

## The influence of tobacco smoking on the enzyme activity of serum gamma glutamyl transferas (GGT)

Received: 2/7/2012

Accepted: 14/11/2012

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### Abstract

**Background and objective:** The gamma glutamyl transferase GGT is a cell-surface protein contributing to the extracellular catabolism of glutathione. The enzyme is produced in many tissues, but most GGT in serum is derived from the liver so that it is currently the most sensitive enzymatic indicator of liver disease. To evaluate the effect of smoking on liver diseases through assessing the enzyme activity of serum GGT.

**Methods:** A cross-sectional study was carried out from January to June 2012 by clinical analysis department in College of Pharmacy at Hawler Medical University on (30) individuals of smokers, and (30) non-smokers. Serum GGT activity was measured using enzymatic colorimetric method.

**Results:** The mean value of serum GGT activity was significantly higher in smokers ( $20.89 \pm 6.79$  IU/L) (Mean  $\pm$  SD) than that of non-smokers ( $14.51 \pm 6.46$  IU/L) (Mean  $\pm$  SD) ( $p < 0.01$ ).

**Conclusion:** Significant increased activity of GGT in smokers seems to support the harmful effects of cigarette smoking.

**Keywords:** Serum Gamma Glutamyl Transferase (GGT), smoking, liver disease.

### Introduction

The liver enzyme Gamma Glutamyl Transferase (GGT) is a sensitive indicator of hepatobiliary disorders<sup>1-4</sup>. Present on the surface of most cells and serum, mediates cellular glutathione uptake which is an important element of intracellular protective antioxidant mechanisms, but at the same time is also regarded as a "pro-oxidant"<sup>5</sup>. Serum GGT is partly absorbed on low density lipoprotein LDL particles by which it may be delivered into the plaques<sup>6</sup>. GGT is present in the liver and bile duct, in the serum is mainly from hepatic. Clinical studies have shown that GGT is highly sensitive indicator of liver damage. Increased activity is observed in acute and chronic liver disease, biliary tract and pancreas<sup>7</sup>. Elevation in serum GGT activity can predict morbidity and mortality of liver disease<sup>8</sup>. Cigarette smoking is a world-wide major cause of morbidity and mortality<sup>9</sup>. This factor may have additional

roles in the etiology or progression of the hepatocellular carcinoma<sup>10</sup>. The possible association between GGT and inflammatory process, it should be considered that GGT has a key role in the interconversion of the glutathione containing inflammatory mediator leukotriene C4 into leukotriene<sup>11-13</sup>. Gamma glutamyl transferase metabolism of glutathione gamma glutamyl-cisteinyl-glycine (GSH) within the plaque. The hydrolysis of GSH originates cisteinyl-glycine, which is a powerful reductant of  $Fe^{3+}$ , able to simultaneously generate  $Fe^{2+}$  and a free thiyl radical. Thereafter, oxygen reactive species, by the same reaction, contribute to a pro-oxidant effect, leading to LDL oxidation and likely contributing to other processes<sup>12</sup>. Serum GGT level has shown a strong graded relationship with incident diabetes, suggesting a role in the pathogenesis of diabetes. Although serum GGT activity has been commonly used as a

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marker for excessive alcohol consumption or liver disease, neither alcohol consumption nor liver dysfunction likely explained these associations<sup>13-15</sup>. The relationship between cellular GGT and serum GGT is not known. Cellular GGT has been known to play an important role in antioxidant defense systems<sup>16</sup>; paradoxically, cellular GGT may also be involved in the generation of reactive oxygen species in the presence of iron or other transition metals<sup>17</sup>. Modest increases within normal range may be an early marker of cellular oxidative stress and explain the strong associations of serum GGT with many cardiovascular risk factors and disease<sup>8, 18, 19</sup>. Better understanding factors determining individual GGT levels thus have become a quest of high relevance to public health<sup>20</sup>. Few studies have described effects of smoking status on GGT, with increasing exposure to smoking being associated with higher serum concentrations, particularly in women. In contrast, GGT sensitivity appears to be higher in men, but the evidence base for this observation also is rather limited<sup>21</sup>. The aim of this study is to evaluate the serum activity of GGT in smokers and non-smokers

## Methods

**Subjects:** This study was conducted over a period of six months, from January to June 2012, by clinical analysis department in College of Pharmacy at Hawler Medical University. The subjects included (30) individual smoker volunteers (29 males and 1 female), (group 1), and (30) non-smoker volunteers (to recruit), (27 males and 3 females), control group (group2) a sample size used for logistical reasons and all the cases in both groups (1 and 2), were apparently healthy, and Informed -consent was obtained verbally from all participants and they were interviewed to obtain their smoking histories, including the number of cigarettes smoked / day and the number of years of smoking. Inclusion criteria were as follows: > 18 years of age, smoking 10-20 cigarettes /day (smokers only) accounted

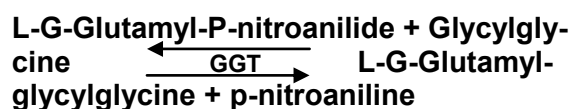
as moderate smoker<sup>9</sup>. Exclusion criteria were chronic diseases, regular use of medicine, use of vitamins or other dietary supplements and smoking within the last 2 years (non smokers only). The data collection in this research from female smokers was not easy and not present adequately, extra efforts to involve female participants has failed, because they are ashamed of participating in the research, for this reason the majority of participants were male smokers.

**Serum Sampling:** Four to six mls. venous blood was withdrawn from each individual using disposable syringes. The samples were immediately centrifuged for (10) minutes at 3000 round per minute, and the serum obtained was analyzed directly.

**Estimation of Serum GGT activity:** Serum GGT activity was determined by kits (Biolabo, France) for the two groups according to the method which is described by (Szasz, Rosalki and Tarlow method)<sup>22, 23</sup>.

**Principle of the Method:**

Gamma glutamyl transferase is easily assayed by reaction scheme as follows:



The rate of formation of p-nitroaniline, directly propotional to GGT activity in the specimen, is measured at 405 nm.

**Reagents:-**

Vial R1; Buffer:

Glycylglycine 62 mmol/L

TRIS PH 8.1 95 mmol/L

Preservative

Vial R2; Substrate:

L-G-Glutamyl-p-nitroanilide(GPNA) 2 mmol/L

**Procedure:**

**Reagent preparation:-**

Adding promptly the contents of Substrate into Buffer. And let stand reagents and specimens at room temperature.

**Table 1:** The method of the GGT test.

Pipette into 1 cm pathlength thermostated cuvette

**Reagent** 1 mL

Bring to 37 °C then add:

**Specimen or calibrator** 50 µL

Mix. Read initial absorbance after 30 seconds, record absorbance at 405 nm every minute during 3 minutes.

Calculate absorbance change per minute ( $\Delta$ Abs/min).

Calculation:-

Calculate the result as follows:

$$\text{IU/L} = (\Delta\text{Abs/min}) \times 2121$$

2121= Theoretical factor.

IU/L = International unit per liter can be defined as the quantity of enzyme.

Normal Value:-

Men (IU/L) 2-30

Female (IU/L) 1-24

Statistical Analysis:-

The statistical evaluation of the results [mean, standard deviation (SD) and standard error of mean (SEM)] were calculated using the SPSS (Statistical Package for Social Science) software version 13.0 for windows. The different variables were compared to each other; and tested with the unpaired student test [t-test]. Only  $p < 0.05$  is regarded as significant<sup>24</sup>.

## Results

Table 2 provides the age and number of cigarette smoking per day in smokers and non-smoker groups. The results obtained indicated that the smoker group classified as moderate smoker group.

**Table 2:** the (Mean  $\pm$  SEM) of age and no. of cigarette smoking by smoker and non-smoker groups.

| Groups               | No. | Age (Mean $\pm$ SEM) | No. of cigarettes /day |
|----------------------|-----|----------------------|------------------------|
| Smoker (Group 1)     | 30  | 39.73 $\pm$ 2.84     | 10 – 20                |
| Non-smoker (Group 2) | 30  | 35.0 $\pm$ 2.61      | 0                      |

The activity of serum Gamma Glutamyl Transferase (GGT):-

Table 3 provides the mean S.GGT activity in the smokers and non-smoker groups. The results obtained indicated that the mean S.GGT activity was (20.89  $\pm$  6.79 IU/L) (Mean  $\pm$  SD), in smokers group. This value was higher significantly than that obtained in non-smokers group (14.51  $\pm$  6.46 IU/L) ( $p < 0.003$ ).

**Table 3:** The Mean  $\pm$  SD of S. GGT activity in smoker and non-smoker groups.

| Groups            | No. | S. GGT (IU/L) (Mean $\pm$ SD) |
|-------------------|-----|-------------------------------|
| Smoker (Group 1)  | 30  | 20.89 $\pm$ 6.79              |
| Control (Group 2) | 30  | 14.51 $\pm$ 6.46              |

Serum GGT of smoker versus control group ; t test= 3.258 ,  $P < 0.01$

## Discussion

In this study, demonstrate a relationship of serum GGT to cigarette smoking. Preventive means should be intensified on all possible levels to encourage subjects to refrain from this risk, this way reducing the likelihood of various chronic diseases and mortality. The mean activity of serum GGT in cigarette smokers was significantly higher than that of non-smoker individuals. Our results are in agreement with those obtained by Brenner, et al<sup>1</sup>, Lee, et al<sup>15</sup>, Ruttmann, et al<sup>18</sup>, Lutz P. Breitling, et al<sup>20</sup>, Breitling, et al<sup>21</sup>, Haren, et al<sup>25</sup>, and

Whitehead, et al<sup>26</sup>. But in disagreement with Nakanishi, et al<sup>2</sup> and Khedmat, et al<sup>27</sup>, because they conducted a study to evaluate the influence of smoking on lifestyle choosing BMI, cigarette smoking, eating breakfast, and physical exercise and found no significant difference between smokers and non smokers. Furthermore, other studies gave an indication that cigarette smoking may adversely affect the public health. The elevation of serum level of GGT has been reported to be associated with liver diseases; this indicates that liver function enzymes can be used as biomarkers for the assessment of hepatobiliary disease<sup>11</sup>. Particularly, GGT is reported to have emerged as the clinically useful diagnostic test<sup>3</sup>. The observations made from the result of this present study therefore gave an indication that cigarette smoking may be considered to be one of the predisposing lifestyle risk factor for liver disorders. It has previously been pointed out that elevated GGT levels may be induced by glutathione depletion and oxidative stress. Likewise, the cardiovascular effects of tobacco smoking seem to be mediated at least to some extent by smoking-related oxidative stress<sup>16</sup>. The results showed a significant rise in serum GGT activity in moderate cigarette smokers when compared to control group. Previous studies also has reported a significant rise in serum GGT activity despite that in other studies showed that other liver enzymes like serum ALT, AST and ALP activity were no significant elevation in moderate cigarette smokers when compared to control group<sup>9</sup>. Till now, there is no previous study that concerned with the full investigation of the effect of cigarette smoke and dose exposure to tobacco smoke on liver tissue and liver functions in Iraq. Breitling, *et al*<sup>21</sup> found that the estimated individual effects of smoking on serum GGT were very similar in men and women. And the data collection in this research from female smokers was not easy and not present adequately, extra efforts to involve female participants has failed, because they are

ashamed of participating in the research, for this reason the majority of participants were male smokers. There is limitation in this study, selected factor for raised serum GGT was cigarette smoking. However, other factors that were not evaluated in this study could be those related to diet and lifestyle.

### Conclusion

Significant increased activity of GGT in smokers seems to support the harmful effects of cigarette smoking. Further studies are needed to clarify the association between dietary and lifestyle factors and serum GGT activity.

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