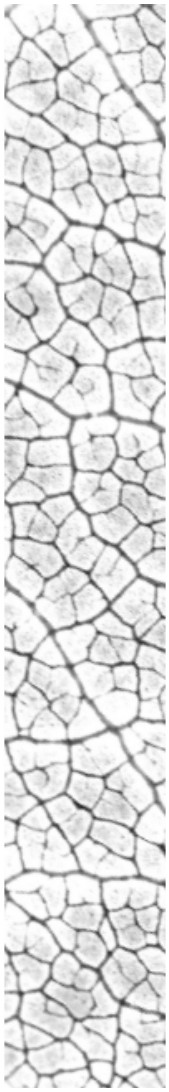

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Study of topoisomerase I (Topo I) level and some biochemical parameters in diabetic patients

Nagham Q. Kadhim [1*], Saif M.H. Al-faraji [1], Firas T. Maher [1]

[1] Department of Chemistry, College of Science, Tikrit University, IRAQ

Abstract

This study was done to determine level of Topoisomerase I and other biochemical parameters such as (Glucose, HbA1c, Insulin, C-Peptide, Cholesterol, Triglyceride, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) in Diabetic patient. The study included (70) patients of both sexes with diabetes for both types (type 1 and type 2 diabetes mellitus) at age (27-79) year, and included (30) apparently healthy persons of both sexes as a control groups. The Results showed that there was a highly significant decrease ($p \leq 0.01$) in the level of Topoisomerase I in Diabetic patients compared with control group, no significant differences between both sexes and age groups studied and there were no significant differences in enzyme levels concerning mass body index (BMI) between patients and people with history of disease and without history of disease and smoking and non-smoking. Results also showed that there are a highly significant increase ($p \leq 0.001$) in the levels of glucose and HbA1c in patients group compared to control group and significant increase in insulin levels in type2 diabetic patients compared with control group, and a significant decrease in the levels of insulin in type1 diabetic patients compared with control group and a significant increase in C-peptide level in type 2 diabetic patients compared with control group while there was a significant decrease in type 1 diabetic patients compared with control group. There was a significant increase ($p \leq 0.01$) in cholesterol, triglyceride and VLDL levels in patients groups compared with control group, and no significant difference in HDL and LDL level.

Keywords: Topoisomerase I, Diabetes Mellitus, C-Peptide

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INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia due to derangement in carbohydrate, fat, and protein metabolism. Diabetes mellitus is associated with absolute or relative deficiencies in insulin secretion, insulin action or both. The global figure of people suffering from diabetes mellitus is estimated to rise from current estimate of 415 million to 642 million by 2040 (American Diabetes Association 2016, 2017, International Diabetes Federation 2016, Razana et al. 2017).

DNA topoisomerases (Top) helps to solve topological problems by introducing single or double transient strand breaks followed by a relegation of the scissile strands; in this way they change the linking number and the supercoiling state of DNA (Chan et al. 2018). The reaction catalized by topoisomerases consist of a cleavage that occurs with an initial transesterification reaction between a tyrosine of the active site of the protein and a phosphate group of the DNA; this lead to the formation of a covalent intermediate enzyme-DNA complex. Then DNA is released through a second transesterification reaction. There are topoisomerases that only relax negative supercoils, others relax both signs and some even introduces negative (such as bacterial DNA gyrase) or positive (reverse gyrase) supercoils. The classification of topoisomerases is based on the number of DNA strands cleaved during the catalytic reaction. Topoisomerases that cleaves only one strand are defined as type I, those that cleaves both strands, generating a cleaved staggered duplex filament, are classified as type II topoisomerases (Harvey and Ferrier 2014).

Type I topoisomerases are monomeric (M.W 67KD) and are further classified into two subfamilies: IA and IB. The IA family introduces positive supercoils, decatenate single-stranded DNA and unwind supercoiled DNA through covalent binding to the 5' end of the scissile strand, leaving a free 3'-OH strand. Top IB family relax both negatively and positively supercoiled DNA, establishing temporary phosphotyrosine bonds with the 3' end and leaving a free 5'-OH strand (Ang et al. 2016, Ireton et al. 2000, Lima et al. 1994, Liu et al. 1980, Pommier et al. 2010).

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Table 1. mean ±SD of topoisomerase I concentration in studied groups with some factors

Sex	Level of Topoisomerase I ng/ml (Mean ±SD)		
	Diabetic type 1	Diabetic type 2	Control
Male	0.35631±0.01283	0.43044 ±0.0491	0.85302±0.0792
Female	0.43559±0.1794	0.32871± 0.0593	0.6938±0.21130
Total	0.40132±0.13036*	0.38463 ±0.1052*	0.72606±0.09806

Age/ year	Diabetic Patients	Control
27 - 38	0.40132 ± 0.13036*	0.692905 ± 0.09524
39 - 50	0.35540 ± 0.02427*	0.56712 ± 0.02665
50 >	0.406358 ± 0.0232*	0.765485 ± 0.10369

Genetic history of disease		Living		Smoking		BMI Kg/m²		
Found	Non found	City	Village	Smoker	No smoker	Normal 19-24	Over weight 25-29	Obese 30 ≤
0.3757± 0.104	0.3956± 0.1162	0.3855± 0.1291	0.3947± 0.1069	0.39935± 0.13095	0.38384± 0.10205	0.3845± 0.02325	0.37762± 0.10321	0.3894 ± 0.1132

* a highly significant decrease

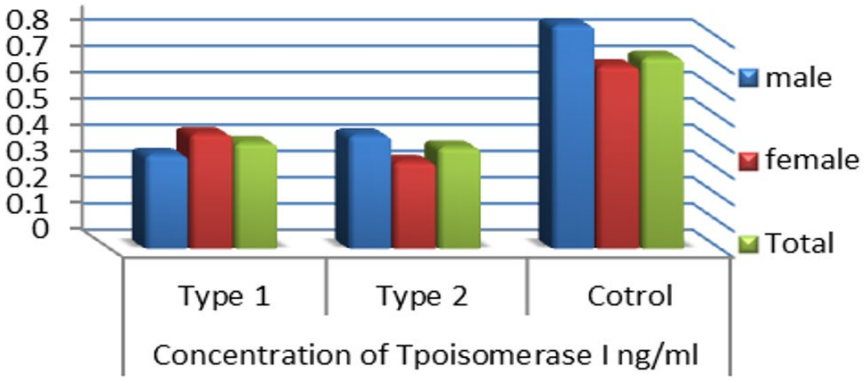

Fig. 1. Level of Topoisomerase I in diabetes patients with type I , II and control

Insulin and C-peptide are synthesized in the h cells of the islets of Langerhans by enzymatic cleavage of proinsulin and are both released into the circulation in equimolar amounts. In contrast to insulin, C-peptide physiologic function is not clearly known. However, C-peptide has a longer half-life in plasma than insulin and therefore can be used as a marker to reflect more accurately insulin secretion of a given individual (Gadre et al. 2015).

Lipids are hetrogeneous group of compounds which are relatively insoluble in water but dissolve in non-polar organic solvents such as chloroform. The major lipids found in the blood are cholesterol, triacylglycerol, phospholipids and nonesterified fatty acids. Lipoprotein types are: Chylomicrons, very low density lipoproteins cholesterol (VLDL-C), low-density lipoproteins cholesterol (LDL-C), intermediate-density lipoproteins cholesterol (IDL-C) and high-density lipoproteins cholesterol (HDL-C). They have many structural, metabolic and nutrient functions in the biological system (Nagham 2015).

MATERIALS AND METHODS

Seventy patients suffering from of diabetes mellitus and thirty normal healthy were participated in the present study. Their ages ranged from 27 to 75 years. Samples were collected from the Al - Askari Hospital in Salah Al - Din Governorate. All patients had blood glucose level > 200 mg/dl. Five milliliters of venous blood were drown from each patient and healthy control individuals. Serum was obtained and kept into small Eppendorf tubes capacity 1.5 ml at -20C° until time of analysis. The topoisomerase 1 assay employs the quantitative sandwich enzyme immunoassay technique (Cloud-Clone Corps 2017) by kit supplier by Cloud-Clone Corp company. Insulin and c-peptide were determined by cobase instruments (Sonksen and Sonksen 2000). HbA1C was measured by STANBIO-USA kit. Glucose, HBA1C, cholesterol, TG and HDL-Ch levels were determined by enzymatic colorimetric methods (Burtis and Ashwood 1999, Gonen and Rubenstein 1978, Tietz 2006, Martia et al. 1997), and the LDL-Ch and VLDL-Ch was estimated by Friedewald formula (Friedewald et al. 1972). BMI was calculated using the formula weight in kgs/height (m2) (Vittal et al. 2010).

Statistical analysis: The significance of difference between mean values were estimated by student T-test. The probability is considered as significant when $p<0.05$ and highly significant when ($p<0.001$).

RESULTS & DISCUSSION

Topoisomerase I

The mean (±SD) of topoisomerase I concentration in serum of control group (normal individuals) and serum of patients with diabetes mellitus are illustrated in **Table 1** and **Fig. 1**. There are a highly significant decrease ($p<0.001$) in the serum levels of topoisomerase I in

Table 2. Level of glucose, Glycolysed hemoglobin(HbA1c) , Insulin and C-Peptide in diabetic patient and control

Parameters	Mean ±SD		
	Diabetic type 1	Diabetic type 2	Control
GLU mg/dl	266.42 ± 70.957	271.20 ± 64.732	117.2±9.0985
HbA1C%	6.2 ± 1.5032	8.4 ± 1.6786	4.229 ± 0.2322
Insulin µU/ml	2.59873±1.2358	12.18125±2.1314	5.8432±2.1032
C-Peptide ng/ml	0.0962 0.4712±	0.43732 2.6625±	1.6679±0.1527

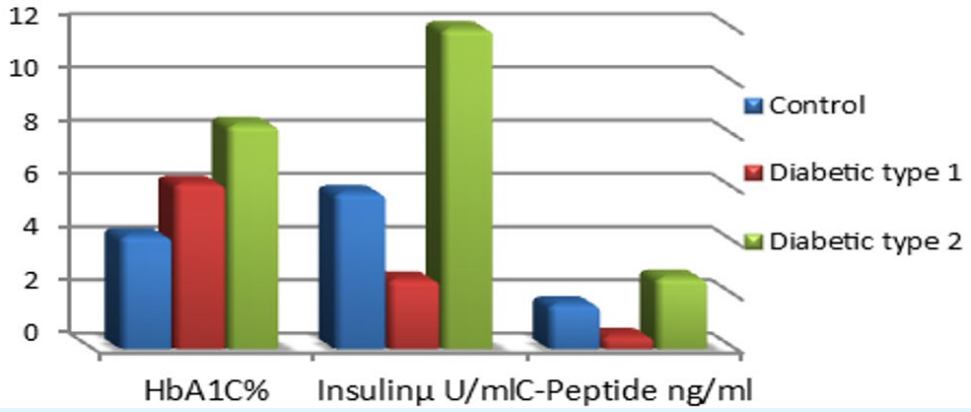

Fig. 2. Level of HbA1c , Insulin and C-Peptide in diabetes patients with type I , II and control

patients with diabetes mellitus group when compared with control group, while no significant different between type 1 and 2 diabetes mellitus group, or between other factors (Genetic history of disease, Living style, Smoking, BMI). There are no significant differences between age groups in patients, and also there are a significant increase ($p<0.05$) in the serum levels of topoisomerase I in age groups of patients with diabetes mellitus when compared with of control group (normal individuals)

Our results agree with other researches which found reduced topoisomerase I in diabetic (Itzhake et al. 2012, Wolf and Ziyadeh 1999). The reduction in topo I protein might be due to protein degradation or reduction in topo I expression. Long-term hyperglycemia leads to the development of diabetes-specific microvascular and macrovascular complications, through changes in both gene expression and protein function (Girach et al. 2006). This reduction in the activity was accompanied by a decrease in the enzyme protein level, in diabetic tissues.

Other study found that; The decrease in topoisomerase I protein level was not a consequence of the reduction in total protein level in the organs derived from diabetic rats, since the level of structural proteins such as β-actin was not altered in these organs compared with those derived from nondiabetic rats. Moreover, it was reported that in diabetic kidney a decreased turnover of proteins is observed (Wolf and Ziyadeh 1999). Therefore, these results suggest that topo I protein is not stable in the diabetic organs and its amount reduced probably due to protein degradation.

Glucose, HBA1C, Insulin and C-peptide

The mean (±SD) of glucose, HBA1C, insulin and C-peptide levels in serum of control group (normal individuals) and serum of patients with diabetes mellitus are illustrated in **Table 2** and **Fig. 2**.

There are a significant increase ($p<0.05$) in the serum levels of glucose and HbA1C, and a highly significant increase ($p<0.001$) in the serum levels of insulin in patients with type2 diabetes mellitus when compared with control group, and a highly significant decrease ($p<0.001$) in the serum levels of insulin in patients with type1 diabetes mellitus when compared with control group. Also, There are a significant increase ($p<0.001$) in the serum levels of C-peptide in patients with type 2 diabetes mellitus when compared with control group, while there is a highly significant decrease ($p<0.001$) in the serum levels of C-peptide in patients with type1 diabetes mellitus when compared with control group.

Insulin resistance is one of the primary defects in the majority of patients with DM.

Our result agreement with study conducted by Shim et al. (2006) and others (Khursheed 2018) also states that fasting c-peptide levels decreases with increase in duration of diabetes and fasting glucose, postprandial glucose and HbA1c levels were increased with the increase in duration of diabetes.

Glucotoxicity caused by elevated plasma glucose levels has been (Thunander et al. 2012) implicated as a primary cause of beta cell dysfunction. Increased glucose levels activate the hexosamine pathway and contribute to the excess generation of reactive oxygen species, resulting in inhibition of insulin gene transcription and insulin secretion (Zhou et al. 2017). Lower levels of c-peptide and decreased beta cell function have been linked to greater levels of glucose variability and glucose variability is known to be associated with increased complications and mortality in patients with diabetes.

Table 3. Level of Lipids and Lipoprotein in diabetic patient and control

Parameters	Mean ± SD			
	Total Diabetic	Diabetic type 1	Diabetic type 2	Control
Cholesterol mg/dl	250.6024 ± 70.21725	258.3684 ± 62.72622	248.9269 ± 72.59251	220.4250 ± 18.54601
TG mg/dl	119.9759 ± 72.2627	121.3684 ± 71.8959	120.8594 ± 72.59251	88.850 ± 9.62451
HDL mg/dl	46.53375 ± 4.35693	50.26429 ± 8.36692	45.4892 ± 8.2165	47.3112 ± 8.19215
LDL mg/dl	188.2452 ± 61.5707	172.9929 ± 22.73735	185.1156 ± 65.60081	170.659 ± 23.0963
VLDL mg/dl	26.34217 ± 12.81213	26.61053 ± 12.71559	26.2625± 12.75112	17.7575 ± 9.89801

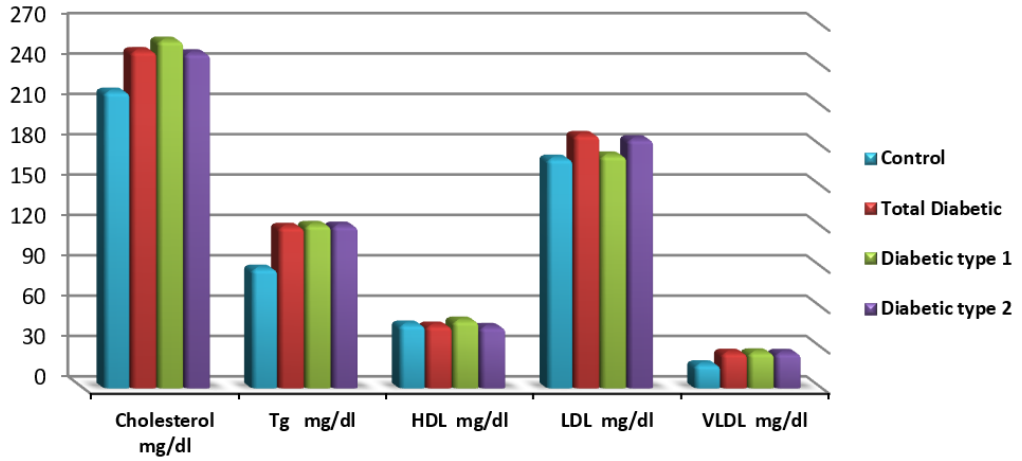

Fig. 4. FigureCaptionWillBeHere

The decline in beta-cell dysfunction in type 2 diabetes was most probably caused by progressive loss of beta cell mass. Similar results are reported by other authors reported that there is inverse correlation between duration of diabetes and fasting c-peptide levels (Sonwane et al. 2018). Zangeneh et al. (2006) states that insulin secretion decreases over time in many patients with type 2 diabetes mellitus.

C-peptide is much more than a byproduct of insulin synthesis and has several biological actions such as hypoglycemic, antilipolytic and vasodilator effects. These biological effects suggest that it may act as a hormone to contribute in fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. Others have shown no effect by C-peptide on blood glucose level in healthy subjects or patients with type 1 diabetes. However, infusion of physiological concentrations of C-peptide to patients with type 1 diabetes augments whole body glucose utilization by approximately 25% (Johansson et al. 1992, Ghorbani and Shafiee-Nick 2015).

Lipid Profile

The mean (±SD) of cholesterol, triglyceride, HDL, LDL and VLDL concentration in serum of control group (normal individuals) and serum of patients with diabetes mellitus are illustrated in **Table 3** and **Fig. 3**. There are a significant increase ($p<0.05$) in the serum levels of cholesterol, triglyceride, LDL and VLDL, and significant decrease ($p<0.05$) in the serum levels of HDL in patients patients with diabetes mellitus, while no significant difference between type 1 and 2 diabetes mellitus.

Lipid profile and diabetes have been shown to be the important predictors for metabolic disturbances including dyslipidemia, hypertension, and cardiovascular diseases. The abnormalities of carbohydrate metabolism observed in diabetes mellitus may lead to affect other metabolism also especially lipid metabolism (AL-Hamadan 2002). The degree of variations in lipid profile of diabetic patients may not generalized to all region and should be individualized to specific regions as ethnic, hereditary and environmental factors influence lipid profile.

These findings are consistent with those of King (2014), and Senthilkumar et al. (2018); they found that increased TG & decreased HDL-C plasma concentration are common features of dyslipidemia in T2DM (Akar et al. 2018, Çoban and Ediger 2018, Dhanotiya and Sharma 2014).

CONCLUSIONS

Topo I is an important enzyme, which regulates gene expression , thus, the regulation of its DNA relaxation activity by high glucose levels may contribute to the complications seen in organs under diabetic state and could mark this enzyme as a therapeutic target for preventing and/or reducing the development of diabetic complications.

REFERENCES

Akar E, Batçık KE, Acar C, Ton Ö, Canaz H, Baydın S, et al. (2018) A Comparative Analysis of the Effects of Melatonin and Nimodipine on Vasospasm. J Clin Exp Invest., 9(3): 113-8. https://doi.org/10.5799/jcei.458757

AL-Hamadan RY (2002) Pattern of Dyslipidemia in diabetic patients. J. Basic. Med, 1(2): 107–10.

American Diabetes Association (2016) Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes. Diabetes Care, 39(1): 13–28.

American Diabetes Association (2017) Report of the Expert Committee on the Diagnosis and classification of Diabetes Mellitus. Diabetes Care, 40(I): 11–24. https://doi.org/10.2337/dc17-S005

Ang RB, Teoh LS, Chan MK, Miswan N, Khoo BY (2016) Comparing the expression of human DNA topoisomerase I in KM71H and X33 strains of Pichia pastoris. Electronic Journal of Biotechnology, 21: 9-17. https://doi.org/10.1016/j.ejbt.2016.01.007

Burtis CA, Ashwood ER (1999) Text Book of Clinical Chemistry, 3rd edn. USA. W.B. Saunders Company, pp. 490, 482, 500.

Chan M, et al. (2018) Expression of stable and active human DNA topoisomerase I in Pichia pastoris. Journal of Protein Expression and Purification, 141(1): 52-62. https://doi.org/10.1016/j.pep.2017.09.003

Cloud-Clone Corps (2017) Enzyme linked Immunosorbent assay kit for Topoisomerase I (Top 1). Guide,12th edn. SE234Hu, Katy, Tx 77494, USA.

Çoban H, Ediger D (2018) Control of asthma, quality of life, anxiety and depression symptoms among Turkish patients with asthma. Electronic Journal of General Medicine, 15(5): em71. https://doi.org/10.29333/ejgm/91403

Dhanotiya A, Sharma V (2014) Power reduction in digital VLSI circuits. International Journal of Research in IT, Management and Engineering, 4(6): 13-23.

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18(6): 499-502.

Gadre S, Bajaj A, Patil S, Sukumaran S (2015) C-peptide and its correlation to parameters of insulin resistance and obesity in Type 2 diabetes. Indian Journal of Basic and Applied Medical Research, 5(1): 285-91.

Ghorbani A, Shafiee-Nick R (2015) Pathological consequences of C-peptide deficiency in insulin-dependent diabetes mellitus. World J Diabetes, 6(1): 145-50. https://doi.org/10.4239/wjd.v6.i1.145

Girach A, Manner D, Porta M (2006) Diabetic microvascular complications: Can patients at risk be identified? A review. Int J Clin Pract., 60: 1471–83. https://doi.org/10.1111/j.1742-1241.2006.01175.x

Gonen E, Rubenstein AH (1978) Determination of Glycohemoglobin. Diabetologia, 15: 1. https://doi.org/10.1007/BF01219319

Harvey RA, Ferrier DR (2014) Lippincott`s illustrated Reviews: Biochemistry, 6th edn. Lippincott Williams & Wilkins, a Wolters Kluwer business, Philadelphia.

International Diabetes Federation (2016). IDF Diabetes Atlas, 7 edn. Int Diabetes Fed.

Ireton GC, Stewart L, Parker LH, Champoux JJ (2000) Expression of human topoisomerase I with a partial deletion of the linker region yields monomeric and dimeric enzymes that respond differently to camptothecin. Journal of Biological Chem, 275: 25820–30. https://doi.org/10.1074/jbc.M002144200

Itzhak L, Yael S, Esther P (2012) Type 1 diabetes affects topoisomerase I activity and GlcNAcylation in rat organs: kidney, Liver and pancreas. Glycobilogy Journal, 22(5): 704-13. https://doi.org/10.1093/glycob/cws008

Johansson BL, Sjöberg S, Wahren J (1992) The influence of human C-peptide on renal function and glucose utilization in type 1 (insulin-dependent) diabetic patients. Diabetologia, 35: 121-8. https://doi.org/10.1007/BF00402543

Khursheed CG (2018) Effect of Ghrelin, Adiponectin and some biochemical markers on patients with diabetic type 2. thesis of High Diploma in Medical Analysis, Tikrit University, Tikrit, Iraq: 60p.

King MW (2014) Medical Biochemistry. Internal Journal Science Stud, 4(9): 163-77.

Lima CD, Wang JC, Mondragón A (1994) Three-dimensional structure of the 67K N-terminal fragment of E. coli DNA topoisomerase I. Journal of Nature, 367: 138–46. https://doi.org/10.1038/367138a0

Liu LF, Liu CC, Alberts BM (1980) Type II DNA topoisomerases: enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. Cell J, 19: 697–707. https://doi.org/10.1016/S0092-8674(80)80046-8

Martia TA, et al. (1997) Cholesterol lowering effects of soybean, potato and rice protein depend on their low methionine contents. J. Nutr, 127: 470-7. https://doi.org/10.1093/jn/127.3.470

Nagham QK (2015) Hormonal and Biochemical Study of Patients with Cardiorenal Syndrome, PhD [dissertation]. Salah AL-din, University of Tikrit.

Pommier Y, Leo E, Zhang HL, Marchand C (2010) DNA Topoisomerases and their poisoning by anticancer and antibacterial drugs. J. Chem. Biol., 17(5): 421-33. https://doi.org/10.1016/j.chembiol.2010.04.012

Razana A, et al. (2017) Guidelines for the Management of Type 2 diabetes mellitus. Journal of Endocrinology, Metabolism and Diabetes of South Africa, 22(1): 1-196.

Senthilkumer GP, et al. (2018) Role of omentin 1 and IL-6 in type 2 diabetes mellitus patients with diabetic nephropathy. Journal of Diabetic & metabolic syndrome: Clinical Research & Reviews, 12: 23-6. https://doi.org/10.1016/j.dsx.2017.08.005

Shim WS, Kim SK, Kim HJ, Kang ES, Ahn CW, Lim KS, et al. (2006) Decrement of postprandial insulin secretion determines the progressive nature of type-2 diabetes. European Journal of Endocrinol, 155: 615-622. https://doi.org/10.1530/eje.1.02249

Sonksen P, Sonksen J (2000) Insulin: understanding its action in health and disease. Br J Anaesth, 85: 69-79. https://doi.org/10.1093/bja/85.1.69

Sonwane A, Sharma LR, Naorem S, Prasad L, Ranabir S (2018). Fasting c-peptide level and its correlation with body mass index and duration of diabetes in type 2 diabetes. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), 17(1): 22-5.

Thunander M, Torn C, et al. (2012) Levels of c-peptide, BMI, and age, and their utility for classification of diabetes in relation to autoimmunity, in adults with newly diagnosed diabetes in kronoberg, Sweden. 2012 EJE-11-0797-R2

Tietz NW (2006) Clinical Guide to Laboratory Tests. 7th edn. WA Saunders, Philadelphia: 457-8.

Vittal BG, Praveen G, Deepak PA (2010) Study Of Body Mass Index In Healthy Individuals And Its Relationship With Fasting Blood Sugar. Journal of clinical and Diagnostic research, 4(6).

Wolf G, Ziyadeh FN (1999) Molecular mechanisms of diabetic renal hypertrophy. Kidney Int., 56: 393–405. https://doi.org/10.1046/j.1523-1755.1999.00590.x

Zangeneh F, Arora PS, Dyck PJ, Bekris L, Lernmark A, Achenbach SJ (2006) Effects of duration of diabetes on insulin secretion. Endocr. Pract., 12(4): 388-93. https://doi.org/10.4158/EP.12.4.388

Zhou S, Meng X, et al. (2017) A 3- year follow up study of B-cell function in patients with early-onset type 2 diabetes; Experimental and therapeutic medicine 2016;12: 1097-102 Leighton E, Sainsbury CAR, Jones GC. A practical review of c-peptide testing in diabetes. Diabetes Ther, 8(3): 475-87. https://doi.org/10.3892/etm.2016.3394