A Cross-sectional study on oxidative stress markers in type 2 Diabetes Mellitus and their correlation with blood sugar levels.

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Abstract

Background: Among major metabolic disorders of glucose metabolism, diabetes mellitus is the most common one, in which Insulin deficiency and insulin resistance are the common observations. It is a renowned fact that long-standing hyperglycaemia is associated with oxidative stress, caused by an increase in the reactive oxygen species. Adenosine deaminase(ADA) is a purine metabolic enzyme that degrades adenosine. Gamma-glutamyl transferase (GGT) maintains antioxidant levels by maintaining reduced glutathione in the cells. Ceruloplasmin is a known acute phase reactant.

Aim: 1. To Study and compare the levels of Serum FBS, PPBS, ADA, GGT and Ceruloplasmin in T2DM patients and nondiabetic subjects.

2. To study the correlation between these parameters and blood sugar levels in cases.

Materials and methods: A descriptive cross-sectional study was done at Subbaiah medical college in Shimoga, taking 50 T2DM patients and 50 controls. Serum levels of fasting blood sugar(FBS), Postprandial blood sugar (PPBS), Adenosine deaminase, Gamma-glutamyl transferase and Ceruloplasmin were estimated. Data were analysed in SPSS software 17 using independent student t test. $p < 0.01$ was considered significant.

Results: Increased levels of ADA, GGT and ceruloplasmin in cases were found, and they were statistically significant. Pearson correlation of these inflammatory markers with FBS and PPBS showed a positive significant correlation.

Key words: Type 2 Diabetes mellitus, Adenosine deaminase, GGT, Ceruloplasmin, oxidative stress.

Introduction

Of the major non-communicable diseases, the incidence of Diabetes mellitus (DM) is rising alarmingly all the world over. Incidence in India has been steadily rising, with India having the second largest number of people with diabetes mellitus[1]. As per the WHO statistics, the prevalence is rising rapidly among the low and middle-income countries in comparison to the developed nations. Mortality directly caused by diabetes is also on the rise. In 2019 it was the ninth leading cause of death with an estimated 1.5million people dying[2].

Due to this increasing prevalence, diabetes has become a topic of interest for many researchers who are coming up with new theories regarding molecular mechanisms in the pathogenesis of diabetes. Patients with long term diabetes are known to suffer from diseases and dysfunction of various organs. Insulin deficiency or resistance, either of which arises from autoimmune destruction of beta cells of the pancreas, is the most common etiological factor. Low-grade inflammation induced by oxidative stress mediators could be one of the causes of insulin resistance[3]. Many recent studies have shown that oxidative stress is one of the main culprits in the development of DM, with theories of altered immunological mechanisms and altered T cell functions gaining prominence.

Adenosine deaminase (ADA) is the enzyme of

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purine nucleoside metabolism which catalyses the deamination of adenosine to inosine. This enzyme is essential for the differentiation and proliferation of T lymphocytes, monocyte and macrophages. Thus, ADA is considered as a marker of cell-mediated immunity and also a marker of many immunological diseases[4,5,6]. Adenosine acts as an important modulator of insulin at various levels of glucose metabolism by stimulating its action as well as on glucose transport and lipid synthesis[7]. Adenosine helps in increasing glucose uptake into cells. It not only regulates the pro and anti-inflammatory cytokine release, but also regulates the free radicals production and release such as hydrogen per-oxide from stimulated neutrophils. This in process protects the vascular endothelium from damage. ADA decreases intracellular adenosine and is involved in the production of reactive oxygen species. Also, it acts as a stimulator of lipid peroxidation in DM. ADA has therefore been proven to be an important biomarker in various disease conditions, including DM[7]. Thus, more ADA activity causes decrease glucose uptake by cells[8].

Gamma-glutamyl transferase (GGT) is an enzyme involved in the catabolism of glutathione (GSH) which is a major antioxidant. Most of the GGT in serum is derived from the liver[9]. Elevated levels of GGT are found in various conditions associated with oxidative stress and may be considered as a hallmark of oxidative stress. GGT is associated with excessive fat deposition in the liver, resulting in non-alcoholic fatty liver disease and is known to cause insulin resistance. Thus, GGT might serve as a marker of insulin resistance[9,10]. According to some studies obesity, insulin resistance, and type 2 diabetes are associated with abnormal hepatocyte function. Serum GGT acts as a marker of visceral and hepatic fat deposition which can lead to hepatic insulin resistance and long-term hepatic insulin resistance may lead to T2DM[11]

Transition metal ions such as copper and iron have a role to play in oxidative stress[12]. Ceruloplasmin is a copper carrying protein that plays a major role in iron metabolism. It has ferroxidase activity, which helps in the oxidation of ferrous ions into ferric ions, thus helping its transport in the plasma along with transferrin[13].

In conditions of elevated oxidative stress, ceruloplasmin may act as a pro-oxidant by donating free copper ions, which induces reactive oxygen species (ROS) formation and low-density lipoprotein (LDL) oxidation[12]. Increased serum ceruloplasmin could generate excess oxidized LDL, and cause vascular injury by generating free radicals such as hydrogen peroxide[14]. Thus, Ceruloplasmin could be one of the inflammatory biomarkers elevated in DM.

Objectives:

1. To Study and compare the levels of Serum FBS, PPBS, ADA, GGT and Ceruloplasmin in T2DM patients and nondiabetic subjects.
2. To study the correlation between these parameters and blood sugar levels in cases.

Materials and methodology:

This study was planned and carried out at Subbaiah Institute of Medical Sciences and hospital, Purale, Shimoga. Before starting the study institutional ethical committee clearance was obtained. Informed consent was taken from all the participants. The sample size was calculated by convenient sampling method. The cases chosen were 50 diagnosed Type 2 DM patients without any complications and controls were 50 age and sex-matched healthy volunteers. A simple random sampling technique was used. The study was carried out from September 2018 to March 2019.

Inclusion criteria:

Cases were diagnosed T2DM patients, diagnosis was done by using American diabetes association criteria[15] and clinical history, without any complications, aged between 35 years to 65 years, on oral hypoglycaemics, not on insulin and willing to participate in the study.

Controls were age and sex-matched healthy volunteers.

Exclusion criteria:

1. Patients with diabetes having complications.
2. Individuals with severe inflammatory diseases, infections, hepatic or renal diseases and persons on drugs that would affect blood glucose levels.
3. Chronic alcoholics, thyroid dysfunction.
4. Pregnant and lactating women.
5. Persons not willing to participate in the study.

Information was obtained by a preformed questionnaire. Basic investigations using standard protocol were done to rule out diabetes among controls and complications of diabetes among cases. Informed and written consent was taken. 3 ml venous blood was collected in a vacutainer, allowed to clot and serum was separated by centrifugation. A blood sample was collected using standard aseptic precautions. Following tests were done,

- FBS, PPBS - glucose oxidase peroxidase method[16]. (kits supplied by Robonik)

- Serum ADA - Giusti and Galanti method[17] (kits supplied by Proton Diagnostics limited)
- Serum GGT - Szasz enzymatic method[18] (kits supplied by Robonik)
- Serum Ceruloplasmin - Ravin's p-phenylenediamine method[19]. (Spectrophotometric method)

FBS, PPBS, ADA, GGT were estimated in fully automated analyser Autora.

Statistics:

Data entry was done in MS Excel Worksheet. Data were expressed in terms of Mean ± SD. Analyzed by descriptive statistics and independent student 't' test using SPSS software version 17. $p < 0.01$ was considered significant.

Results :

Table 1 showing the comparison of age and sex in cases and controls.

Parameter	Controls (n = 52)		Cases (n=52)	
Sex	28M	24F	28M	24F
Mean age in years	47.40 ± 10.26		53.33 ± 10.09	

Table 2 showing the comparison of FBS, PPBS, serum ADA and GGT in cases and controls.

Parameter	Controls	Cases	p value
FBS(mg/dl)	84.25 ± 13.67	158.67± 52.26	<0.001**
PPBS(mg/dl)	116.71 ± 8.14	225.96 ± 81.71	<0.001**
ADA(U/L)	19 ± 3.95	25.48 ± 6.43	<0.001**
GGT(U/L)	15.76 ± 4.54	30.05 ± 15.23	<0.001**
Ceruloplasmin (mg/dl)	30.45 ± 6.66	47.77 ± 11.99	<0.001**

** Statistically highly significant.

Table 3 showing the correlation between ADA, GGT, Ceruloplasmin with FBS.

Parameter	Pearson correlation coefficient (r)	p value
FBS and ADA	+ 0.454	<0.001**
FBS and GGT	+ 0.372	<0.001**
FBS and Ceruloplasmin	+ 0.448	<0.001**

** Statistically highly significant.

Table 4 showing the correlation between ADA, GGT, Ceruloplasmin with PPBS.

Parameter	Pearson correlation coefficient (r)	p value	Correlation
PPBS and ADA	+ 0.436	<0.001**	Positive
PPBS and GGT	+ 0.409	<0.001**	Positive
PPBS and Ceruloplasmin	+ 0.413	<0.001**	Positive

**Statistically highly significant.

Figure 1 : Correlation of FBS and ADA.

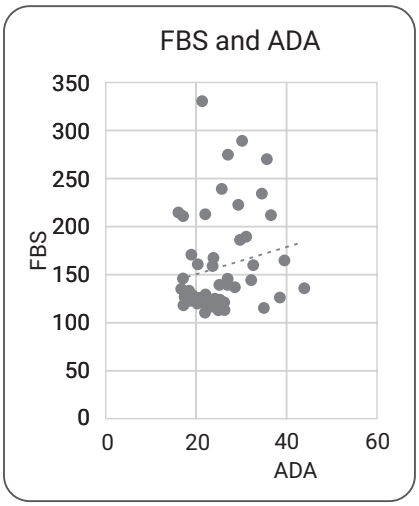


Figure 2: Correlation of FBS and GGT.

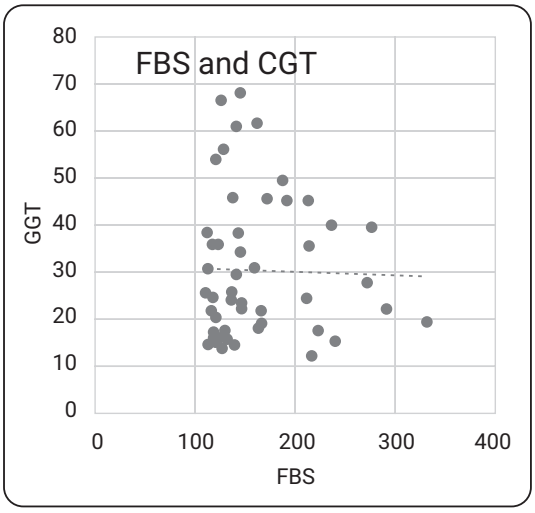


Figure 3: Correlation of PPBS and ADA.

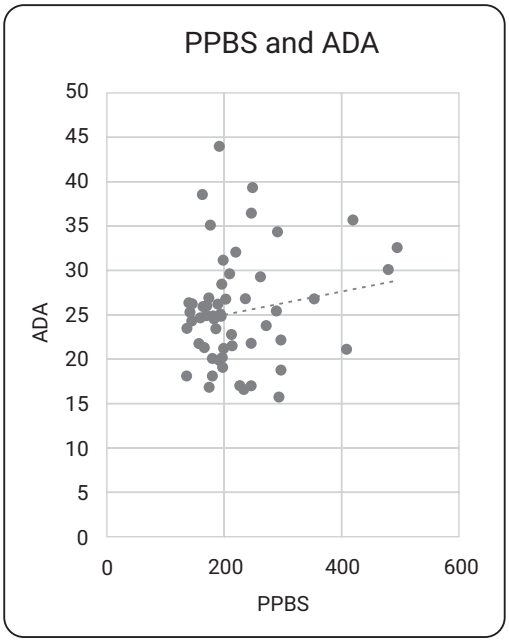
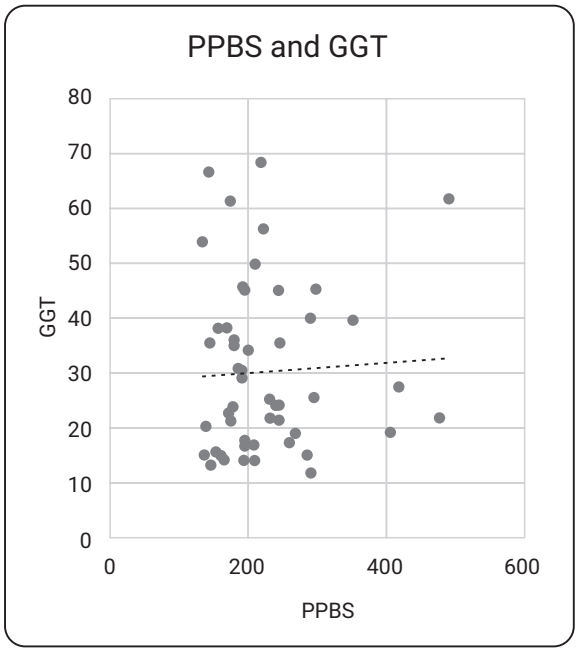


Figure 4: Correlation of PPBS and GGT.



Discussion

An impaired biological response to insulin stimulation on target tissues like liver, adipose tissue and muscle is considered as insulin resistance. This impairs the disposal of glucose resulting in increased secretion of insulin from beta cells of the pancreas causing hyperinsulinemia. Insulin resistance might lead to consequences like hyperglycemia, dyslipidemia and a rise in inflammatory markers[20].

Insulin resistance in T2DM leads to many complications through various mechanisms including the formation of ROS. This present study shows that ADA levels were raised in the diabetic group significantly as compared to that of the control group. The Pearson's correlation analysis in cases ADA shows a significant positive correlation between FBS and PPBS levels. An increase in ADA activity in T2DM patients has also been reported in other studies[2,5,7,21,22]. Adenosine is found to be having insulin-like activity on glucose and lipid metabolism. ADA can regulate adenosine concentration in cells[7]. ADA is found to be producing ROS and stimulates lipid peroxidation[1]. Chronic hyperglycemia leads to increased oxidative stress by forming free radicals and an increase in ADA levels, both leading to Insulin resistance. GLUT4 receptors are downregulated in the absence of adenosine. This is one of the reasons for Insulin resistance. Thus, if ADA activity is suppressed, insulin sensitivity may be improved. Our study is in accordance with other studies by different researchers[21,22]

In our study, serum GGT levels were significantly elevated in subjects with diabetes compared to controls which is in accordance with other studies[10]. There are several mechanisms by which GGT levels are increased in DM. GGT plays imp role in the antioxidant mechanisms of the body. It maintains glutathione levels in cells. Thus increased GGT means increased oxidative stress, which might play a role in DM. An increase in GGT activity can be a response to oxidative stress, facilitating increased transport of Glutathione precursors into cells[23]. We also observed a significant positive correlation between GGT and blood sugar levels. Hence, raised GGT concentrations could be a marker of oxidative stress and subclinical inflammation, in the cause and development of diabetes. Several studies showed similar results[23].

Ceruloplasmin is an acute phase protein that reflects underlying inflammation[24]. In our study ceruloplasmin levels are increased in cases. Similar results are found in other studies like Min Jung Lee et al, Vinayak Gaware et al[24,25]. High levels of ceruloplasmin reflect inflammation by hyperglycaemia. High levels of ceruloplasmin might lead to LDL oxidation which can inhibit the action of Nitrous oxide, causing endothelial dysfunction and atherosclerosis in DM[25]. However, some studies showed decreased ceruloplasmin levels[12,13,26].

Conclusion: Understanding the mechanism of pathogenesis in DM has changed in recent years. Many researchers are still coming up with different findings. Inflammatory mechanisms in DM have been proved many times by researchers. Our study also showed increased levels of different inflammatory markers like ADA, GGT, Ceruloplasmin. Adequate control of inflammation in DM might delay the progression and complications of DM. New treatment strategies aimed at decreasing oxidative stress might help in patients with DM.

Limitations: Our sample size was less. Follow up study with more sample size and with cases with complications can be considered. Our study did not consider glycaemic status. The prediabetes group was not studied.

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