, Vasanthakumar R, Mohan V, et al (2014) Increased IL-12 and decreased IL-33 serum levels are associated with increased Th1 and suppressed Th2 cytokine profile in patients with diabetic nephropathy (CURES-134). Int J Clin Exp Pathol 7:8008–8015

51. Wu H-P, Chen C-H, Hsieh H-C, Liu Y-C (2008) Effects of Insulin and Glucose on Cytokine Production from Peripheral Blood Mononuclear Cells. Chang Gung Med J 31:7

52. Wu H-P, Kuo S-F, Wu S-Y, Chuang D-Y (2010) High interleukin-12 production from stimulated peripheral blood mononuclear cells of type 2 diabetes patients. Cytokine 51:298–304. https://doi.org/10.1016/j.cyto.2010.06.014

53. Tsiavou A, Degiannis D, Hatziagelaki E, et al (2004) Intracellular IFN-γ production and IL-12 serum levels in latent autoimmune diabetes of adults (LADA) and in type 2 diabetes. Journal of interferon & cytokine research. Journal of Interferon & Cytokine Research 24:381–387. https://doi.org/10.1089/107999004153566 5

Table 1: Biochemical, clinical and inflammatory parameters in both diabetic patients and control

Parameters	Patients (n= 84)	control (n=105)	*P* value
Anthropometric and clinical parameters of cases and control			
Age (years)	36.6±12.9	39.5±11.7	>0.05
Gender (m/f)	56/28	31/20	>0.05
Duration of disease (years)	10.5±4.8	--	--
Body mass index (kg/m2)	26.8±6.6	24.7±4.5	>0.05
Systolic blood pressure (mmHg)	124.70±17.91	113.40±15.67	>0.05
Diastolic blood Pressure (mmHg)	75.31±13.58	74.41±9.79	>0.05
Biochemical parameters of cases and control			
Triglycerides (mg/dl)	192±38.52	131.51±43.12	<0.05*
Cholesterol (mg/dl)	200±45.81	185.78±33.87	>0.05
HDL cholesterol (mg/dl)	42.37±9.16	56.63±8.42	>0.05
LDL cholesterol (mg/dl)	136.30±17.47	128.78±12.56	>0.05
Hemoglobin (g/dl)	13.56±3.65	12.56±3.22	>0.05
White blood cells (10^3/cm)	9.3 ± 2.96	5.3 ± 2.1	<0.05*
Red blood cells (10^6/cm)	4.6±1.22	4.8±1.8	>0.05
Platelets (10^3/cm)	324±67.98	369±80.7	>0.05
Clinical parameters related to blood glucose and insulin status			
Hba1C (%)	8.9±2.5	4.93±0.8	<0.001***
Fasting glucose (mg/dl)	228.83 ± 70.93	98.4 ± 11.097	<0.001***
Fasting insulin (µU/ml)	28.43±8.00	7.97±2.98	<0.001***
Homa-IR	4.80±1.584	1.82±0.77	<0.01**
Homa-β	345.85±187.53	171.54±68.72	<0.01**
QUICKI	0.26±0.03	0.37±0.01	<0.05*
Inflammatory parameters			
CRP (mg/L)	9.8±0.2	3.2±0.13	<0.05*
IL-2 (pg/ml)	169.17 ± 51.47	206.7 ± 75.32	>0.05
IL-12 (pg/ml)	146.11 ± 62.66	66.38 ± 25.55	>0.05

All data are represented as mean ± S.

* $P < 0.05$ is significant, ** $P < 0.01$ is very high significant, and *** $P < 0.001$ is very high significant

HDL = high density lipoprotein, LDL =low density lipoprotein, TG= triglyceride, CRP = C- reactive protein, , IL-2= Interleukin-2, IL-12= Interleukin-12, Hba1c= glycohemoglobin, HOMA-IR (Homeostasis Model =Assessment- Insulin Resistance) = [fasting insulin ((µ U/ml) x fasting glucose (mg/dl)]/405, HOMA-β (Homeostasis Model Assessment-β) = (fasting insulin (µ U/ml) x 360) / (fasting glucose (mg/dl) - 63), QUICKI (Quantitative insulin sensitivity check index) = 1/ [log (fasting insulin (µ U/ml)) + log (fasting glucose (mg/dl)].

Table 2: Comparison between diabetic patients as regards the duration of illness, age, and gender, body mass index, diabetes control (Hba1C)

	Duration of disease			
	5-10 years (n=43)	10-20 years (n=27)	> 20 years (n= 14)	***p***
Glucose (mg/dl)	161.5±54.8	195.8±76.9	240.6±40.9	0.035*
Hba1c %	7.1±3.6	8.2± 3.8	9.6±4.67	0.024*
IL-2 (pg/ml)	67.9±30.7	255.8±105.8	406.9± 187.9	0.044*
IL-12 (pg/ml)	35.7±14.5	90.7± 34.5	168.9± 56.8	0.039*
	Age			
	15-35 years (n= 39)	35-50 years (n=21)	> 50 years (n= 34)	***p***
Glucose (mg/dl)	192.9± 54.9	189.9±67.9	235.8± 70.7	0.054
Hba1c %	7.34± 2.5	7.99± 3.5	9.45± 4.1	0.064
IL-2 (pg/ml)	99.8±45.7	145.8±45.8	189.9±67.9	0.22
IL-12 (pg/ml)	68.9±38.7	97.9±31.3	188.9± 76.2	0.063
	Gender			
	Males (n= 56)	Females (n= 28)		***p***
Glucose (mg/dl)	198.7± 67.8	191.5± 80.6		0.764
Hba1c %	7.9± 4.2	8.4±3.1		0.567
IL-2 (pg/ml)	145.6±67.9	163.9±68.9		0.98
IL-12 (pg/ml)	156.7±78.9	148.9±88.9		0.74
	Body mass index			
	Non obese < 25 kg/m^2 (n= 33)	overweight 25-30 kg/m^2 (n=45)	Obese > 30 kg/m^2 (n=6)	***p***
Glucose (mg/dl)	188.7± 57.8	191.5± 80.6	206.9±90.8	0.325
Hba1c %	8.1± 3.2	8.7±3.7	8.6± 4.8	0.146
IL-2 (pg/ml)	148.7±57.9	183.9±69.7	203.9±79.1	0.179
IL-12 (pg/ml)	146.8±88.2	168.9±89.2	199.9±89.2	0.097
	Diabetes control			
	Good control Hba1c 6-7.9% (n = 25)	Fair control Hba1c 8-9.9% (n = 41)	Poor control Hba1c ≥10% (n =18)	***p***
Glucose (mg/dl)	148.9± 34.9	187.8± 54.9	239.8± 78.9	0.023*
IL-2 (pg/ml)	109.8±55.7	165.8±48.8	209.9±67.9	0.054
IL-12 (pg/ml)	88.9±78.7	107.9±71.3	288.9± 76.2	0.052
	Fasting glucose			
	101-200 mg/dl (n= 62)	201-300 mg/dl (n=15)	> 300 mg/dl (n= 7)	***p***
Hba1C (%)	7.5 ± 3.7	8.9± 3.7	10.6± 4.8	0.047*
IL-2 (pg/ml)	102.6±51.7	175.8±38.8	229.9±57.9	0.055
IL-12 (pg/ml)	108.9±78.7	157.9±71.3	297.9± 86.2	0.069
	Homa-IR			
	2-3 (n= 55)	3.1-5 (n= 21)	> 5 (n= 8)	***p***
Fasting glucose	165.8± 43.6	199.8± 23.7	255.8± 78.9	0.043*
Hba1C (%)	7.75± 4.3	8.7± 4.6	10.1± 4.9	0.039*
IL-2 (pg/ml)	92.6±41.7	185.8±34.2	289.9±56.2	0.059
IL-12 (pg/ml)	103.9±61.7	187.9±51.2	277.9± 79.2	0.066

All data are represented as mean ± S. * $P < 0.05$ is significant

IL-2= Interleukin-2, IL-12= Interleukin-12, Hba1c= glycohemoglobin, Homa-IR= hemostasis model of assessment for insulin resistance.

Table 3: Correlation between biomedical parameters in the study

Correlation	**IL-12**				
		r		p	
IL-2		0.86		0.049	
	IL-2			**IL-12**	
		r	p	r	p
Fasting Glucose		-0.283	0.272	-0.007	0.989
CRP		-0.092	0.403	0.115	0.3
Homa-IR		-0.093	0.4	-0.198	0.104

P < 0.05 is significant

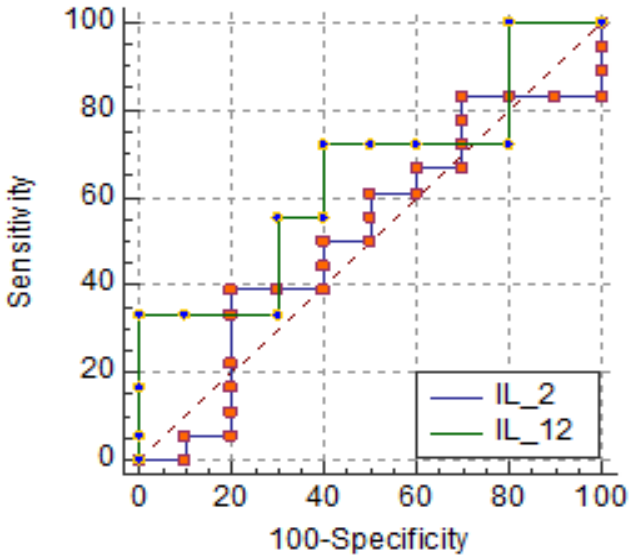

Fig 1: Receiver Operating Curves for the IL-2 and IL-12