Estimation of Interleukin-4 (IL-4) and Interleukin-6 (IL-6) Levels in Sera From Patients with Type 2 Diabetes Mellitus

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Abstract

Type-2 diabetes mellitus is the most common chronic metabolic disorder characterized by elevation of blood glucose concentration (hyperglycemia) due to defect in carbohydrate, lipid and protein metabolism. Cytokines have an important role in impairing insulin signaling and selective destruction of insulin-producing beta cells. Thus, the second type of diabetes can be characterized as an immune-mediated disease. This study aimed to evaluate the serum levels of Interleukin-4 (IL-4) and Interleukin-6 (IL-6) of patients with type 2 diabetes mellitus among cases from Wasit province-Iraq. A total of 90 randomly selected subjects from Wasit province – Iraq: (60) patients with T2DM, and (30) apparently healthy subjects with normal fasting blood sugar as a control group. Enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of interleukin-4 IL-4 and interleukin -6 in sera from patients with type-2 diabetes mellitus. The results of this study showed that IL-4 concentrations had a non-significant difference when compared patients with type-2 diabetes mellitus with the control group (154 ± 7.00 versus (vs) 151.49 ± 21, P-value (P) = > 0.05). While patients with T2DM revealed elevated serum levels of IL-6 compared to control group (B 637.1 ± 355.9 versus 266.3 ± 128.8, P = < 0.001).

Keywords: *IL-6, interleukin-6; IL-4, interleukin-4; T2DM, type 2 diabetes mellitus, ELISA, the enzyme-linked immunosorbent assay.*

Introduction

Recently, diabetes is classified as an epidemic disease due to its worldwide spread in varying proportions[1]. Diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder caused by hyperglycemia derived from either insulin action deficiency or impaired insulin secretion or both[2], which alters carbohydrate, protein, and fat metabolism[3]. To date, the mortality rate of diabetes has increased to 1.5 million people making diabetes the 1st leading cause of death in the world[4]. At a local setting, the prevalence of the disease in Iraq in 2012 was 10.9% depicting a serious rise in the number of T2DM patients[5].

One of the most common diseases in the world is type-2 diabetes mellitus. The increased acute immune response and pro-inflammatory cytokines were detected in diabetics in 1997[6]. Since then, emerging evidence has shown that T2DM is a chronic inflammatory disease in which various stimuli, such as genetic or fatal metabolic pre-programming, over-nutrition or increased age, can increase levels of cytokines expressed[7]. In which, the pattern of cytokine expression is changed[8, 9], therefore, pro-inflammatory cytokines cause damage to pancreatic islet cells resulting in pro-inflammatory and protective cytokines imbalance[9].

Current studies are suggesting the possibility of inflammation being an important contributor to diabetes. This is due to the fact that inflammation can provoke changes in diabetes predominantly at the cellular

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level, altering the functionality of tissues and cells demonstrating reactions of the inflammation including regulators, mediators, fibrinogen hs-CRP high sensitivity C reactive protein[10].

One family member of the cytokines is Interleukin 4 (IL-4) which is a typical cytokine of T helper type-2 (Th2) cells, could inhibit effect on the inflammation, decrease the production of pro-inflammatory cytokines and reduce the destructive enzymes through monocytes[11], and also plays a crucial role in the pathophysiology of T2DM. In addition to Interleukin-4 (IL-4), Interleukin-6 (IL-6) is a proinflammatory mediator cytokine biosynthesized by T-lymphocytes, macrophages, adipocytes and other sources such as endothelial cells, fibroblasts, and skeletal muscles[12]. On the other hand, IL-6 is responsible for many tasks such as controlling the activation and differentiation of T-lymphocyte responses and proinflammatory responses and also plays a role in the pathogenesis of autoimmune and inflammatory diseases, in the regulation of body weight, and in lipid metabolism[12].

This study aims to investigate the possible relationship between some cytokines (interleukin-4 and interleukin-6) and type 2 diabetes.

Material and Methods

Subjects

Patients group consist of 60 subjects with T2DM from Wasit province/Iraq (30 males and 30 females). Their ages ranged between 45–75 years. Control group comprised of 30 individuals (15 males and 15 females), apparently healthy subjects with normal fasting blood sugar and the controls were selected from Al- Karama Teaching Hospital (the local community of Wasit province – Iraq). Written consent was obtained by Al-Karama Teaching Hospital.

The criteria of the American Diabetes Association have been adopted in the diagnosis of T2DM in patients. This study excluded subjects with autoimmune diseases, cardiovascular diseases, acute and chronic inflammation[13,14].

Blood sampling

Venous blood (10 ml) has been collected in the infected tubes of patients and controls under sterile conditions between 08:30–10:30 am. Then the serum has been quickly frozen at (−20°C) and stored until further processed (Estimating the concentration of interleukins (IL-4 and IL-6) from patients with T2DM).

Estimation of IL-4 and IL-6 levels

Interleukin-4 and Interleukin-6 concentrations in sera were measured by ELISA using Human-IL-4-Mini ABTS-ELISA Development-Kit (Pepro-Tech, France) and Human IL-6 Mini ABTS-ELISA Development Kit (Pepro-Tech, France) as per the manufacturer's instructions.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) or median (interquartile range). Differences between groups were tested with the Student's t-test. The values of $P < 0.05$ were considered significant.

Results

The results of this determination as shown in Table 1, revealed that serum IL-4 concentrations displayed a non-significant difference in T2DM patients when compared with the control group (Diabetic patients 154.48 ± 7.00 compared with control 151.49 ± 6.21, P-value = 0.052). While, the serum concentrations of IL-6 revealed a significant difference in T2DM patients in comparison to controls (Diabetic patients 637.1 ± 355.9 compared with control 266.3 ± 128.8, P-value = 0.00013).

Table 1: Concentration of IL-4 and IL-6 in patients with T2DM and controls

Groups	IL-4 concentration pg/ml a	IL-6 concentration pg/ml
Control	151.49±6.21	266.3±128.8
Diabetic Patients	154.48±7.00 ns	637.1±355.9 b
Probability P-value	0.052	0.00013
LSD c	6.3	11.43
Significant level	Non-significant	Significant

a, Concentration of interleukin in pictogram / milliliter. ' Values are given as mean ±standard deviation of the mean (SD). *b,* significant P = 0.00013 when compared diabetic patients group with control group. *c,* LSD (11.43) Least significant difference is the value at a particular level of statistical probability (e.g. P≤0.01- means with 99% accuracy) when exceeded by the difference between two varietal means for a particular characteristic. *ns,* non-significant P-value = 0.052 when compared diabetic patients group with control group**.**

Regarding to IL-4 concentrations, males and females of diabetic patients displayed no significant differences when compared with controls (155.16 ± 8.15 versus 151.89 ± 6.75, P-value = 0.733; 153.79 ± 5.68 versus 151.10 ± 5.84, -value = 0.463) as in Table 2. Regarding IL-6 concentrations in T2DM males for this study showed significant difference comparing to controls (808.3 ± 296.0 versus 241.8 ± 9.0, P-value = 0.00022). No significant difference was showed in relation to female diabetic patients IL-6 levels in comparison to controls (466.0 ± 331.0 versus 290.9 ± 181.6, P-value = 0.065) as in Table 2.

Table 2: Concentration of serum IL-4 and IL-6 on the basis of gender (males and females) in diabetic and control groups.

Groups	Gender/ IL-4 concentration pg/ml a		Gender/ IL-6 concentration pg/ml a	
	Male	Female	Male	Female
Control	151.89±6.75	151.10±5.84	241.8± 9.0	290.9±181.6
Diabetic Patients	155.16±8.15 ns1	153.79±5.680 ns2	808.3± 296.0 s	466.0±331.0
P-value	0.733	0.463	0.00022	0.065 ns3
Significance	Non-significant	Non-significant	Significant	Non-significant

a*,** Concentration of interleukin in pictogram/ milliliter. Values are given as mean ± standard deviation of the mean (SD), ***ns1, nonsignificant P-value = 0.733 when compared diabetic male patients group with control male group, ***ns2:*** nonsignificant P-value = 0.463 when compared diabetic female patients group with control female group. ***ns3,*** non-significant P-value = 0.065 when compared diabetic female patients group with control female group. ***s,*** significant P-value = 0.00022 when compared diabetic male patients group with control male group

Discussion

Type 2 diabetes is a metabolic disease caused by insulin resistance and characterized by abnormal metabolism of glucose, proteins, and lipids. In fact, Type 2 diabetes is described as an inflammatory disease, with cytokines playing an important role in its diseases[15]. Inflammatory processes influence the development of insulin resistance and reduced insulin secretion by pancreatic beta cells[6]. Cytokines act as signaling molecules for immune cells, especially in autoimmune diseases, so cytokines play their role in developing and activating these cells[16].

Table 1 revealed a non-significant difference of IL-4 levels when compared patients with T2DM to control. These results are in agreement with a study conducted by Nuhair and others (2018) on the residents of Nassryain population in Thi-Qar/ Iraq[1]. Festa and others 2002, also mentioned the decreased production of IL-4 in human T-cells in diabetic patients. Moreover, the levels of IL-4 in the blood of diabetic patients demonstrated decreased values[17], this finding are disagreed with our results related to IL-4.

The differentiation activity of IL-4 plays an important role in the production of immunoglobulin, also IL-4 is described as a growth factor for β-cells. IL-4 developed by CD+4T-lymphocytes type Th2, after activation by antigen binding to the T-cell receptor, and also by activated mast cells and basophils[18]. IL-4 down-regulates the production of IFN-γ by Th1 CD4 + T-lymphocytes on the β-cells[19], IL-4 has a growth factor role mediated through the development of soluble CD23. On monocytes, because of its pleiotropic activity, IL-4 induces an increased number of major histocompatibility complex (MHC) class II antigens[20].

The results of this study revealed elevated serum levels of IL-6 in patients with T2DM. These results are in agreement with Nuhair et al (2018), who mentioned that there was a significant increase in the levels of IL-6 among diabetic patients as compared with controls in a study conducted on the Nassryain population in Thi-Qar/ Iraq[1]. Vidhate et al (2013) also mentioned that there was a significant increase in the levels of IL-6 among diabetic patients as compared with controls in a study conducted on Indian Population from Navi Mumbai[21]. In addition, several studies have found an increase in serum IL-6 concentrations[22, 1], however some studies reported no difference[23], or even decreased IL-6 levels[24].

IL-6 might play a significant role in IDDM etiopathogenesis[25]. In general, high levels of IL-6 are usually observed in the blood of diabetics, which is known to play an important role in the development of certain vascular diseases and atherosclerosis in addition to its main role in increasing inflammation[26].

Chronic low-grade inflammation in obese people plays a significant role in the subsequent development of insulin resistance. This results in a triple increase in systemic cytokine levels including IL-6, and thus becomes a risk factor for T2DM[27, 28, 29].

Some studies point to the important role that IL-6 plays in fat metabolism in general[30]. In humans, the action of IL-6 is associated with increased plasma free-fatty acids (FFAs). Due to high concentrations of IL-6 and C-reactive-protein, which is a surrogate marker for IL-6 activity, these associations seem significant. In obesity, increased concentrations of IL-6 are observed[31]. Further, an increase in the release of IL-6 by visceral adipose tissue was observed three to four times more than subcutaneous fat taken from obese and non-diabetic patients[32]. Therefore the main sources of elevated plasma concentration IL-6 up to 2-3g/mL-1 in obese patients and T2DM are adipocytes and macrophages present in adipose tissue[27].

The primary cell kinds engaged in regulating peripheral insulin sensitivity and homeostasis of glucose, hepatocytes, skeletal muscle cells, and adipocytes, react differently to IL-6. Strong experimental proof supports IL-6's capacity to decrease insulin sensitivity in hepatocytes by interfering with insulin signaling, while findings on adipocytes and skeletal muscle cells

are not always coherent[33]. Compared to other kinds of cells, the greater responsiveness of hepatocytes may be associated with the existence of membrane-bound IL-6R. It should be observed that these experiments are carried out primarily in vitro on cell lines and for a brief period, using supraphysiological of IL-6 levels much greater than those engaged in low-grade chronic inflammation[27].

In addition, the experimental circumstances may not strongly match acute pathophysiological circumstances in which IL-6 operates in synergy with other cytokines and during inflammatory responses mediates crosstalks between distinct kinds of cells and tissues. Moreover, the level of IL-6R and sgp130 is not assessed in targeted pathological models in these early studies, nor is IL-6 trans-signaling taken into consideration, which is particularly critical in the investigation of cells that lack membrane-bound IL-6R[27].

Further, Serum IL-6 levels have also been found to be associated with insulin resistance and diabetes. In nondiabetic older populations[34], and healthy, middle-aged, white populations. Schultz and others showed that higher IL-6 serum concentrations associated with enhanced opposition to insulin[35]. Liu and others in 2007 showed that serum IL-6 levels were also found to be higher in people with impaired glucose tolerance, T2DM, or cardiovascular syndrome relative to those with ordinary glucose tolerance or those who did not fulfill metabolic syndrome standards[36], Recently, a big prospective study of postmenopausal females who participated in the WHI (Women's Health Initiative) in the United States has revealed that high concentrations of IL-6 are also correlated with an increased risk of clinical diabetes[36]. Emphasis was placed on interleukin-6 discussion very broadly due to the high concentrations of this interleukin in the serum of patients with type 2 diabetes. Thus, it is necessary to clarify the immune and physiological roles of this interleukin, and as far as we know there are very few studies that explain the reason for the high levels of interleukin-6 in patients with type 2 diabetes.

Regarding to IL-4 concentrations, males and females of diabetic patients displayed no significant differences when compared with controls (155.16 ± 8.15 versus 151.89 ± 6.75, P-value = 0.733; 153.79 ± 5.680 versus 151.10 ± 5.84, P-value = 0.463) as in Table 4.2. It is traditionally accepted that IL-4 has anti-inflammatory functions but may have multiple actions[37]. IL-4 did not differ significantly between ChP and T2DM. Lower levels of IL-4 were observed in ChP + T2DM which is similar to another study[38], although IL-4 and TNF-α in their investigation correlated positively with each other in T2DM. Hence, a conclusive anti-inflammatory role for IL-4 is not forthcoming. To the best of our knowledge, the lack previous studies in people with T2DM on the basis of gender related to IL-4 and IL-6 levels or prediabetes does not permit direct comparisons with similar samples[39].

Concentrations of IL-6 in T2DM males showed significant difference comparing to controls (808.3 ± 296.0 versus 241.8 ± 9, (P-value = 0.00022). No significant difference was showed in relation to female diabetic patients IL-6 levels in comparison to controls (466.0 ± 331.0 versus 290.9 ± 181.6, P-value = 0.065) as in Table 2.

Serum IL-6 levels have also been established to be associated with insulin resistance and diabetes. In nondiabetic older populations and healthy, middle-aged, white populations[40]. In females, it is possible that elevated IL-6 levels may largely reflect adipocyte activation. For instance, IL-6 and downstream CRP production may be associated with the corelease of other pathogenic substances arising from otherwise stimulated adipocytes. Other potential mediators of insulin resistance deriving from adipose stores include tumor necrosis factor-a [41, 42], leptin[43, 44], free fatty acids[45], and resistin[31]. Nonetheless, under the assumption that elevated levels of IL-6 and CRP purely reflect altered adipocyte function, the ready availability of reliable and sensitive markers of this process may represent a novel approach for early identification of both obese and nonobese individuals at increased risk for the clinical development of this disease[46].

Conclusions

In this study, we have determined the concentrations of two interleukins (Interleukin-4 (IL-4) and Interleukin-6 (IL-6)) in sera from patients with type-2 diabetes mellitus using enzyme-linked immunosorbent assay (ELISA). From this study, we concluded that elevated levels of IL-6 may be related with type 2 diabetes mellitus whereas the levels of IL-4 may not be

ass0ciated with the disease.

This case-control study is investigating the association between type-2 diabetes mellitus and two important interleukins; Interleukin-4 and Interleukin-6. Determination of these interleukins in sera of type-2 diabetes mellitus patients is considered a vital assay to assess this association. Elevated levels of interleukins might play a significant role in type-2 diabetes mellitus etiopathogenesis. High levels of IL-6 may be associated with insulin resistance and diabetes. For a deeper view of the relationship between interleukins and type 2 diabetes mellitus, we propose large scale studies involving different regions of Iraq.

Conflicts of Interest : The authors declare complete freedom of any issue concerning conflict of interests related to this work.

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Ethical Clearance: The authors declare that the approval of the completion of the research before the start of work by the Scientific Committee for the Biology Department in the Faculty of Sciences and Mathematics at the Universiti Pendidikan Sultan Idris (UPSI) and took the consent of all patients to conduct the research.

References

1. Nuhiar, R. S., Salman, A. N., & AL-Rekaby, H. R. The some cytokines Levels (TGFβ1, IL-4, IL-6, and IL-17) in sera Patients with Diabetes Mellitus Type1, Type 2 in Nassiriya city. *Journal of Thi-qar Science*, 2018, 6(4), 42-48.
2. Punthakee, Z., Goldenberg, R., & Katz, P. (2018). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian Journal of Diabetes, 42, S10-S15.
3. Al-Goblan, A. S., Al-Alfi, M. A., & Khan, M. Z. Mechanism linking diabetes mellitus and obesity. Diabetes, Metabolic Syndrome and Obesity: *Targets and Therapy*, 2014, 7, 587.
4. Ates, I. Type 2 diabetes mechanisms, role of cytokines and their variations in disease development. *FABAD Journal of Pharmaceutical Sciences*, 2018, 43(1), 31-40.
5. Hussain, M. K., Deli, F. A., Algenabi, A. H. A., et al Adiponectin gene polymorphisms as a predictor for development of type 2 diabetes mellitus in Iraqi population. *Gene*, 2018, 662, 118-122.
6. Shih, Y. L., Ho, K. T., Tsao, C. H., et al., Role of cyotkines in metabolism and type 2 diabetes mellitus. *International Journal of Biomedical Laboratory Science*, 2013 2(1), 1-6.
7. Saxena, M., Agrawal, C. G., Srivastava, N., et al., Interleukin-6 (IL-6)-597 A/G (rs1800797) &-174 G/C (rs1800795) gene polymorphisms in type 2 diabetes. *The Indian Journal of Medical Research*, 2014 140(1), 60.
8. Banerjee, M., & Saxena, M. Genetic Polymorphisms of Cytokine Genes in Type 2 Diabetes Mellitus. *World Journal of Diabetes*, 2014 5 5(4): 493.
9. Cieślak, Marek, Andrzej Wojtczak, and Michał Cieślak. Role of Pro-Inflammatory Cytokines of Pancreatic Islets and Prospects of Elaboration of New Methods for the Diabetes Treatment. *Acta Biochimica Polonica*, 2015, 62(1): 15–21.
10. Malenica, M., Šilar, M., Dujić, T., et al., Importance of inflammatory markers and IL-6 for diagnosis and follow up of patients with type 2 diabetes mellitus. *Medicinski Glasnik*, 2017, 14(2).
11. Wei, H., Feng, G., Pei, W., et al., Association between interleukin-4 genetic polymorphisms and the risk of cerebral infarction in a population of China. *Biomedical Research*, 2017, 28(21), 9198-9203.
12. Galavi, H. R., Saravani, R., Alamdari, A. R., et al., Evaluating the effect of the rs2229238 and the rs4845625 interleukin 6 receptor gene polymorphisms on body mass index and the risk of type 2 diabetes in an iranian study population. *International Journal of High Risk Behaviors and Addiction*, 2016, 5(4).
13. Helaly, M. A. H., Hatata, E. S. Z., Abu-Elmagd, et al., Association of IL-10 and IL-6 Gene

Polymorphisms with Type 2 Diabetes Mellitus among Egyptian Patients. *European Journal of General Medicine*, 2013, 10(3).

14. Rodrigues, K. F., Pietrani, N. T., Bosco, A. A., et al., IL-6, TNF-α, and IL-10 levels/polymorphisms and their association with type 2 diabetes mellitus and obesity in Brazilian individuals. *Archives of Endocrinology and Metabolism*, 2017, 61(5), 438-446.
15. Tavangar, A., Khozeimeh, F., Ghoreishian, F., et al., Serum level of Interleukin-8 in subjects with diabetes, diabetes plus oral lichen planus, and oral lichen planus: A biochemical study. *Dental research journal*, 2016, 13(5), 413.
16. Gomes, K. B. IL-6 and type 1 diabetes mellitus: T cell responses and increase in IL-6 receptor surface expression. *Annals of Translational Medicine*, 2017, 5(1).
17. Festa, A., D'Agostino, R., Tracy, R. P., et al., Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*, 2002, 51(4), 1131-1137.
18. Elbe-BuÈrger, A., Olt, S., Stingl, G., et al., Overexpression of IL-4 alters the homeostasis in the skin. *Journal of Investigative Dermatology*, 2002, 118(5), 767-778.
19. Kiani, A., García-Cózar, F. J., Habermann, I., et al., Regulation of interferon-γ gene expression by nuclear factor of activated T cells. *Blood*, 2001, 98(5), 1480-1488.
20. Pesu, M., Takaluoma, K., Aittomäki, S., et al., Interleukin-4-induced transcriptional activation by stat6 involves multiple serine/threonine kinase pathways and serine phosphorylation of stat6. *Blood*, 2000, 95(2), 494-502.
21. Vidhate, D. A., Thomas, J., & Gupte, A. M., Association of IL-6 with diabetes mellitus in Indian population from Navi Mumbai. *International Journal of Recent Trends in Science and Technology*, 2013, 8(2), 100-102.
22. Erbağci, A. B., Tarakçioğlu, M., Coşkun, Y., et al., Mediators of inflammation in children with type 1 diabetes mellitus: cytokines in type I diabetic children. *Clinical Biochemistry*, 2001, 34(8), 645-650.
23. Kulseng, B., Skjåk□Braek, G., Følling, I., et al., TNF production from peripheral blood mononuclear cells in diabetic patients after stimulation with alginate and lipopolysaccharide. *Scandinavian Journal of Immunology*, 1996, 43(3), 335-340.
24. Geerlings, S. E., Brouwer, E. C., Van Kessel, K. C., et al., Cytokine secretion is impaired in women with diabetes mellitus. *European Journal of Clinical Investigation*, 2000, 30(11), 995-1001.
25. Wędrychowicz, A., Dziatkowiak, H., & Sztefko, K. Interleukin-6 (IL-6) and IGF-IGFBP system in children and adolescents with type 1 diabetes mellitus. *Experimental and Clinical Endocrinology and Diabetes*, 2004, 112(08), 435-439.
26. Jain, S. K., Kannan, K., Lim, G., et al., Elevated blood interleukin-6 levels in hyperketonemic type 1 diabetic patients and secretion by acetoacetate-treated cultured U937 monocytes. *Diabetes Care*, 2003, 26(7), 2139-2143.
27. Qu, D., Liu, J., Lau, C. W., & Huang, Y. IL-6 in Diabetes and Cardiovascular Complications. *British Journal of Pharmacology*, 2014, 171: 3595–3603.
28. Spranger, J., Kroke, A., Möhlig, M., et al., Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes*, 2003, 52(3), 812-817.
29. Lowe, G., Woodward, M., Hillis, G., et al., Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: the ADVANCE study. *Diabetes*, 2014, 63(3), 1115-1123.
30. Hashizume, M., & Mihara, M. IL-6 and lipid metabolism. *Inflammation and Regeneration*, 2011, 31(3), 325-333.
31. Steppan, C. M., Bailey, S. T., Bhat, S., et al., The hormone resistin links obesity to diabetes. Nature, 2001, 409(6818), 307.
32. Papaetis, Georgios S, Panagiotis Papakyriakou, and Themistoklis N Panagiotou. Central obesity, type 2 diabetes and insulin : exploring a pathway full of thorns. *Archives of Medical Science: AMS*, 2015, 11(3), 463.
33. Kristiansen, O. P., & Mandrup-Poulsen, T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes*, 2005, 54(suppl 2), S114-S124.

34. Hak, A. E., Pols, H. A., Stehouwer, C. D., et al., Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: the Rotterdam study. *The Journal of Clinical Endocrinology & Metabolism*, 2001, 86(9), 4398-4405.

35. Schultz, O., Oberhauser, F., Saech, J., et al., Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. *PloS One*, 2010, 5(12), e14328.

36. Liu, S., Tinker, L., Song, Y., et al., A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Archives of Internal Medicine*, 2007, 167(15): 1676–85.

37. Acharya, A. B., Thakur, S., Muddapur, M. V., et al., Tumor necrosis factor-α, interleukin-4 and-6 in the serum of health, chronic periodontitis, and type 2 diabetes mellitus. *Journal of Indian Society of Periodontology*, 2016, 20(5), 509.

38. Vieira Ribeiro, F., de Mendonça, A. C., Santos, V. R., et al., Cytokines and bone□related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. *Journal of Periodontology*, 2011, 82(8), 1187-1196.

39. Panagi, L., Poole, L., Hackett, R. A., et al., Sex differences in interleukin□6 stress responses in people with Type 2 diabetes. *Psychophysiology*, 2019, 56(6), e13334.

40. Abbatecola, A. M., Ferrucci, L., Grella, R., et al., Diverse effect of inflammatory markers on insulin resistance and insulin□resistance syndrome in the elderly. *Journal of the American Geriatrics Society*, 2004, 52(3), 399-404.

41. Hotamisligil, G. S., Arner, P., Caro, J. F., et al., Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *The Journal of Clinical Investigation*, 1995, 95(5), 2409-2415.

42. Hotamisligil, G. S., Peraldi, P., Budavari, A., et al., IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. Science, 1996, 271(5249), 665-670.

43. McNeely, M. J., Boyko, E. J., Weigle, D. S., et al., Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care*, 1999, 22(1), 65-70.

44. Kieffer, T. J., Heller, R. S., Leech, C. A., et al., Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic β-cells. *Diabetes*, 1997, 46(6), 1087-1093.

45. Groop, L. C., Saloranta, C., Shank, M., Bonadonna, R. C., et al., The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism*, 1999, 72(1), 96-107.

46. Pradhan, A. D., Manson, J. E., Rifai, N., Buring, et al., C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama*, 2001, 286(3), 327-334.