Comparison of Liver Enzymes (ALT, AST & GGT) in Patients with Newly Diagnosed and Established Type-2 Diabetes Mellitus in Rural Population of Northern India

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Abstract
Background: Work on the comparison of liver enzymes between newly diagnosed type-2 diabetes mellitus (T2DM) and established T2DM can be an indicator of insulin resistance.
Objective: To compare the serum levels of alanine amino transferase (ALT), aspartate amino transferase (AST), and γ-glutamyl transferase (GGT) in patients with newly diagnosed and established T2DM in rural Jaipur (India).
Material and Methods: A cross-sectional study comprises with 240 subjects, divided into three groups of 80 each including control. (a): 80-newly diagnosed type-2 DM, (b): 80-established T2DM and (c): 80-healthy age and sex matched controls. Fully automated analyser (HUMAN) was used for biochemical analysis of FPG, ALT, AST, GGT. Chi square ($\chi^2$) test and Z-test were used for statistical analysis. P<0.05 was considered as significant.
Results: Highly significant association was found between newly diagnosed T2DM with raised ALT/raised GGT, and no significant association was found between newly diagnosed T2DM and raised AST with compare to established T2DM.
Conclusion: Rise of ALT and GGT in newly diagnosed T2DM represents insulin resistance due to accumulation of fat in hepatocytes and indicates more oxidative stress due to lipid per-oxidation, β-oxidation. Rise in AST is nonspecific to liver involvement in T2DM and might be due to other manifestations.
Keywords: Liver enzymes ALT, AST, GGT, T2DM.

Introduction
Diabetes mellitus (DM) is a major public health problem in India and worldwide. There are two main types of DM. Type-1 DM and Type-2 DM, Where type-1 DM is caused by autoimmune destruction of insulin producing β-cells in the pancreas result in absolute insulin deficiency and type-2 DM is characterised by raised insulin resistance, raised glucose production, abnormal fat metabolism[1]. The prevalence of T2DM is 11% in urban and 2.4% in rural area and is on persistently increasing trend. As a result of insulin resistance...
in adipose tissue, lipolysis and free fatty acid flux from adipocytes are increased, leading to increased lipid (VLDL, TG) storage in hepatocytes[2]. This excessive lipid storage in the liver becomes toxic to hepatocytes leading to hepatocyte injury. Insulin resistance state and steatosis also causes increase in pro-inflammatory cytokines such as tumor necrosis factor (TNF,) which causes hepatocellular injury, resulting in increased leakage of liver enzyme in the blood[3]. Mild elevation of liver enzymes are frequently found in pre diabetic and T2DM patients[4,5] but chronic persistent and progressive rise in liver enzymes in T2DM may suggest other cause of chronic liver disease[6].

Material and Methods
A cross-sectional study comprise with 240 subjects, divided into three groups 80 in each including control.
(a): 80-newly diagnosed T2DM,
(b): 80-established T2DM and
(c): 80-healthy age and sex matched controls.
All 240 subjects were selected for this study after filled consent form was signed by the patients, and then history was taken followed by clinical & routine examination. Diabetes could be provisionally diagnosed with any one of the three criteria:
1) Fasting plasma glucose (FPG) >126mg/dl (Fasting: no caloric intake for at least 8 hours).
2) Random plasma glucose (RPG)>200mg/dl with classic diabetes symptoms (↑ Urination, ↑ Thirst, ↓ weight)
3) Post prandial plasma glucose (PPPG) after 2 hrs of meal >200mg/dl.
Estimation of glucose was carried out by spectrophotometric glucose oxidase peroxidise (GOD-POD) method (using human kit for fully auto analyser)[7]. AST (Ref range:10-40U/L), ALT (Ref range: 8-50U/L) and GGT (Ref range: 15-75U/L) were assessed with a Trivitron Nanolab 150™ fully automated spectrophotometer using the original kits (Centronic, Gm BH, Wartenberg, Germany).Statistical analysis was carried out by using Chi-square ($\chi^{2}$) test and Z-test. P value <0.05 was considered as statistically significant.

Results
Highly significant association was found between newly diagnosed and established T2DM with raised ALT/ raised GGT. No significant association was found between newly diagnosed and established T2DM with raised AST (Table-1).

Table-1: Comparison of liver enzymes (ALT, AST and GGT) in newly diagnosed and established T2DM patients.

| Test Group | Liver Enzymes  | Test | Control | Total | $\chi^{2}$ | P-value | Significance |
|------------|----------------|------|---------|-------|------------|---------|-------------|
| New Dig. T-2DM | S ALT N=80 | ↑   | 18 | 22.5 | N 06.25 | 023 14.37 | 8.5814 | 0.003396 | Highly Significant |
|         | Normal        |      | 62 | 72.5 | 75 93.75 | 137 85.63 |           |            |               |
|         | S.AST N=80    | ↑   | 10 | 12.5 | N 05  | 14 08.75 | 2.818 | 0.093212 | No Significant |
|         | Normal        |      | 70 | 87.5 | 76 95   | 146 91.25 |           |            |               |
|         | S.GGT N=80    | ↑   | 25 | 31.25 | 03 03.75 | 028 17.5 | 20.9524 | 0.00005 | Highly Significant |
|         | Normal        |      | 55 | 68.75 | 77 96.25 | 132 82.5 |           |            |               |
| Esta. T-2DM | S ALT N=80 | ↑   | 13 | 16.25 | 05 06.25 | 018 11.25 | 4.0063 | 0.045332 | Highly Significant |
|         | Normal        |      | 67 | 83.75 | 75 93.75 | 142 88.75 |           |            |               |
|         | S.AST N=80    | ↑   | 07 | 08.75 | 04 05   | 011 13.75 | 0.8786 | 0.34859 | No Significant |
|         | Normal        |      | 73 | 91.25 | 76 95   | 149 86.25 |           |            |               |
|         | S.GGT N=80    | ↑   | 20 | 25  | 03 03.75 | 023 14.38 | 14.6747 | 0.000128 | Highly Significant |
|         | Normal        |      | 60 | 75  | 77 96.25 | 137 85.62 |           |            |               |

Highly significant difference was found between the mean of serum ALT and GGT levels in newly diagnosed and established T2DM ($P<0.05$). There is no significance difference was found between the mean of serum AST in newly diagnosed and established T2DM ($P>0.05$) (Tabl-2, Graph-1).
Table-2: Mean ±SD of Serum ALT, AST, GGT in newly diagnosed T2DM, Established T2DM and Control group

| Liver Enzymes | New Diag. T2DM | Established T2DM | Control | Z-test | Sig. |
|---------------|----------------|------------------|---------|--------|------|
| S.ALT         | Mean ±SD       | Mean ±SD         | Mean ±SD|        |      |
| S.ALT         | 32.43 ± 10.44  | 31.19 ± 9.367    | 28.94 ± 9.22 | 2.456  | HS   |
| S.AST         | 34.52 ± 6.06   | 31.96 ± 7.09     | 28.54 ± 8.22 | 0.794  | NS   |
| S.GGT         | 78.22 ± 9.176  | 72.5 ± 8.45      | 41.71 ± 19.29 | 4.106  | HS   |

HS-highly significant, NS-no significant

Graph-1: Comparison of mean±SD of liver enzymes in all T2DM study groups

Discussion

ALT: In the present study, 22.5% patients of newly diagnosed T2DM were found raised serum ALT. It compared with established T2DM and controls, 16.25% patients and 6.25% subjects were found raised ALT respectively. The present study showed consistent result with a study done by Shreyas et al. they concluded, a well-defined population of newly diagnosed T2DM with high incidence of abnormal serum ALT levels which was associated with features of the metabolic syndrome but not glycemic control[8]. Sattar et al. found ALT as continuous variable which was associated with new onset type-2 diabetes. Mean±SD of ALT was 20.9±7.6U/L thus elevated ALT levels predict incident diabetes[9]. The present study showed consistent results with this study.

AST: In the present study, 12.5% patients of newly diagnosed T2DM were found raised serum AST levels. It compared with established T2DM and controls, 8.75% patients and 5% subjects were found raised serum AST respectively. The AST level was not significantly associated with type 2 DM both newly diagnosed and established. Our study is in accordance with a study done by Ahn et al. they suggested that the serum ALT concentrations were independently associated with type 2 diabetes established cases in both sexes. It also showed that there was no linear relationship between AST and type 2 diabetes[10].

GGT: In the present study, 31.25% subjects of newly diagnosed T2DM were found raised serum GGT levels. It compared with established T2DM and controls 25% patients and 3.75% subjects were found raised serum GGT respectively. Our study also showed consistent result with a study done by Sabanayagam et al. they showed that higher serum GGT levels were positively associated with type-2 diabetes mellitus, independent of alcohol consumption, body mass index, hypertension and other confounders[11].
Conclusion
The present study concluded that elevated serum ALT and GGT is less than two times the upper limit of normal in T2DM as compared to controls. Serum levels of ALT and GGT were found to be marginally raised in newly diagnosed as compared to established T2DM. It represents insulin resistance due to accumulation of fat in hepatocytes which is known to be directly toxic to hepatocytes. Raised ALT and GGT in newly diagnosed as compared to established T2DM indicates more oxidative stress due to lipid peroxidation, β-oxidation and accumulation of inflammatory mediators in view of initial stage of developing insulin resistance. Rise in AST is concluded to be nonspecific to liver involvement in T2DM and might be due to other manifestations.

References
1. Mittendorfer B, Origins of metabolic complications in obesity: adipose tissue and free fatty acid trafficking. Current opinion in clinical nutrition and metabolic care. 2011;14(6):535-40.
2. Mohan V, Sandeep S, Deepa R, Dhah B, Varghees C. Epidemiology of type 2 diabetes : Indian scenario. India journal of medical research. 2007;125(3):217-21.
3. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology. 1997; 26(1):143-46.
4. Nguyen QM, Srinivasan SR, XuJH, ChenW, Hassing S, Rice J, et al. Elevated liver function enzyme are related to the development of prediabetes and type 2 diabetes in younger adults the Bogalusa heart study. Diabetes care. 2011; 34(12):2603-07.
5. Salmela PI, Sotaniemi EA, Niemi M, Maentausta O. Liver function tests in diabetic patients. Diabetes care.1984; 7(3):248-54.
6. Harris EH. Elevated liver function tests in type 2 diabetes. Clinical diabetes.2005; 23(3):115-19.
7. Trinder P, Determination of glucose using an oxidaseperoxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology.1969;22:158-61.
8. Saligram S, Williams EJ, Masding MG, Raised liver enzymes in newly diagnosed type 2 diabetes are associated with weight and lipids, but not glycemic control. Indian journal of endocrinology and metabolism 2012;16(6):1012-14.
9. Sattar N, Acherbakova O, Ford I, O’Reilly DS, Stanely A, Forrest E, et al. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. Diabetes.2004;53(11):2855-60.
10. Ahn HR, Shin MH, Nam HS, Park KS, LeeYH, Jeong SK, et al. The association between liver enzymes and risk of type 2 diabetes: the Namwon study. Diabetology & metabolic syndrome. 2014; 6(14):1-8.
11. Sabanayagam C, Shankar A, Li J, Pollard C, Ducatman A. Serum gamma-glutamyl transferase level and diabetes mellitus among US adults. European jortal of epidemiology. 2009; 24(7):369-73.