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Original Research

Expression of toll-like receptor 4 and its connection with type 2 diabetes mellitus

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Abstract: Toll-like receptor 4 (TLR4) plays an important role in modulating innate immunity. Type 2 diabetes mellitus (T2DM) is a chronic disease that is characterized by impaired insulin resistance and abnormal immune response. Genetic background and consequently genetic factors might have a key role in both onset and progression of T2DM-related complications. The aim of this work was to study the role of toll-like receptor 4 (TLR4) in the development of type 2 diabetes mellitus (T2DM). This study was carried out on 90 subjects, 30 type 2 diabetic patients, 30 patients with impaired glucose tolerance and 30 age and gender matched healthy controls. mRNA expression of (TLR4) was assessed by reverse transcriptase PCR (RT-PCR) using real time PCR.. Results showed significant statistical difference between the three studied groups regarding BMI, serum FBG, HDL, TGs, TC, LDL, HOMA -IR and mRNA expression of TLR4 with highest level of TLR4 mRNA expression in T2DM patients. From this study, it might be concluded that high expression of (TLR4) is associated with T2DM.

Key words: Type2 diabetes mellitus; Toll-like receptor 4; Gene expression.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, and its incidence is growing worldwide. Long-term hyperglycemia is the fundamental factor that promotes vascular lesions and dysfunction, leading to a variety of complications of DM (1). Type 2 DM, which accounts for 90–95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, represents individuals who have insulin resistance and usually have relative insulin deficiency (2).Although T2DM constitutes a major emerging health problem worldwide and the clinical symptoms and pathophysiology of this disease are well understood, some aspects of molecular mechanisms underlying the developing complications have remained unexplained yet (3).

TLR4 is a key protein molecule involved in nonspecific immunity and is also a bridge between nonspecific and specific immunity. TLR4 is a single trans membrane non catalytic protein that could be used to identify the molecules with conservative structure from microorganisms (4). Toll-like receptor 4 is a protein that in humans is encoded by the *TLR4* gene. TLR4 is a trans membrane protein, member of the toll-like receptor family, which belongs to the pattern recognition receptor (PRR) family. Its activation leads to an intracellular signaling pathway NF-κB and inflammatory cytokine production which is responsible for activating the innate immune system (5). In the recent years, it has been also proposed that T2DM may be the consequence of the stimulation of Toll-like receptors (TLRs), a family of pattern-recognition receptors able to detect microbial conserved components and trigger protective host responses, and implicated in mediating chronic inflammatory diseases, including obesity and diabetes (6). The activation of TLR4, one of the best known TLR member, expressed in several tissue cells, such as cells of the pancreatic islets (i.e.,β -cells and resident macrophages), can induce both insulin resistance, pancreatic cell dysfunction, and alteration of glucose homeostasis (7). The TLR4 activation seems also to be exacerbated by the low-grade of circulating endotoxin (circulating lipopolysaccharide-LPS) correlated with the altered gut microbiota, which characterizes subjects with metabolic diseases, such as T2DM (6). The aim of this work was to study the role of toll-like receptor 4 (TLR4) in the development of type 2 diabetes mellitus (T2DM).

Materials and Methods

Subjects

This study was carried out in Medical Biochemistry and internal medicine Departments, Faculty of Medicine, Menoufia University. 90 subjects were enrolled in the study; they were 30 type 2 diabetic patients (males 21 and 9 females) with mean age of 50.57±6.51, 30 patients with impaired glucose tolerance (18 males and 12 females) with mean age of 48.27±5.71 and 30 age and gender matched healthy controls (19 males and 11 females) with mean age of 50.77±7.20.

The study was approved by ethical committee of Faculty of Medicine, Menoufia University. A written informed consent was obtained from all subjects. The diagnosis was based on the American Diabetes Association (ADA) criteria (8). All subjects were subjected to complete history taking, physical examination. Including estimation of body mass index [BMI] was done by

dividing body weight in kilograms by (height in meter^2) **(9).** Estimation of lipid profile; serum total cholesterol (TC), high density lipoprotein cholesterol (HDLc), triglycerides (TG) and low density lipoprotein cholesterol (LDLc) were done. Glycated hemoglobin (HbA1c), fasting and 2 hours post prandial blood glucose levels and fasting serum insulin was measured. Insulin resistance was assessed by homeostatic model assessment of insulin resistance (HOMAIR) (10).Measurement of TLR4 mRNA expression was performed using reverse transcriptase PCR (RT-PCR) using real time PCR. After 12 hours overnight fasting, 9 ml of venous blood were withdrawn from each subject and divided into three tubes; 4 ml of blood were transferred into two EDTA tubes: one ml was used for quantitative colorimetric determination of glycated hemoglobin as percent of total hemoglobin using kits supplied by Teco diagnostics, USA (11), the other tube was used for RNA extraction and further molecular analysis.

One ml of blood was transferred into sodium fluoride tube and another sample of blood was collected after 2 hours for enzymatic colorimetric determination of blood glucose. Blood glucose was determined by enzymatic colorimetric test, using Spinreact kit, SPAIN (12).

Four ml of blood was transferred into a plain tube and allowed to clot at 37°C, then centrifuged for 10 minutes at 3000 r.p.m. The clear supernatant serum was separated from the clot and kept at -80°C until colorimetric determination of serum TC (13), HDLc (14), TG (15), LDLc (16) ,fasting serum insulin was determined by enzyme linked immunosorbent assay method using DRG® Insulin ELISA kit ,GERMANY (17). Estimation of insulin resistance was done by homeostatic model assessment (HOMA) according to Matthews et al.,1985 (10). HOMA- IR = fasting glucose (mg/dl) x fasting insulin (µIU/mL) / constant (405).Determination of *Reverse transcriptase PCR (RT-PCR):*RNA was isolated from peripheral blood leukocytes using QIAamp RNA Blood MiniKit (Qiagen, USA, 2013), then assuring RNA concentration and purity by Nano drop. First step-PCR: Complementary DNA was synthesized using QuantiTect Reverse Transcription Kit (Qiagen,Applied Biosystems, USA, 2012),second step- PCR (real time PCR step): it was performed using QuantiTect SYBR Green PCR Kit with readymade quantiTect Primer Assay, Qiagen.For measurement of TLR4 mRNA levels, The cDNA was used in SYBR green based quantitative real-time PCR for quantification of TLR4 gene expression by (SensiFAST TM SYBR Lo-ROX Kit from USA), using the following designed primers .Forward and reverse primers of human TLR4, 5- CAGAGTTTCC-TGCAATGGATCA-3 and 5- GCT TATCTGAAGG-TGTTGCACAT-3, respectively; forward and reverse primers for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 5-CATGAGAAGTATGACAA-CAGC-3 and 5- AGTCCTTCCACGATACCAAAG-3, respectively. Each reaction was performed in a final volume of 20 µL, containing 10 µL SYBR Green 2x QuantiTect PCR Master Mix, 3µL cDNA, 1 µL forward primer, 1 µL reverse primer and 5 µl RNase-free H2O. PCR was conducted under the following conditions: initial incubation at 50°C for 2 min, followed by 10 min denaturation at 95°C and 60 cycles at 95°C for 15 s, 60°C for 1 min and 72°C for 40 s. Data analysis was done in Applied Biosystem 7500 software version 2.0.1. The relative quantification (RQ) of gene expression performed using comparative ΔCt method (18) figure (1). TLR4 was normalized to the mRNA levels of housekeeping gene GAPDH. Melting curve was done to confirm specificity of the amplification and absence of primer dimers.

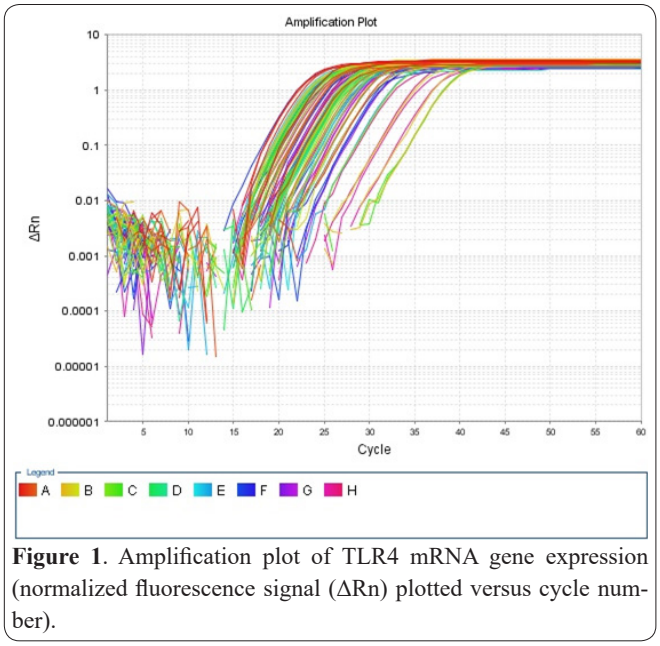

Figure 1. Amplification plot of TLR4 mRNA gene expression (normalized fluorescence signal (ΔRn) plotted versus cycle number).

Statistical analysis

The data collected was tabulated and analyzed by SPSS version 20. Chi-square test is used to study the association between two qualitative variables. Kruskal Wallis Test was used for nonparametric data. Spearman's correlation was used for skewed distributed quantitative variables. Odds ratio (OR) describes the probability that people who are exposed to a certain factor disease compared to people who are not exposed to the factor. Values less than 0.05 were considered significant.

Results

There was no significant statistical difference between studied groups regarding age and gender and There was a statistically significant difference between the three studied groups regarding; serum FBG,HBA1C, TC,TG ,LDLc, HDLc , BMI ,2HPP, fasting Insulin, HOMA-IR and TLR4 mRNA expression (table 1 and table 2).

There were significant positive correlations between TLR4 mRNA expression and FBG, 2HPP, fasting Insulin and HOMA-IR. While there was significant negative correlation between TLR4 mRNA expression and HDL within T2DM group (table 3 and figure 2).

There were significant positive correlations between TLR4 mRNA expression and FBG, 2HPP, fasting Insulin and HOMA-IR within IGT group (table 4 and figure3).

Multivariate logistic regression for risk of T2DM showed that the most common risk factor is TLR4 mRNA expression OR; 9.568(3.080-25.991), followed by LDL OR; 0.852(0.789-0.919) (Table 5).

Multivariate logistic regression for risk of IGT showed that the most common risk factor is TLR4 mRNA expression OR; 6.597(1.904-22.859), followed

Table 1. Comparison between control group, T2DM group and IGT group regarding demographic and laboratory data.

Studied variable	Control Group (No.=30)	T2DM Group (No.=30)	IGT Group (No.=30)	Test value	P-value	Post-hoc
Age Mean ± SD	50.77±7.20	50.57±6.51	48.27±5.71	1.372*	0.259	P1=0.905 P2=0.140 P3=0.174
Gender No. (%) -Male -Female	19(63.30%) 11(36.70%)	21(70.00%) 9(30.00%)	18(60.00%) 12(40.00%)	0.679***	0.712	--------
BMI Mean ± SD	21.62±1.80	26.12±1.72	23.10±1.39	51.725**	0.0001	P1=0.0001 P2=0.002 P3=0.0001
FBG mg/dl Mean ± SD	84.57±7.65	231.07±53.40	119.17±4.73	79.171**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
2Hpp Mean ± SD	87.17±8.67	272.90±57.62	160.17±14.59	79.157**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
HbA1c (%) Mean ± SD	5.05±0.67	8.46±1.56	6.05±0.30	65.594**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
HDL Mean ± SD	48.53±1.36	31.17±1.49	37.70±1.47	79.62**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
TGs Mean ± SD	92.27±5.08	165.07±11.63	111.70±5.63	79.218**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
Cholesterol Mean ± SD	171.50±9.15	202.03±18.52	206.78±10.95	53.063**	0.0001	P1=0.0001 P2=0.0001 P3=0.551
LDL Mean ± SD	104.47±8.49	137.37±18.34	146.80±11.08	58.540**	0.0001	P1=0.0001 P2=0.0001 P3=0.058
Fasting insulin (IU/ml) Mean ± SD	4.09±0.54	23.10±3.45	17.58±5.71	72.407**	0.0001	P1=0.001 P2=0.0001 P3=0.986
HOMA- IR Mean ± SD	0.83±0.11	11.90±2.85	4.63±0.75	79.197**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001
Family history No. (%) -Negative -Positive	30(100.00%) 0(0.0%)	3(10.00%) 27(90.00%)	11(36.70%) 19(63.30%)	51.314***	0.0001	--------
TLR4 mRNA expression Mean ± SD	0.05±0.02	1.43±0.18	0.57±0.21	79.566**	0.0001	P1=0.0001 P2=0.0001 P3=0.0001

*:Anova test. **::Kruskal Wallis Test. ***: Chi—square test. P1: Control group and T2DM group. P2:Control group and IGT group. P3: T2DMgroup and IGT group.

Table 2. Comparison between control group, T2DM group and IGT group regarding TLR4 mRNA expression.

Studied variable	Control Group (No.=30)	T2DM Group (No.=30)	IGT Group (No.=30)	Kruskal Wallis Test		Post-hoc
				Test value	P-value	
TLR4 mRNA expression -Mean ± SD -Median(range)	0.05±0.02 0.05(0.03-0.08)	1.43±0.18 1.40(1.20-2.00)	0.57±0.21 0.50(0.20-0.90)	79.566	0.0001	P1=0.0001 P2=0.0001 P3=0.0001

-P1: Control group and T2DM group. P2: Control group and IGT group. P3: T2DM group and IGT group.

by age OR; 1.096(1.005-1.196) followed by family history OR; 0.091(0.022-0.378) (Table 6).

Discussion

Type2 diabetes mellitus is a multifactorial disorder characterized by chronic hyperglycemia, insulin resistance and impaired insulin secretion. Type 2 diabetes mellitus (T2DM) is becoming a common worldwide disease with epidemic proportions in many populations (19). Hyperglycemia has adverse effects on β cells, as the chronic elevation of blood glucose level has been shown to impair β cell function (glucotoxicity) (20). Subjects with IGT are now referred to as having "pre diabetes" indicating the relatively high risk for development of diabetes in these patients (21). IGT is an intermediate category between normal glucose tolerance and overt diabetes. Individuals with IGT are defined as having 2hours post prandial plasma glucose values in the OGTT >140 mg/dl (>7.8 mmol/l) but <200 mg/

Table 3. Correlation between TLR4 mRNA expression and other parameters within T2DM group.

Studied parameters	TLR4 mRNA expression	
	Correlation coefficient (r) (spearman's correlation)	P-value
Age (years)	0.022	0.908
BMI (kg/m2)	0.100	0.600
FBG (mg/dl)	0.852	**0.0001**
2Hpp (mg/dl)	0.926	**0.0001**
HbA1c (%)	0.120	0.526
HDLc (mg/dl)	-0.412	**0.024**
TGs (mg/dl)	-0.085	0.656
Cholesterol (mg/dl)	0.203	0.281
LDLc (mg/dl)	0.234	0.212
Fasting insulin (IU/ml)	0.838	**0.0001**
HOMA- IR	0.795	**0.0001**

Table 4. Correlation between TLR4 mRNA expression and other parameters within IGT group.

Studied parameters	TLR4 mRNA expression	
	Correlation coefficient (r) (spearman's correlation)	P-value
Age (years)	0.024	0.901
BMI (kg/m2)	0.103	0.588
FBG (mg/dl)	0.859	**0.0001**
2Hpp (mg/dl)	0.856	**0.0001**
HbA1c (%)	0.310	0.095
HDLc (mg/dl)	-0.133	0.483
TGs (mg/dl)	0.110	0.563
Cholesterol (mg/dl)	-0.233	0.215
LDLc (mg/dl)	-0.253	0.177
Fasting insulin (IU/ml)	0.752	**0.0001**
HOMA- IR	0.745	**0.0001**

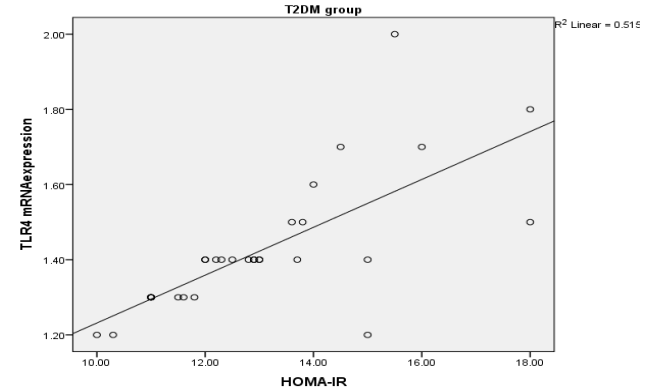

Figure 2. Correlation between TLR4 mRNA expression and HOMA- IR in T2DM.

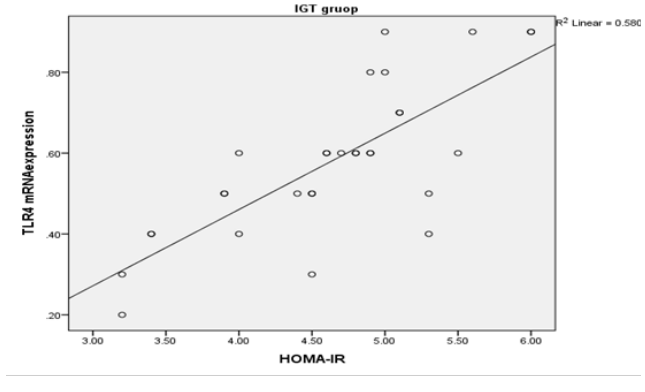

Figure 3. Correlation between TLR4 mRNA expression and HOMA- IR in IGT.

Table 5. Multivariate logistic regression for risk of T2DM.

Logistic regression of risk factors of type 2 DM	P-value	OR (95% C.I.)
Age(years)	0.293	1.073(0.941-1.224)
TLR4 mRNA expression	**0.007**	9.568(3.080-25.991)
Family history	0.390	0.417(0.057-3.069)
Sex	0.436	0.527(0.106-2.634)
BMI (kg/m2)	0.177	1.377(0.866-2.190)
LDLc (mg/dl)	**0.0001**	0.852(0.789-0.919)

Table 6. Multivariate logistic regression for risk of IGT.

Logistic regression of risk factors of IGT	P-value	OR (95% C.I.)
Sex	0.383	1.585(0.564-4.458)
Age (years)	**0.039**	1.096(1.005-1.196)
Family history	**0.001**	0.091(0.022-0.378)
TLR4 mRNA expression	**0.003**	6.597(1.904-22.859)

dl (<11.1 mmol/l) (22). TLR4 seems to have the major role in the incidence of chronic inflammation observed in T2DM complications (23).

In this study**,** there is no significant statistical difference between the three studied groups regarding age and gender. In this study, fasting, 2 hours post prandial blood glucose levels and HbA1c were significantly higher in both T2DM and IGT groups compared to control group. This is in agreement with the results obtained by (24,25).

The present study reported that total cholesterol, triacylglycerol (TG) and LDLc were significantly higher in T2DM group and IGT group compared to the control group. Whereas serum HDLc was significantly lower. This comes in line with Rizzo M and Berneis K, 2007 (26) who reported that in patients with T2DM.

It was showed that patients with insulin resistance (IR) have an increased expression of hepatic lipase which acts on HDLc , resulting in smaller HDL particles that are more rapidly catabolized by the kidney leading to, lower plasma HDLc. Also, an enzyme called endothelial lipase, is up regulated in IR states (27) .

Sorrentino, (2005) (28) reported that, patients with insulin resistance tend to have impaired fasting plasma glucose levels, which increase the prevalence of more atherogenic, low density lipoprotein (LDL) particles. The other associated abnormalities include, elevated levels of triglyceride (TG) rich lipoproteins along with low levels of high density lipoprotein cholesterol (HDLc). The present study showed that, fasting insulin was significantly higher in IGT and T2DM groups compared with the control group. This is in agreement with the results obtained by Henze et al.,2010(29) and Ballard,2006(30) who stated that , the β cell increases insulin secretion, resulting in hyperinsulinemia in an attempt to overcome insulin resistance, which is able to maintain relatively normal glucose tolerance and overcomes hyperglycemia. In some people, this hyperinsulinemic response is insufficient to fully compensate for insulin resistance, IGT and T2DM thus develop.

The present study showed that fasting insulin and HOMA-IR were significantly higher in T2DM and IGT groups compared to control group. This is in agreement with the results obtained by Henze et al., 2010 (29).

In this study, BMI was significantly higher in IGT and T2DM groups compared with the control group. Obesity is closely linked to a wide array of pathophysiologic consequences including insulin resistance (IR), hypertension, hyperlipidemia and atherosclerosis. The major basis for its link to T2DM is the ability of obesity to cause IR (31). Circulating FFAs derived from adipocytes are elevated in many insulin resistance states and have been suggested to be a main underlying mechanisms of IR in obesity associated T2DM (32).

Tissues insensitivity to insulin has been noted in most patients with type 2 diabetes irrespective of weight. It was attributed to genetic factor that is aggravated in time by further enhancers of insulin resistance such as aging, sedentary life and abdominal visceral obesity. In addition to insulin resistance, there is an accompanying deficiency in the response of pancreatic β cells to glucose (33).

In the current study mRNA expression of TLR4 was significantly higher in T2DM and IGT groups compared to the control group. This is in accordance to results obtained by Dasu et al who reported that T2DM patients had significantly increased TLR2, TLR4 mRNA, and protein in monocytes compared with control participants. They concluded that TLR2 and TLR4 expression and their ligands, as well as their signaling and functional activation, are increased in recently diagnosed T2DM (34).

Activation of the innate immune system via toll-like receptors (TLRs) is implicated in the pathogenesis of insulin resistance, diabetes, and atherosclerosis (35). Complementary genetic studies link toll-like receptor 4 (TLR4) polymorphisms to T2DM, suggesting a causal relationship between TLR function and diabetes and its complications (36).

The present study observed that mRNA expression of TLR4 was significant positively correlated with and FBG, 2HPP, fasting Insulin and HOMA-IR within IGT and T2DM groups. This is in agreement with results obtained by Dasu et al who reported that increased TLR2 and TLR4 expression correlated with FBG, , HbA1c,fasting insulin in addition to homeostasis model assessment - insulin resistance (HOMA-IR) in diabetic patients (34).

Studies have also shown that TLR2 and TLR4 expression levels are elevated in patients with T2DM. TLR4 has been shown to play an important role in the pathogenesis of atherosclerosis, diet-induced obesity, and the related insulin resistance (37).

Shi et al. (38) and Wong et al. (39) also reported consistent observations confirming the implication of TLR4 in the pathogenesis of insulin resistance and T2DM. Similar results, but in T1DM, were obtained by Devaraj *et al*. (39) who examined TLR2 and TLR4 expression in monocytes from 31 T1DM patients and 31 controls. They found that TLR2 and TLR4 surface expression and mRNA were significantly increased in T1DM monocytes compared with controls (40).

In the current study the Multivariate logistic regression for risk of T2DM and IGT showed that the most common risk factor is TLR4 mRNA expression.

TLR4 is abundantly expressed in insulin target tissues such as adipose tissue, liver and skeletal muscle and is now accepted as a key player in obesity-induced insulin resistance and T2DM (41). Earlier studies suggested that the stimulation of TLR4 seen in obesity/ insulin resistance/T2DM (42).

In conclusion, the mRNA expression level of TLR4 was significantly increased in T2DM.It might be a genetic risk factor for incidence of T2DM. TLR4 should be included in the list of molecules affecting control of insulin homeostasis.

References

1. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clin Sci (Lond) 2013; 124: 153–164.

2.Landon M.B, Spong C.Y, Thom E, Carpenter M.W, Ramin S.M, CaseyB, Wapner R.J. Eunice kennedy shriver national institute of child health and human development maternal-fetal medicine units network. A multicenter, randomized trial of treatment for mild gestational diabetes. N.Engl. J. Med; 2009; 6: 94–108.

3. Ewa Żurawska-Płaksej , Ewa Maria Kratz, Mirosława Ferens-Sieczkowska, Maria Knapik-Kordecka,Agnieszka Piwowar: Changes in glycosylation of human blood plasma chitotriosidase in patients with type 2 diabetes. Glycoconj J; 2016; 33:29–39.

4. Weissgerber TL, Mudd LM. Preeclampsia and diabetes. Curr Diab Rep. 2015;15:9.

5.Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. Frontiers in Immunology. 2014;5: 316.

6. Fan Yaochun1, Ou Xia2, Wang Wenrui, Tian Xiaoling, Yan Shaohong, Hu Nan et al., Identification and analysis of toll like receptor 4 (TLR4) level changes in vascular dementia patients related type 2 diabetes mellitus. Biomedical Research.2017; 28, Issue 10

7. H. M. Garay-Malpartida, R. F. Mourão, M. Mantovani, I. A. Santos, M. C. Sogayar, and A. C. Goldberg. Toll-like receptor 4 (TLR4) expression in human and murine pancreatic beta-cells affects cell viability and insulin homeostasis, BMC Immunology. 2011; vol. 12, article 18.

8. Standards of medical care in diabetes-2014. Diabetes Care 2014; 37 [1], S14-S79.

9. Enyioma O., Michael PT ., Abd-Ishakur A ., Mustapha S and Mona A . Leptin, lipid and lipid metabolism related hormones in chronic renal failure in Arabia. Nephrology, 2002; 7:115-119.

10. Matthews D, Hosker J, Rudenski A, Naylor B,Treacher D and Turner R. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28:412-419.
11. Gonen B and Rubenstien AH. Determination of glycohemoglobin. Diabetologia 1978;1: 1 5.
12.Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. J Ann Clin Biochem 1969; 6: 24-25.
13. Rifai N and Warnick R. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. In: Tietz Textbook of Clinical Chemistry and Molecular Diagnosis. Carl, A. B,Edward, R. A and David, E. B [edrs.]. Saunders. [4th edition] 2006; Ch 26. PP. 918-922.
14.Gordon T, Zidek W and Amer M. Determination of high density lipoprotein cholesterol. J. Med 1977; 42: 707:710.
15. Fossati P and Prenciphe L. Determination of serum triglyceride. Clin Chem 1982; 28: 207-210.
16. Friedewald W, Levy R and Fredrickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem.1972; 18:499- 502
17. Judzewitch R, Pfeifer M, Best J, Halter J and Port D: Chronic chlorpropamide therapy of noninsulin-dependent diabetes augments basal and stimulated insulin secretion by increasing islet sensitivity to glucose. J Clin Endocrinol Metab. 1982 Aug; 55(2):321-8.
18. Dorak M. Real-time PCR. Clinical Chemistry. 2004;50:1680.
19.Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW:Obesity and type 2 diabetes: what can be unified and what needs to be individualized? J Clin Endocrinol Metab; 2011; 96:1654±63.
20. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA: Glucose induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets. J Clin Invest.2002; 110:851±860
21.Fonseca V, Desouza C, Asnani S and Jialal I. Non traditional risk factors for cardiovascular disease in diabetes. Endocrine Reviews. 2007;25: 153-175.
22.Singh R ,Barden A and Mori T: Advanced glycation end products. Diabetologia; 2009;44: 129 -146.
23.Carmela Rita Balistreri, Anna Rita Bonfigli, Massimo Boemi, Fabiola Olivieri, Antonio Ceriello, Stefano Genovese, Claudio Franceschi et al. Evidences of +896 A/G TLR4 Polymorphism as an Indicative of Prevalence of Complications in T2DM Patients. Mediators of Inflammation Volume 2014, Article ID 973139, 8 pages .
24. Cho YM , Youn BS , Lee H , Lee N ,Min SS , Kwak SH , Lee H and Park K. Plasma retinol binding protein 4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care. 2006; 29:2457–2461.
25.Graham TE ,Yang Q , Bluher M, Hammarstedt A , Ciaraldi TP and Henry RR et al. Retinol binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med. 2006; 354:2552-2563.
26.Rizzo M and Berneis K: Small, dense low density lipoproteins and the metabolic syndrome. Diabetes Metab Res Rev.2007; 23: 14-20.
27.Daniel A , Toth P , Marz W and Fried, S. High density lipoproteins and cardiovascular risk. Circulation.2006;109: 1809-1818.
28. Sorrentino MJ. Implications of the Metabolic Syndrome: the new epidemic. Am J Cardiol. 2005;96:3E–7E.
29.Henze A ,Simone K , Frey M , Raila J ,Scholze A and Spranger J. Alterations of retinol binding protein 4 species in patients with different stages of chronic kidney disease and their relation to lipid parameters. Biochemical and Biophysical Research Communications. 2010; 393:79–83.
30.Ballard A: Pathophysiology of diabetes. Diabetes Care. 2006;23 :381-389.
31. Marcelo Macedo Rogero and Philip C. Calder. Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients 2018, 10(4), 432
32.Saltiel AR and Kahn CR: Insulin signaling and regulation of glucose and lipid metabolism. Nature. 2001; 414: 799-806.
33. Umesh M and Michael G: Pancreatic hormones and diabetes mellitus. In: greenspan's Basic and clinical Endocrinology. David G and Dolores SH (edrs.). Mc Graw-hill. 2007;8th ed. Ch 18. P. 662-747.
34.Dasu MR, Devaraj S, Park S and Jialal I. Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects. Diabetes Care. 2010; 33 :861-868.
35. Wifi, Mohamed-Naguib Abdalla, Assem, Maha, Elsherif, Rasha Hamed, El-Azab, Hameda Abdel-Fattah and Saif, Aasem. Toll-like receptors-2 and -9 (TLR2 and TLR9) gene polymorphism in patients with type 2 diabetes and diabetic foot. Medicine. April 2017; 96,17 - p e6760
36.Bagarolli RA, Saad MJ and Saad ST. Toll-like receptor 4 and inducible nitric oxide synthase gene polymorphisms are associated with type 2 diabetes. J Diabetes Complications. 2010; 24 :192-198.
37.Ghanim H, Mohanty P, Deopurkar R, Sia CL, Korzeniewski K and Abuaysheh S, et al.,. Acute modulation of toll-like receptors by insulin. Diabetes care. 2008;31:1827–1831.
38.Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H and Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116 :3015-3025
39.Wong FS, Hu C, Zhang L, Du W, Alexopoulou and Flavell RA. The role of Toll-like receptors 3 and 9 in the development of autoimmune diabetes in NOD mice. Ann N Y Acad Sci 2008; 1150 :146-148.
40.Devaraj S, Dasu MR, Rockwood J, Winter W, Griffen SC and Jialal I. Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. J Clin Endocrinol Metab 2008; 93 :578-583.
41. Jialal I, Kaur H and Devaraj S . Toll-like receptor status in obesity and metabolic syndrome: a translational perspective. Journal of Clinical Endocrinology and Metabolism.2014; 99 39–48.
42.Velloso LA, Folli F & Saad MJ. TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. Endocrine Review.2015; 36 245–271.