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Role of Serum γ-Glutamyl Transpeptidase (GGT) Level as a Marker for the Detection of Type-2 Diabetes Mellitus

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Abstract: ***Introduction**: Diabetes Mellitus is one of the World's leading public-health problems.It is a metabolic disorder resulting either from deficiency of insulin or resistance to its action. GGT might reflect metabolic disorder and could serve as a marker for insulin resistance syndrome. Emerging evidence suggests that GGT is a predictor of incident diabetes. Raised serum GGT activity observed in diabetic patients may be in response to oxidative stress that occurs during the course of the disease. Thus, this study will be performed to examine whether GGT, an oxidative stress marker is useful marker for the detection of diabetic patients i.e., DM type2. Materials & Methods: A total of 50 individuals were selected out of which 25 each included adults individuals suffered from DM type2 & normal adults. Glucose Oxidase- Peroxidase Method and Fluoroscence Immunoassay meter methods applied for the determination of DM type 2 and Hb_{A1C} of the respectively. Also, Carboxy substrate Kinetic method used for the estimation of GGT in DM type 2 patients. All the above mentions methods were significant for the estimation of both DM type2 and GGT. Result: The study showed a significant increase in Hb_{A1C} levels in the study group when compared to control group. Also, the level of GGT increased in the case of DM type2 patients as compared with that of control group. Serum GGT, a marker of oxidative stress plays a central role in glutathione homeostasis. High levels of GGT are associated with insulin resistance and also involved in the development of type 2 diabetes mellitus. Conclusion: Study suggested the level of Fasting Blood Sugar, Post Prandial Blood Sugar,Hb_{A1C} increased in the DM type2 patients and the serum level of those individuals having high level of GGT as compared with that of normal persons. Hence, GGT which is a marker of Oxidative stress also raised in case of DM type2.*

Keywords: Diabetes Melllitus (DM), Gamma-Glutamyl Transpeptidase (GGT), Methylated Haemoglobin (Hb_{A1C}).

1. Introduction

Diabetes is a common clinical condition affecting above 10% of the general population (more prevalent after the age of 50) but nowadays it is becoming prevalent among age groups between 30 to 55 years also. It is a metabolic disorder resulting either from deficiency of insulin or resistance to its action causing increased blood glucose level (hyperglycemia) which leads to several systemic complications. Insulin resistance is defined as a decreased biological response to normal levels of circulating insulin[1]. Factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production[2]. Type 2 diabetes mellitus is on track to become one of the major global public health challenges of the 21st century[2]. About 90% of diabetes patients belong to this category. It usually affects the individual after 40 years of age (hence it is also called adult-onset diabetes). Earlier it was said thatsuch patients are not dependent on insulin (hence it was formerly known as non-insulin dependent diabetes mellitus-NIDDM)[1]. Diagnostic criteria by the American Diabetes Association (ADA) include the following:

- A fasting plasma glucose (FPG) level of 126 mg/dl (7.0 mmol/L) or higher on two occasions, or
- A 2-hour post glucose load plasma glucose level of 200 mg/dl (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), or
- A random plasma glucose of 200 mg/dl (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.
- Whether hemoglobin A1c (HbA1c) level of 6.5% or higher should be a primary diagnostic criterion or an optimal criterion remains a point of controversy[4].

Serum Gamma-Glutamyl Transpeptidase (GGT) is well known as a marker of alcohol-induced liver disease. GGT might reflect metabolic disorder and could serve as a marker for insulin resistance syndrome. GGT also plays an important role in antioxidant systems[3]. GGT has a central role in glutathione homeostasis by catalyzing breakdown of extracellular glutathione, a critical antioxidant defence for the cell[4]. Enzyme GGT catalyses the transfer of the gamma-glutamyl residue of glutathione (GSH) to other substrates[5]. Emerging evidence suggests that GGT is a predictor of incident diabetes.

2. Materials & Methods

Determination of Glucose in Serum (FBS, PPBG) by Glucose Oxidase- Peroxidase Method

Principle: Glucose Oxidase (GOD) acts on glucose to produce gluconic acid and hydrogen peroxide. Hydrogen peroxide produces nascent oxygen by peroxidase (POD). Nascent oxygen further reacts with a chromogen to produce colored product, which is estimated colorimetrically.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2\ (\text{Hydrogen Peroxide})$$

$$H_2O_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Reagents:
1) Enzyme Reagent: Glucose Oxidase, Peroxidase, 4-aminoantipyrine and Phenol in Phosphate Buffer.
2) Glucose Standard: 100 mg/dl

Test Procedure:
Clean and dry test tubes were labeled as Blank (B), Standard (S) and Test (T) and contents were added into the tubes according to the table given below:

Table 1: Test Procedure of GOD-POD Method

Addition Sequence	Blank	Standard	Test
Glucose Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	-	-
Glucose Standard	-	10 µl	-
Plasma	-	-	10 µl

1) Contents of the tubes were mixed well and incubated at 37°C for 10 min.
2) The absorbance of Standard (Abs. S) and Test Sample (Abs. T) was measured against the blank at 505 nm.
3) Color was stable for 45 minutes when protected from light, so absorbance was measured within that period.

Calculations:
Glucose in mg / dl of Test Specimen = Abs. T ÷ Abs. S × 100

Reference Values of Normal Ranges

Table 2: Normal range of FBS & PP

Serum / Plasma (Fasting)	70 – 110 mg / dl
(PP)	Upto 140 mg / dl

Determination of Glycated Haemoglobin (HbA1C) by Fluoroscence Immunoassay Meter.

Determination of Gamma-Glutamyl Transpeptidase (GGT) by Carboxy substrate Kinetic method

Principle: γ-glutamyl transpeptidase (TGG) catalyses the transfer of γ-glutamyl group from γ-glutamyl-p-nitroanilide to acceptor glycylglycine according to the following reaction:

$$\gamma\text{-L-Glutamyl-3-carboxy-4-nitroanilide} + \text{Glycylglycine} \xrightarrow{\text{GGT}} \gamma\text{-L-Glutamyl-glycylglycine} + \text{2-nitro-5-aminobenzoic acid}$$

The rate of 2-nitro-5-aminobenzoic acid formation, measured photometrically, is proportional to the catalytic concentration of TGG present in the sample.

Reagents:

Table 3: Reagent used for Carboxy substrate Kinetic Method

R1 Buffer	MES Buffer Glycylglycine	4 mmol/L 170 mmol/L
R2 Substrate	L-γ-glutamyl-3-carboxy-4 nitroanilide Stabilizers and inactive ingredients	3 mmol/L

Test Procedure
1) The working reagent was pre-warmed to 37°C before use.
2) The assay was performed as given below:
   - Working Reagent ⟶ 2.0ml
   - Serum/Plasma ⟶ 100µl
3) These two were mixed and transferred to thermostated cuvette.
4) Absorbance was recorded at 60th , 120th , 180th , 240th sec. (60 sec. interval) as A1, A2, A3, A4.
5) ΔA was calculated using the formula: ΔA = A4 – A1.

Calculation
GGT (IU/L) = ΔA / 60 × 2201

Reference Values for Normal Range

Table 4: Normal Range of Serum GGT

Gender	Men	Women
GGT (IU/L)	8 – 61	8 – 35

Inclusion Criteria:
- Adult Diabetic Patients

Exclusion Criteria:
- Surgical Patients
- Pregnant Women
- Children
- Liver disease
- Non- Alcoholic

3. Observations & Results

Table 5: Comparison of the Age (yrs.) between CONTROL group and DIABETIC group.

			P Value
AGE (years) (Mean±SD)	Control Group	Diabetic Group	> 0.05
	49.16±9.22	52.00±9.93	

* Non-Significant

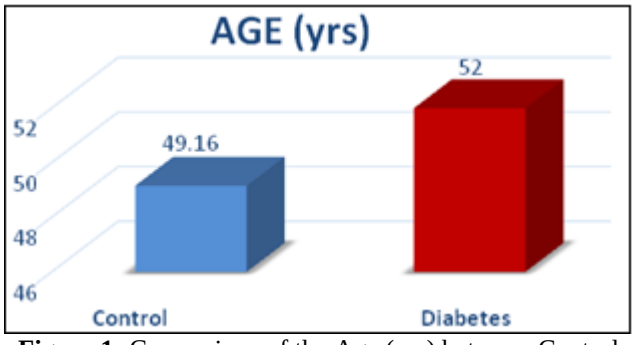

Figure 1: Comparison of the Age (yrs) between Control group and Diabetic group

Comparison of the AGE (yrs) between the two groups shows that AGE (yrs) difference in Diabetic group and control group is statistically non- significant with a p value of > 0.05.

Table 6: Comparison of the Fasting Blood Sugar (mg/dl) between Control group and Diabetic group

			P Value
Fasting Blood Sugar (mg/dl) (Mean±SD)	Control Group	Diabetic Group	< 0.001
	99.00±9.71	191.36±53.40	

* Significant

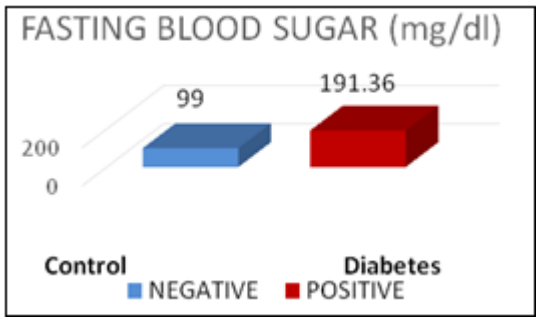

Figure 2: Comparison of the Fasting Blood Sugar (mg/dl) between Control and Diabetic group.

Comparison of the FASTING BLOOD SUGAR (mg/dl) between the two groups shows that FASTING BLOOD SUGAR (mg/dl) is higher in Diabetic group and is statistically significant with a p value of <0.001**.**

Table 7: Comparison of the Post Prandial Blood Sugar (mg/dl) between Control and Diabetic group

			P Value
Post Prandial Blood Sugar (mg/dl) (Mean±SD)	Control Group	Diabetic Group	< 0.001
	125.12±17.21	284.12±66.24	

* Significant

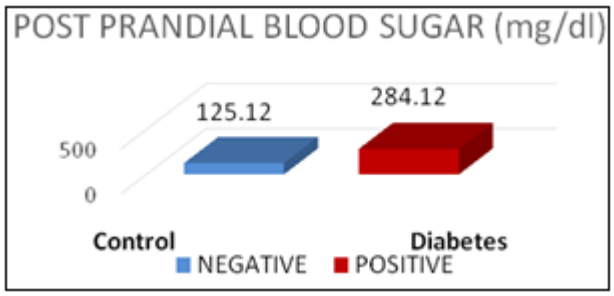

Figure 3: Comparison of the Post Prandial Blood Sugar (mg/dl) between Control and Diabetic group

Comparison of the POST PRANDIAL BLOOD SUGAR (mg/dl) between the two groups shows that POST PRANDIAL BLOOD SUGAR (mg/dl) is higher in Diabetic group and is statistically significant with a p value of <0.001.

Table 8: Comparison of the HbA1C (%) between control and diabetic group.

			P Value
HbA1C (%) (Mean±SD)	Control Group	Diabetic Group	< 0.001
	5.46±0.31	8.80±1.44	

*Significant

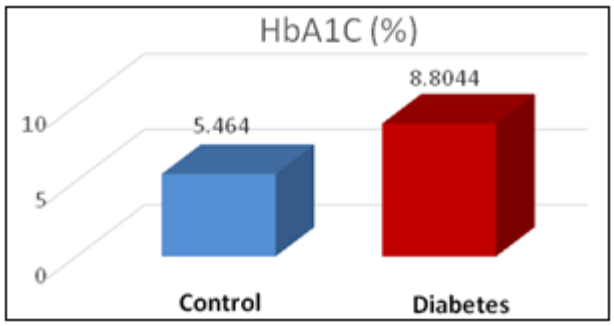

Figure 4: Comparison of the HbA1C (%) between Control and Diabetic group

Comparison of the HbA1C (%) between the two groups shows that HbA1C (%) is higher in Diabetic group and is statistically significant with a p value of <0.001.

Table 9: Comparison of the Gamma-Glutamyl Transpeptidase (IU/L) between control and diabetic group

			P Value
Gamma-Glutamyl Transpeptidase (IU/L) (Mean±SD)	Control	Diabetic	<0.001
	22.31±6.68	51.83±17.24	

*Significant

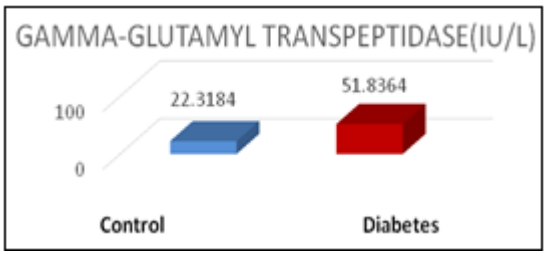

Figure 5: Comparison of the Gamma-Glutamyl Transpeptidase (IU/L) between Control and Diabetic group

Comparison of the GAMMA-GLUTAMYL TRANSPEPTIDASE(IU/L) between the two groups shows that GAMMA-GLUTAMYL TRANSPEPTIDASE(IU/L) is higher in Diabetic group and is statistically significant with a p value of <0.001.

4. Discussion

A total of 25 normal and same number of individuals having DM type 2 has been taken for the study in NIMS Hospital & Research Center, Jaipur. The age of the both the group was 30 to 55 years. The study showed a significant increase in HbA1C levels in the study group when compared to control group[6]. Studies also suggested that the Serum GGT, a marker of oxidative stress plays a central role in glutathione homeostasis and is widely distributed in various cells. High levels of GGT are associated with insulin resistance and also involved in the development of type 2 diabetes mellitus[7,8,9]. In the present study, even though the serum GGT levels in few cases were within the physiological range, the results showed a significantly high (p<0.001) increase in the levels of serum GGT in type 2 diabetics.

5. Conclusion

The FBS levels were significantly increased (with a p value of <0.001) in type 2 diabetics when compared to controls.

The Post Prandial Bood Sugar levels were significantly increased (with a p value of <0.001) type 2 diabetics when compared to controls. The HbA1C levels were significantly increased (with a p value of <0.001) in type 2 diabetics when compared to controls. The GGT levels were significantly increased (with a p value of<0.001) in type-2 diabetic patients when compared to controls. The present study help to investigate the DM type2 more precisely when it's related with GGT which help to diagnosed the condition and also help to prevention and treatment of DM type2.

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