

## SERUM GAMMA-GLUTAMYL TRANSFERASE AS A BIOMARKER OF TYPE-2 DM AMONG CIGARETTE SMOKERS

K. Suganthy<sup>1</sup>, P. K. Mohanty<sup>2</sup>

<sup>1</sup>Associate Professor, Department of Biochemistry, Velammal Medical College, Hospital and Research Institute, Madurai.

<sup>2</sup>Professor, Department of Biochemistry, Velammal Medical College, Hospital and Research Institute, Madurai.

### ABSTRACT

#### BACKGROUND

Smoking is one of the most common addictions of modern times and needs to be studied in a community as a public health issue. Also, smoking is a modifiable risk factor for type-2 DM. The smoking-related diseases share common pathophysiologies of imbalance of systemic oxidants and antioxidant status, increased inflammatory reactions, insulin resistance and dyslipidaemia. Biochemical assay of serum Gamma-Glutamyl Transferase (GGT) activity is a low cost and highly sensitive laboratory test. Studies have indicated GGT is moderately elevated before the onset of other traditional risk factors for type-2 DM. So, among hepatic markers, the baseline GGT analysis can be an early risk marker of type 2 diabetes in cigarette smokers has to be studied.

#### MATERIALS AND METHODS

This is a case-control study on male cigarette smokers. 57 smokers were studied clinically and biochemically for plasma insulin, glucose and liver enzymes including GGT using standard biochemical methods and compared with 42 age and sex matched non-smokers as controls.

#### RESULTS

The mean serum GGT in smokers ( $25.45 \pm 10.8$ ) was increased compared to non-smokers ( $18.8 \pm 5.8$ ). Smokers GGT ( $r=0.396$ ) and HOMA-IR ( $r=0.352$ ) showed significant positive association with duration of smoking ( $p<0.05$ ) than fasting blood glucose. Multiple regression analysis showed only duration of smoking ( $p=0.001$ ) as a dependable factor on GGT. 24.5% (14/57) smokers showed an increased GGT  $>24$  IU/L. Regression analysis showed none of the diabetic risk factors were observed to be dependent on GGT including other liver enzymes. Regression analysis showed GGT is not an independent risk factor for DM. Although, the mean fasting blood glucose ( $91.4 \pm 21.3$ ), BMI ( $26.1 \pm 9.3$ ) and HOMA-IR ( $7.3 \pm 2.3$ ) was increased among cigarette smokers with GGT  $>24$  IU/L.

#### CONCLUSION

The baseline GGT assay in cigarette smokers might be associated with the proinflammatory status or be a marker of oxidative stress of smoke toxins. Smokers with baseline GGT  $>24$  IU/L develop insulin resistance should be investigated in future longitudinal studies for prediabetes to consider cigarette smoking as an important modifiable risk factor of type-2 DM.

#### KEYWORDS

Cigarette, Smokers, Gamma-Glutamyl Transferase, Insulin Resistance, Diabetes Mellitus.

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#### BACKGROUND

Serum GGT has been a marker for various clinical conditions like alcohol consumption, body fat content, plasma lipids, glucose levels and medications. A number of studies have shown that the serum level of GGT directly correlates with increased risk of obesity, hypertension, DM, insulin resistance, dyslipidaemia and metabolic syndrome even after adjustment for alcohol consumption and established risk factors.<sup>1</sup>

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*Corresponding Author:*

*Dr. Suganthy K,*  
*Associate Professor, Department of Biochemistry,*  
*Velammal Medical College, Hospital and Research Institute,*  
*Madurai.*

*E-mail: drksuganthy@gmail.com*

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In addition, serum GGT serves as a clinical marker of overall hyperinsulinaemia, hepatic and systemic insulin resistance.<sup>2</sup> GGT can serve as an independent risk predictor of type-2 DM because of its strong association with insulin resistance syndrome and also independent with respect to other confounding factors like age, alcohol intake, physical activity, family history of diabetes and fatty liver indices.<sup>3</sup>

Smoking generates proinflammatory molecules causing systemic inflammation and insulin resistance through nicotine receptor.<sup>4</sup> Currently, smoking has been associated with 20-40% of increased risk of DM of which 99% is type-2 DM.<sup>5</sup> Therefore, determination of serum GGT can predict the development of diabetes among smokers.

As type-2 DM is a multifactorial disease, search of a low cost and strong biomarker for DM risk continuous among researchers. This study tries to find the association of serum GGT with IR to assess type-2 DM risk among cigarette smokers.

**AIMS AND OBJECTIVES**

To study the association of serum gamma-glutamyl transferase with insulin resistance to assess type-2 DM risk among cigarette smokers.

**MATERIALS AND METHODS**

This is a case-control study on male subjects of 57 cigarette smokers and 42 non-smokers as controls. After getting the informed consent from the selected study subjects, their smoking history was elucidated, clinical examination and anthropometric measurements were taken and recorded in a protocol format.

**Inclusion Criteria**

Cigarette smokers more than one year and smoking minimum of 2 cigarettes/day. Only males were included.

**Exclusion Criteria**

Alcoholic smokers, acute or chronic illness, previously diagnosed type-2 DM, history of liver diseases, treatment with steroid, anti-epileptics or anti-inflammatory drugs.

A 5 mL of blood sample collected after 8-12 hrs. overnight fast in a sterile disposable syringe under aseptic condition and then 3 mL blood was transferred to a dry clean test tube to clot and the remaining 2 mL of blood was transferred to an EDTA coated tube and used for the estimation of insulin and glucose.

Biochemical analysis of plasma insulin was done with ELISA reader of B4B diagnostics by enzyme immunoassay kit obtained from Bio source at 450 nm. Other parameters analysed using semi-auto analyser (photometer 5010v5+). Fasting Blood Glucose (FBG) was measured using the glucose oxidase peroxidase method and serum GGT, AST, ALT and ALP were measured using enzymatic kits by IFCC method.

Calculated parameters were- Insulin resistance was estimated by the Homeostasis Model Assessment Insulin Resistance Score (HOMA-IR score).

HOMA-IR = fasting glucose (mmol/L) x fasting insulin (µIU/mL)/22.5.

Body Mass Index (BMI) = Weight (kg)/Height (m<sup>2</sup>).

Smoking index = Number of cigarettes smoked per day x duration (years).

All quantitative data are presented as mean ± SD and qualitative data are presented as %. Statistical analysis was done by using SPSS (version 17.0). A p-value of <0.05 was considered as statistically significant.

**RESULTS**

57 volunteered male cigarette smokers were studied and compared with 42 age and sex matched non-smokers as controls. The mean age among non-smokers and cigarette smokers was 33.5 and 34.2 years, respectively. Both smokers and non-smokers were normotensive in this study.

The Table 1 shows in this study cigarette smoked on an average of 10.24 cigarettes/day for a duration ranging from 2-40 years with mean of 13.07 years. Smokers and non-smokers showed not much difference in their mean BMI.

Smokers showed mild significant elevation of AST, ALT and GGT with p<0.05. Mean HOMA-IR was significantly increased (p<0.001) in smokers on comparison with controls shown in Table-2.

Smoking Parameters	Range (Min-Max)	Mean ± SD
Number of cigarettes/day	3-45	10.24 ± 7.15
Duration of smoking (years)	2-40	13.07 ± 10.66
Smoking index	10-1440	148.1 ± 60.7

**Table 1. Cigarette Smokers Data**

Parameters (Normal Reference Range)	Non-smokers (n=42) Mean ± SD	Smokers (n=57) Mean ± SD	p-value
BMI (<23 kg/m <sup>2</sup> )	24.7 ± 2.92	23.8 ± 7.2	0.44
FBG (<110 mg/dL)	84.3 ± 11.9	86.3 ± 20.2	0.06
HOMA-IR (<6.4)	4.86 ± 1.02	6.96 ± 2.72	0.001*
AST (5-40 IU/L)	18.45 ± 6.17	23.3 ± 9.50	0.004*
ALT (5-40 IU/L)	20.07 ± 9.94	24.9 ± 12.6	0.04*
ALP (30-120 IU/L)	101.2 ± 25.6	100.65 ± 25.6	0.91
GGT (10-45 IU/L)	18.8 ± 5.88	25.45 ± 10.89	0.0005*

**Table 2. Comparison of Study Parameters Among Non-Smokers and Cigarette Smokers**

(\* p value <0.05 and statistically significant).

The correlation analysis in Table-3 depicts that the BMI, FBG, AST, ALT, ALP and HOMA-IR had positive association with the smoking pattern. Serum GGT showed positive and statistically significant (p<0.001) correlation with number of cigarettes/day (r=0.518) and duration of smoking (r=0.396).

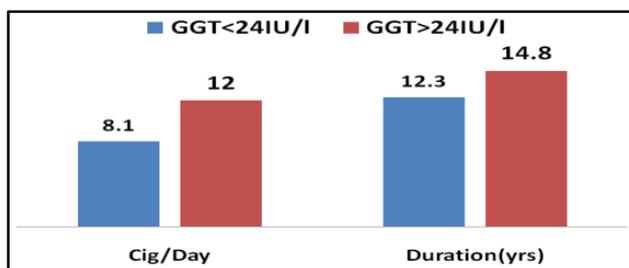
Parameters	Number of Cigarettes/Day r value	Duration of Smoking r value
Age	0.099	0.875*
BMI	0.219	0.04
FBG	0.189	0.112
HOMA-IR	0.158	0.332*
AST	0.219	0.108
ALT	0.212	0.150
ALP	0.233	0.21
GGT	0.518*	0.396*

**Table 3. Correlation of All Parameters with Cigarettes/Day and Duration of Smoking**

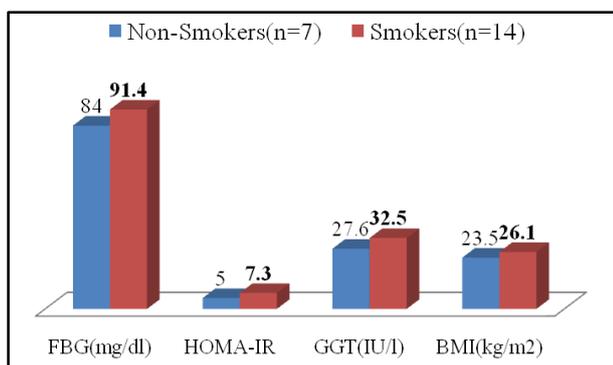
(\* Correlation is significant at the p <0.05 level (2 tailed).

As GGT >24 IU/L is associated with more incidence of DM in Misuza et al study.<sup>6</sup> Therefore, taking GGT >24 IU/L as cut-off value 14/57 (24.5%) smokers and 7/42 (16.6%) non-smokers had elevated serum GGT value. In the Figure 1, smokers with GGT >24 IU/L smoked on an average 12 cigarettes/day and duration of smoking was 14.8 years. Also, in cigarette smokers with GGT >24 IU/L correlation analysis of GGT with number of cigarettes/day (p=0.008) and duration of smoking were positive and statistically significant (p<0.05).

The Figure-2 shows comparison of diabetic risk factors of study subjects with GGT >24 IU/L. Mean BMI, GGT and HOMA-IR (26.1 ± 9.3, 32.5 ± 9.52 and 7.3 ± 2.53) were increased in smokers compared to non-smokers (23.5 ± 2.03, 27.6 ± 3.3 and 5.0 ± 0.96), respectively. Mean FBG, AST, ALT were in the normal range in both the groups with a slight increased value among cigarette smokers, but statistically not significant.



**Figure 1. Comparison of Mean Values of Smoking Pattern in Categorized Smokers Based on Their GGT Value**



**Figure 2. Comparison of Diabetic Risk Factors in Non-Smokers and Cigarettes Smokers with GGT > 24 IU/L**

Multiple regression analysis showed only duration of smoking (p=0.001) as a dependable factor on GGT. Table-4 shows regression analysis that none of the parameters were independently dependent on HOMA-IR in this study.

Parameters	Std. Coefficient Beta	p-value
Age	-0.087	0.169
Number of cigarettes/day	-0.065	0.063
Duration of smoking	0.077	0.194
BMI	-0.007	0.844
Family history of DM	0.002	0.938
AST	0.115	0.159
ALT	-0.120	0.115
ALP	0.006	0.839
GGT	0.005	0.912

**Table 4. Regression Analysis with HOMA-IR as Dependable Variable for DM Risk**

(\* Correlation is significant at the p <0.05).

**DISCUSSION**

The present work analysed the association of serum GGT among smokers with smoking habit, family history of

diabetes, FBG, BMI and insulin resistance on the incidence of risk of type-2 DM.

Smokers on an average smoked 10.24 cigarettes/day for duration of 13.07 years. The majority of them 51/57 (89%) smoked <20 cigarettes/day like Misuza et al study.<sup>6</sup>

Smokers showed a mean age of 34.2 years where as in a study of association of GGT for risk of type-2 DM in general population showed a mean age of 65 (55-69)<sup>6</sup> and of 46 (40-79) years.<sup>2</sup> Majority of our study individuals (64.9%) 37/57 smokers and (88%) 37/42 non-smokers were middle-aged adults between 20-40 years, which observed to be in consistent with systemic review and meta-analysis of active smoking and risk of type-2 DM.<sup>7</sup>

The mean BMI 23.8 kg/m<sup>2</sup> in smokers was less compared to non-smokers 24.7 kg/m<sup>2</sup>. The non-smokers BMI in Hsin-Cheihyeh et al study was 27.6 kg/m<sup>2</sup>.<sup>8</sup> BMI is positively and significantly associated with GGT (r=0.359, p=0.005) among smokers than in non-smokers (r=0.015) like other study.<sup>9,2</sup> In this study, correlation of mean BMI with number of cigarettes smoked per day (r=0.219) and duration of smoking (r=0.04) was not significant confirming the fact that smokers are not always obese and can have weight loss. Smokers with normal BMI tend to have greater risk of abdominal fat accumulation when compared with non-smokers and is not a significant factor to develop type-2 DM.<sup>10</sup>

Liver function enzymes were observed to be in normal limits both in smokers and non-smokers. In our study, mean AST (23.3 ± 9.56) and ALT (24.9 ± 12.66) was similar to AST (24.0 ± 10.5) and ALT (26.5 ± 18.8) in a study on former smokers. But, smokers had mean GGT (24.4 ± 10.8) was less than the study in former smokers of GGT (58.3 ± 6.7 IU/L).<sup>11</sup> In the present study, cigarette smokers GGT was increased and significant (p<0.05) on comparison to non-smokers.

The normal reference range of serum GGT 10-40 IU/L is considered in many studies. To study the interaction of GGT and type-2 DM, GGT concentrations were stratified into cut-off points like 13, 20, 35 and 56 IU/L.<sup>2</sup> Baseline GGT value >24 IU/L shown to be significantly predict the incidence of DM.<sup>6</sup> Therefore, in this study, smokers were categorised based on baseline GGT >24 IU/L value and assess their diabetic risk.

24.5% (14/57) of smokers had GGT >24 IU/L. These categorised smokers mean age, BMI, FBG, IR and liver enzymes showed increased value on comparison to non-smokers with GGT >24 IU/L consistent with the findings of Lee et al study.<sup>9,12</sup> Mean FBG (84 mg/dL) and HOMA-IR (7.53) in smokers was increased than non-smokers with GGT >24 IU/L. GGT raise had positive association with number of cigarette/day (r=0.518, p=0.008) and duration of smoking (r=0.396, p=0.008) simulating a dose and effect relationship.

As duration of smoking increases IR increased shown by positive significant association (r=0.33, p=0.01), whereas the association with number of cigarettes smoked per day (r=0.09) was not significant. Similarly, no association was found between the number of cigarettes smoked per day

and HOMA-IR by Alpersonmez et al.<sup>13</sup> Moreover, it suggests that nicotine and other possible toxins of tobacco smoke maybe harmful particularly in susceptible individuals, but not in all subjects.

GGT in various studies showed to be an important and independent predictor of type-2 DM in general population.<sup>14</sup> The correlation analysis showed positive association of liver enzymes AST ( $r=0.108$ ), ALT ( $r=0.150$ ), ALP ( $r=0.21$ ), GGT ( $r=0.396$ ) with duration of smoking in the present study. Regression analysis of liver enzymes with diabetic risk factors showed only duration of smoking ( $p=0.01$ ) as a dependant factor with serum GGT.

14/42 non-smokers and 13/57 smokers had family history of DM. Mean FBG and HOMA-IR was increased ( $93.2 \pm 27.6$ ,  $6.9 \pm 2.5$ ) in smokers compared to non-smokers ( $83.6 \pm 11.9$ ,  $4.6 \pm 0.5$ ) with family history of DM. Mean GGT and IR was increased in smokers irrespective of family history of DM speculating the fact that smokers develop insulin resistance directly due to the effects of the constituent of the tobacco smoke, altered glucocorticoid homeostasis or increased release of catecholamine, which might reduce the insulin binding sites and also the synthesis of glucose transporters. Thus, the theories of nicotine might have direct or indirect effect via interaction with insulin receptors and for post-receptive events are to be evaluated.<sup>15</sup>

Mean FBG in other studies were 133.3 mg/dL in Vani Gupta et al<sup>16</sup> and 101 mg/dL in Rachael,<sup>17</sup> comparatively less. In this study, smokers showed mean FBG of  $86.3 \pm 20.2$  ( $p=0.6$ ). If FBG  $>110$  mg/dL are considered to be at diabetic risk. 10.52% (6/57) smokers had FBG  $>110$  mg/dL, whereas Rachael study showed 16% were diagnosed to be diabetic. 22.8% (13/57) smokers had positive family history of DM compared to 14% in Rachael study.<sup>17</sup>

The regression analysis to detect the diabetic risk among smokers with insulin resistance as dependable variable showed that age, BMI, family history of DM, liver enzymes including GGT were not independently a risk factor for diabetes mellitus. Still in the present study, smokers with serum GGT in higher normal range, non-obese, FBG and IR increased should be considered high DM risk target population like NHANES study.<sup>18</sup> The cigarette smoke toxins may affect the mechanism involving early steps in insulin action (e.g., signal transduction, glucose transport and/or glucose phosphorylation) or by mechanisms operating simultaneously on different biochemical pathways to increase GGT has to be researched.<sup>16</sup> The GGT increased among smokers might be depicting the antioxidant defense systems to the proinflammatory status or be a marker of oxidative stress of smoke toxins.

## CONCLUSION

This study suggests smokers in middle adult age were not much obese. Smokers GGT and HOMA-IR were definitely increased compared to non-smokers irrespective of their family history of DM. Smokers with baseline GGT  $>24$  IU/L develop insulin resistance at some stage not known. Baseline GGT is more positively associated with number of

cigarettes smoked per day and duration of smoking, but not stands out to be an independent biomarker of DM risk. Thus, GGT maybe a marker of oxidative stress more than insulin resistance marker in cigarette smokers. A simple and cheaper assay of baseline GGT will be helpful in normal fasting plasma glucose levels in smokers than determination of insulin resistance as follow up. Since, the average age of initiation of smoking become earlier in adolescent stage itself, further the role of GGT as type-2 DM marker in cigarette smokers has to be further evaluated by follow up of high-risk suspected subjects.

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