Original Research Article

Analysis of various biochemical parameters of T2DM patients and healthy controls

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International Archives of Integrated Medicine, Vol. 6, Issue 7, July, 2019.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 09-06-2019 **Accepted on:** 14-06-2019
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: Jaiprabu Jayaraj, Pandiamunian Jayabal. Analysis of various biochemical parameters of T2DM patients and healthy controls. IAIM, 2019; 6(7): 1-6.

Abstract

Background: T2DM is a metabolic disease associated with a group of abnormalities including hyperglycemia, dyslipidemia, hypertension, elevated levels of biochemical and inflammatory markers in circulation. This condition predisposes an individual to a number of adverse consequences which include atherosclerotic cardiovascular disease, neuropathy, nephropathy, and retinopathy.
Aim of the study: To compare the various biochemical parameters in T2DM patients and healthy age-matched controls.
Materials and methods: The Study included 22 type of II diabetic patients as cases and 22 normal individuals as controls. Fasting blood sugar (FBS) and Postprandial blood sugar (PPBS complete hematological profile, lipid profile, total protein, c-reactive protein and calcium levels were measured in plasma of T2DM and compare with healthy controls. Fasting blood samples were collected into labeled centrifuge tubes, after an 8–12 h overnight fast, from the subjects by venepuncture. The blood samples were centrifuged at 2000rpm for 10 min using a desktop centrifuge and the serum separated and kept in labeled sample bottles at −70°C until further analysis.
Results: The results showed higher concentrations of RBC, hemoglobin, HCT, and lymphocytes in healthy controls when compared with T2DM patients and lower concentrations of WBC, platelets, MCV, MCH, neutrophils, monocytes and eosinophils in healthy controls when compared with T2DM patients. Serum lipid profiles in plasma of control and T2DM patients. The results showed high levels of serum lipid profiles including cholesterol, HDL-C, LDL-C and total cholesterol ratio in healthy age-matched controls when compared with T2DM patients. In contrast, the levels of triglycerides were found to be lower in healthy controls when compared with T2DM patients. The results showed that levels of plasma glucose, C-reactive protein, HbA1c were significantly ($p<0.05$) higher in T2DM

patients when compared with those without T2DM (healthy controls). There were also significantly ($p<0.01$) low levels of total bilirubin, ALT, total protein, albumin, total calcium in plasma of TDM patients when compared with plasma from healthy age-matched controls.

Conclusion: The findings in this study support the hypothesis that low-grade systemic inflammation is an underlying factor in the pathogenesis of T2DM and also a common antecedent for both T2DM and CVD. The data from this particular study also provide further evidence that inflammatory markers might provide a method for early detection of CVD risk. These data might have many significant implications for the prevention and treatment of T2DM. Modification of lifestyle habits and management of systemic inflammation should be the major targets for the prevention and treatment of CVD in T2DM patients.

Key words

Complete hemogram, Liver function test, Renal function test, Inflammatory markers.

Introduction

Type 2 diabetes mellitus (DMII) is the most common form of diabetes which accounts for about 90-95% of those with diabetes. It is associated with abnormalities of carbohydrate, lipid, and protein metabolism [1]. Chronic exposure to hyperglycemia can result in dysfunction and failure of various organs especially the eyes, kidneys, nerves, and heart and blood vessels [2]. The long-term micro- and macro-vascular complications in DMII are including retinopathy, nephropathy, neuropathy, myocardial infarction, and stroke. According to the American Diabetes Association, cardiovascular disease (CVD) accounts for as many as 75-80% of mortality in DMII patients [3]. The basis of abnormalities in DMII is deficient action of insulin on target tissues due to impairment of insulin secretion, defects in insulin action, or both. Insulin resistance, which represents a reduced physiological response of the peripheral tissues to the action of the normal levels of insulin, is a major finding in several metabolic disorders, including DMII and metabolic syndrome (Mets) [4]. Initially, insulin resistance is compensated by enhanced insulin secretion but later insulin secretion is impaired. In the progression from normal to impaired glucose tolerance and diabetes, insulin secretion deteriorates faster than insulin sensitivity. Insulin resistance is involved in the pathophysiology of diabetic dyslipidemia and commonly occurs as part of the Mets [5]. According to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATPIII) criteria, the Mets is a combination of modifiable risk factors including hyperglycemia, insulin resistance, hypertension, hypertriglyceridemia, decreased high-density lipoprotein cholesterol (HDL-C), and abdominal obesity. The consequences associated with the Mets may be responsible for cardiovascular complication and mortality observed in DMII population [6].

Materials and methods

The Study included 22 types of II diabetic patients as cases and 22 normal individuals as controls. Fasting blood sugar (FBS) and Post-prandial blood sugar (PPBS complete hematological profile, lipid profile, total protein, c-reactive protein and calcium levels were measured in plasma of T2DM and compare with healthy controls. Fasting blood samples were collected into labeled centrifuge tubes, after 8–12 hours overnight fast, from the subjects by venepuncture. The blood samples were centrifuged at 2000 rpm for 10 min using a desktop centrifuge and the serum separated and kept in labeled sample bottles at −70°C until further analysis.

Biochemical assay of parameters [7, 8]

Various biochemical parameters in blood including glucose, insulin, full blood count, cholesterol, serum lipid profile, HbA1c, U and E, and C - reactive protein were measured

according to standard protocols in both T2DM and healthy controls using Pre- modular analytics Inflammatory mediators were measured using microarray kits. Quantitative measurement of 10 human cytokines in the serum samples was done using Quantibody® Human Inflammation Array 1 from Ray Biotech, Inc., USA. The cytokines including IL-1α, IL-1β, IL-4, IL-6, IL-8, Il-10, IL-13, MCP-1, IFNγ, and TNFα were detected by fluorescence measurement of samples using a laser scanner with Cy3 equivalent dye.

Statistical analysis

All the values were expressed as mean ± SD and $P < 0.05$ was considered statistically significant. Statistical significance of the differences between the mean values was analyzed by one way ANOVA test using SPSS 16 statistical analysis software. Correlations between different variables were analyzed using Pearson's correlation coefficients (r). Sample size was calculated using ($\alpha = 0.05$, $\beta = 0.2$, $\sigma = 18$, $d = 11$) formula.

Results

The Study includes 22 types of II diabetic patients as cases and 22 normal individuals as controls. Fasting blood sugar (FBS) and Postprandial blood sugar (PPBS complete hematological profile, lipid profile, total protein, c-reactive protein and calcium levels were measured in plasma of T2DM and compare with healthy controls.

The results showed higher concentrations of RBC, hemoglobin, HCT and lymphocytes in healthy controls when compared with T2DM patients and lower concentrations of WBC, platelets, MCV, MCH, neutrophils, monocytes and eosinophils in healthy controls when compared with T2DM patients of p value <0.005 (**Table – 1**).

Table – 1: Hematological parameters.

Hematological PARAMETERS FULL BLOOD COUNT	**HEALTHY CONTROL (N=22)**	**T2DM (N=21)**	***P* VALUES**
Red blood cells (10*12/L)	5.142±0.145	4.511±0.140	0.05
White blood cells (10*9/L)	6.425±0.360	8.542±0.380	0.05
Platelets (10*9/L)	222.8±13.091	245.388±15.260	0.05
Lymphocytes (10*9/L)	2.284±0.131	2.040±0.168	0.05
Monocytes (10*9/L)	0.412±0.029	0.635±0.038	0.39
Neutrophils (10*9/L)	3.4785±0.296	5.464±0.307	0.05
Eosinophils (10*9/L)	0.2295 ±0.0421	0.2311±0330	0.05
Hemoglobin (g/Dl)	14.83±0.333	13.311±0.317	0.05
HCT (ratio)	0.434±0.008	0.402±0.008	0.05
MCV (fL)	85.07±1.318	89.72±1.311	0.05

Table – 2: Serum lipid profile.

SERUM LIPID PROFILE	**HEALTHY CONTROL (n=22)**	**T2DM (n=21)**	**P value**
Total Cholesterol (ratio)	4.480±0.184	3.965±0.181	-
HDL-C (mmol/L)	1.268±0.094	1.030±0.079	-
LDL-C (mmol/L)	2.584±0.188	1.921±0.200	0.05
Triglycerides (mmol/L)	1.418±0.123	2.223±0.266	0.05
Total Cholesterol ratio	3.913±0.316	3.805±0.232	0.05

Table – 3: Liver function tests and inflammatory markers.

Liver function tests	Healthy control (N=22)	T2DM (n=21)	P-Value
Total bilirubin (umol/L)	11.565±1.449	18, 8±1.275	0.05
Alkaline Phosphatase (u/L)	64.347±3.198	89.722±10.497	0.05
ALT (U/L)	31.090±8.359	20.785±2.727	0.05
GGT (u/L)	20.217±1.506	69.111±17.683	0.05
Total Protein (g/L)	79.304±0.723	74.444±0.682	0.05
Albumin (g/L)	48.565±0.543	45.111±0.675	-
Total Calcium (mmol/L)	2.375±0.016	2.374±0.02	-
Plasma Glucose (mmol/L)	4.726±0.189	11.23±1.337	0.05
HbA1c (DCCT %)	5.547±0.084	8.277±0.407	0.05
HbA1c (IFCCmmol/mol)	37.238±0.940	67.111±4.469	0.05
CRP (mg/L)	1.421±0.209	8.441±2.438	0.05

Table - 2 shows the serum lipid profiles in plasma of control and T2DM patients. The results showed high levels of serum lipid profiles including cholesterol, HDL-C, LDL-C and total cholesterol ratio in healthy age-matched controls when compared with T2DM patients. In contrast, the levels of triglycerides were found to be lower in healthy controls when compared with T2DM patients.

Table - 3 shows the levels of plasma glucose, CRP, HbA1c, urea, creatinine, alkaline phosphatize and GTT in healthy controls and T2DM patients. The results showed that levels of plasma glucose, C-reactive protein, HbA1c were significantly ($p<0.05$) higher in T2DM patients when compared with those without T2DM (healthy controls), GGT in plasma of T2DM patients when compared to plasma from age-matched healthy controls. There were also significantly ($p<0.01$) low levels of total bilirubin, ALT, total protein, albumin, total calcium in plasma of TDM patients when compared with plasma from healthy age-matched controls.

Discussion

There is now much evidence that risks for CVD are higher among T2DM patients compared to age-matched healthy control subjects. Other well-known risk factors including dyslipidemia, hypertension, sedentary lifestyle, smoking, and obesity add to this risk. In fact, 75-80% of mortality rate in T2DM patients are due to a combination of coronary heart disease, peripheral vascular disease, and cerebrovascular diseases [8]. Several research studies have provided evidence that patients with T2DM have several abnormalities in their blood and lipid profiles including U and E‘s, CRP, HbA1c, Glucose, and various other biochemical parameters. The present results are in total agreement with current evidence regarding the association of inflammatory markers with the development of the metabolic disease, CVD and causes of mortality [9]. These prospective results support a possible role for inflammation in T2DM and they go with the hypothesis that T2DM might possibly be an expression of ongoing cytokine-mediated acute phase response which is initiated by the innate system of our body. The present results also show that an elevation of plasma glucose (hyperglycemia) is observed in T2DM patients when compared with those without T2DM [10]. Glucose- related complications such as the formation of advanced glycation end-products might be important in the pathogenesis of diabetic late complications. In most of the previous studies, glycemia and the duration of clinical T2DM did not appear to be very strong risk factors CHD. However, the reason for this has not been considerably understood [11]. Elevated WBC count in T2DM patients might contribute to the development of vascular

complications. Chronic inflammation might play a crucial role in the pathogenesis of CVD in T2DM patients [12]. The close association between WBC and various complications of T2DM provides evidence that inflammation might be a common linking factor and consequently documented as a major risk factor for atherosclerosis [13]. Mononuclear leukocytes are recruited to the site of endothelial injury and form foam cells in the plaque. Inflammatory markers including various cytokines like IL-6, TNF-α are released from activated leukocytes and cause endothelial dysfunction. Interaction between various risk factors and inflammatory responses lead to widespread vascular damage, endothelial dysfunction, and cause complications in T2DM patients. Patients with T2DM have many lipid abnormalities including elevated levels of Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C). The results of this study are found to be consistent with the literature. The results of this study also reveal a high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C, and low HDL-C levels which are well-known risk factors for CVD [14]. The findings of this particular study demonstrate the potential prognostic importance of this biomarker in clinical care. Measurement of CRP, a specific marker of inflammation, is an additional finding in this study. Levels of CRP have been observed to be significantly higher in T2DM patients when compared with healthy controls [14]. CRP is a member of the pentraxin family of oligomeric proteins involved with pattern recognition in innate immunity. The biological mechanism through which CRP increases the risk of T2DM is not well understood [15]. CRP is a marker of low-grade inflammation and influences both insulin resistance and insulin secretion indirectly as a result of heightened systemic inflammation. The positive association between CRP and T2DM reflects underlying endothelial dysfunction and sub-clinical atherosclerosis. Studies should be directed at confirming these findings in animal models. It can be concluded that CRP is a risk marker for cardiovascular disease and could emerge as a mediator in atherogenesis [16, 17].

Conclusion

The best approach to prevent potential CVD in T2DM patients is early recognition of risk factors and aggressive therapy. In this study, it is found that elevated plasma levels of several biochemical parameters and inflammatory markers were independent predictors of CVD in T2DM patients. The findings in this study support the hypothesis that low-grade systemic inflammation is an underlying factor in the pathogenesis of T2DM and also a common antecedent for both T2DM and CVD. The data from this particular study also provide further evidence that inflammatory markers might provide a method for early detection of CVD risk. These data might have many significant implications for the prevention and treatment of T2DM. Modification of lifestyle habits and management of systemic inflammation should be the major targets for the prevention and treatment of CVD in T2DM patients. Pharmacological therapies with anti-inflammatory properties might also play an essential role in T2DM induced CVD. A healthy diet, regular exercise, yoga, and meditation, may also help in both reduction and prevention of T2DM-induced long term cardiac complications.

Acknowledgments

Authors would like to thank the faculty of the Department of Biochemistry, Vinayaka Missions Medical College, Karaikal for their humble support to complete the research work.

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