International Journal of Biochemistry Research & Review
16(1): 1-8, 2017; Article no.IJBCRR.31935
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Hepatic Involvement with Elevated Liver Enzymes in Nepalese Subjects with Type 2 Diabetes Mellitus

Nirajan Shrestha[1,2], Nirmal Prasad Bhatt[1,3], Puja Neopane[4], Sudimna Dahal[5], Prashant Regmi[5], Madhav Khanal[5] and Rojeet Shrestha[6*]

[1]Department of Medical Biochemistry, Nobel College, Kathmandu, Nepal.
[2]Laboratory of Liver Regeneration, Chonbuk National University, South Korea.
[3]Department of Pharmacology, Kangwon National University, South Korea.
[4]Health Sciences University of Hokkaido, Tobetsu, Japan.
[5]Department of Clinical Biochemistry, Nepal Medical College, Kathmandu, Nepal.
[6]Faculty of Health Sciences, Hokkaido University, Sapporo, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study. Authors NS and RS wrote the first draft of the manuscript. Authors PR and PN performed the statistical analysis and authors NS and NPB wrote the protocol. Authors NS, MK, NPB and RS managed literature searches. Authors NS, NPB and SD managed the specimen collection and laboratory analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/31935
Editor(s):
(1) Noureddine Benkeblia, Department of Life Sciences, The University of the West Indies, Jamaica.
Reviewers:
(1) Lorna T. Enerva, Polytechnic University of the Philippines, Philippines.
(2) Anthony E. Ojieh, Delta State University, Abraka, Nigeria.
(3) Atanas Baltadjiev, Medical University-Plovdiv, Bulgaria.
Complete Peer review History: http://www.sciencedomain.org/review-history/18188

Original Research Article

Received 31st January 2017
Accepted 6th March 2017
Published 14th March 2017

ABSTRACT

Background: Diabetes mellitus (DM) is characterized by a broad disturbance in metabolism. Since the liver plays a vital role in the regulation of metabolism, there is evidence of liver dysfunction in DM. There is a high prevalence of non-alcoholic fatty liver disease among diabetic patients in western countries. However, there is a paucity of data showing of such association in Nepalese patients with DM. Therefore, the present study was carried out for a better understanding of alteration of liver function in patients with type 2 DM (T2DM).
Methods: A total of 105 patients (age between 30-74 years, male/female ratio=0.6), newly

**Corresponding author: E-mail: cl.biochem@gmail.com;*

diagnosed as T2DM, without the history of liver disease and other chronic diseases, were recruited from out-patient department of Nepal Medical College Teaching Hospital along with 58 age and sex matched healthy controls (age between 30-71 years, male/female ratio=0.7). After basic anthropometric measurements, fasting venous blood was collected and subjected to analysis for liver enzymes.

Results: We observed marginal, yet statistically significant increase of serum alanine transferase (26.6±0.84 (diabetic) vs 20.0±0.69 (control), p=0.003) and γ-glutamyl transferase (40.4±1.51(diabetic) vs 21.2±1.1(control), p<0.001) in the diabetic patients compared to healthy controls.

Conclusion: The liver enzymes were marginally elevated in T2DM compared to controls. This increment may be associated with early pathological changes in the liver. Hence, regular monitoring of liver function is highly beneficial to prevent advance liver injury in T2DM.

Keywords: Diabetes mellitus; liver enzymes; non-alcoholic fatty liver disease; transaminases.

1. INTRODUCTION

Diabetes mellitus (DM) is a leading cause of mortality of people across the globe causing various secondary complications such as coronary heart disease (CHD), chronic kidney disease (CKD), neuropathy and various ocular disorders [1]. The prevalence of type 2 DM (T2DM) is increasing alarmingly worldwide due to increased life expectancy, change in lifestyle, and increased prevalence of obesity. Around two million people in Nepal are estimated to be affected with DM. The prevalence of T2DM and impaired fasting glucose (IFG) is estimated to be 9.5% and 19.2%, respectively in the semi-urban population of Nepal. Prevalence of T2DM was found to be higher in men (11.8%) than in women (7.9%). Similarly, IFG was noted higher in men (25%) than in women (15.4%) [2]. Several studies from Nepal have shown that patients with DM are at higher risk for impaired renal function [3,4], dyslipidemia [5,6] and associated elevated cardiac risk factors [7,8].

Interestingly, DM is associated with Non-alcoholic fatty liver disease (NAFLD) including its severe form, non-alcoholic steatohepatitis. In the recent years, NAFLD has drawn much attention to itself as a pathogenic factor of insulin resistance and T2DM. Among patients with DM, the risk of chronic liver disease is doubled, independent of alcoholic liver disease or viral hepatitis [9]. This evidence is supported by several cross-sectional studies, showing an association between NAFLD and prevalence of T2DM as well as features of metabolic syndrome, including dyslipidemia and abdominal obesity, which highlights insulin resistance as an important feature of NAFLD. Type 2 diabetic patients have a higher incidence of liver function test abnormalities in comparison to non- diabetic individuals [10]. Circulating liver enzymes, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) are commonly elevated in asymptomatic patients with NAFLD, and widely acceptable serum markers of liver diseases. Even a minor elevation of ALT is a good predictor of mortality from liver disease [11].

The excess in serum non-esterified fatty acid (NEFA) observed in the insulin resistance is known to be directly toxic to hepatocyte. The insulin resistant state is also characterized by an increase in pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which may also contribute to hepatocellular injury [12]. Nearly 70 to 80% of the diabetic subjects have been reported to have hepatic fat accumulation referred as NAFLD [13]. Recently, there is renewed interest related to study of the liver enzyme diabetes. Many foreign studies have been conducted to find association of the liver diseases with DM. Prevalence of NAFLD in T2DM is higher with the elevation of liver enzymes [14-16]. However, there is a paucity of data showing such association in Nepalese patients with DM. The present study was therefore undertaken to investigate the compromise of Liver Function in T2DM amongst Nepalese Patients.

2. MATERIALS AND METHODS

The present study is cross-sectional and prospective study to investigate the circulating levels of serum liver enzymes in T2DM. A total of 105 patients (age between 30-74 years; male/female ratio: 0.6), newly diagnosed as T2DM, were recruited from outpatient department of Nepal Medical College Teaching

Hospital in a period of a year. DM was defined as per ADA guideline [17]. Exclusion criteria were apparent liver diseases, alcoholism, chronic inflammatory diseases and under medication that are known to affect liver functions. Individuals with prior history of any kind of liver disease were excluded from the present study, as the aim of our study is to evaluate the alteration of liver enzymes due to pathogenesis of diabetes mellitus. As healthy controls, 58 age and sex matched subjects (Age between 30-71, male/ female ratio: 0.7) were also recruited, six out of which were later excluded due to possible liver diseases, as indicated by marked elevation of liver enzymes. Written informed consent was taken from the participants and the study design was approved by Institutional Research Committee of Nepal Medical College, Kathmandu, Nepal.

Basic clinical examinations were performed to study subjects. Clinical history including hypertension, family history of DM, dietary habits, smoking, and alcohol drinking status were noted. Weights, heights, and waist circumferences (WC) were measured. Body mass index (BMI) was calculated by dividing weight (kg) by square of height (m). On the basis of BMI, study subjects were classified as underweight (BMI<18.5), normal (BMI of 18.5-24.9), overweight (BMI of 25.0-29.9) and obese (BMI>30.0). Systolic and diastolic blood pressure was measured using mercury sphygmomanometer. Hypertension was defined as either current use of anti-hypertensive medication or blood pressure more than 140/80 mmHg.

Fasting blood was collected and blood sugar was analyzed using hexokinase method (Vitros250, Ortho-clinical Diagnostics) within 30 min of blood collection. Remaining serum samples were stored at -20°c till used (not more than 1 month). Serum ALT, AST, and GGT were analyzed using Vitros 250 (Ortho-clinical Diagnostics, NY, USA). The normal ranges for ALT, AST and GGT are 0-50 IU/L, 0-45 IU/L and 0-55 IU/L respectively. Their intra and inter assay coefficient of variation (C.V.) were 2.6 and 3.9% for glucose, 3.1 and 4.0% for ALT, 2.6 and 3.5% for AST and 2.8 and 3.4% for GGT respectively.

Data were analyzed using SPSS 11.5 for Windows. Data were expressed as mean± SD. The comparison of the mean between different groups was done by ANOVA. All p-value were two-tailed, and those <0.05 (95% CI) were considered as statistically significant.

3. RESULTS

The average age of diabetic patients was 52±1.09 years while the average age and sex matched control was 51±1.53 years.

The mean BMI was not significantly difference among two groups (BMI: 25.9±0.49 (diabetic) vs 25.6±0.66 (control)); however, WC of the diabetic patients were significantly higher than healthy control (WC: 86±1.27 vs 81±1.5 cm, $p<0.001$) (Table 1). As many as 21 (20%) of diabetic subjects were obese and 32 (30%) were overweight. While 52% of controls have normal BMI and only 16% were obese. Our results support that the central and abdominal obesity were associated with T2DM.

There was marginal, yet statistically significant increase in serum ALT (26.6±0.84 vs 20.0±0.69 IU/L, $P<0.05$) and GGT (40.4±1.51 vs 21.2±1.1 IU/L, $P=0.001$) in the diabetic subjects compared to control. However, elevation of serum AST (30.0±1.00 in T2DM vs 28.0±0.79 IU/L in control, $P=0.270$) is statistically insignificant. We classified the liver enzymes level into four groups as <20 IU/L, 20-40 IU/L, 40-60 IU/L and > 60 IU/L. The percentage of diabetic people vs control having ALT level of <20 IU/L, 20-40 IU/L, 40-60 IU/L and > 60 IU/L were 18.1 vs 46.2%, 76.2 vs 51.9%, 5.0 vs 2.0% and 1.0 vs 0.0 % respectively. Similarly, the percentage of diabetic subjects vs healthy controls having AST level of <20IU/L, 20-40 IU/L, 40-60 IU/L and > 60 IU/L were 11.4 vs 7.7%, 79.0 vs 90.4%, 8.6 vs 1.9% and 1.0 vs 0.0%. In the same way, the percentage of diabetic subjects vs healthy controls having GGT level of <20 IU/L, 20-40 IU/L, 40-60 IU/L and > 60 IU/L were 5.2 vs 51.9%, 45.7 vs 44.2%, 41.9 vs 3.8% and 6.7 vs 0.0%. This distribution elucidates that subjects with T2DM are more likely to be associated with increase in the serum liver enzymes compared to non-DM controls.

Another interesting findings of our study include that the WC is significantly associated with GGT level in female diabetic patients but not in male ($r=0.26$, $p<0.05$) i.e. GGT level was elevated in female diabetic subjects with high WC. However, we didn't observe such significant relation of WC with ALT and AST. Blood pressure of female diabetic patient is also significantly associated with serum GGT level (Table 3). In addition, there was no statistically significant association between BMI and all the liver enzymes. Our study showed that more patients with marginal

Table 1. Demographic and clinical characteristics of diabetic patients and controls

Characteristics	Diabetic patients (n=105)	Controls (n=52)
Age (Years)	52 ±1.09	48±1.53
Male/female	39/66	23/29
Waist Circumference (cm)	86±1.27*	81±1.5*
BMI (kg/m^2)	25.9±0.49	25.6±0.66
SBP (mmHg)	121±2.16	120±1.08
DBP (mmHg)	86±1.06	80±1.21*
Fasting blood sugar (mg/dL)	144±5.87**	86±1.75**
Postprandial blood sugar (mg/dL)	215±0.49**	116±1.6**

*Data represent mean ±SE. *p<0.05 and **p<0.01. BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.*

Table 2. Percentage distributions of diabetic patients and control subjects according to classification of enzyme concentration (IU/L)

Liver enzymes	Study subjects	Distribution (%) of study subjects according to enzyme levels (IU/L)				P value
		<20	20-40	40-60	>60	
ALT	T2DM	18.1	76.2	4.8	1.0	
	Control	46.2	51.9	1.9	0.0	0.003
AST	T2DM	11.4	79.0	8.6	1.0	
	Control	7.7	90.4	1.9	0.0	0.270
GGT	T2DM	5.7	45.7	41.9	6.7	
	Control	51.9	44.2	3.8	0.0	0.001

ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyltranspeptidase

Table 3. Pearson's correlation coefficient (r) between liver enzymes and different variables among male and female diabetic patients

	Male		Female	
	r	p value	r	p value
Age vs ALT	-0.291	0.07	-0.063	0.63
Age vs AST	-0.064	0.71	0.027	0.83
Age vs GGT	-0.057	0.76	0.147	0.23
SBP vs ALT	-0.185	0.27	-0.076	0.57
SBP vs AST	-0.140	0.39	0.128	0.30
SBP vs GGT	-0.116	0.50	0.400	0.0009**
DBP vs ALT	-0.047	0.8	-0.088	0.50
DBP vs AST	-0.168	0.37	-0.002	0.90
DBP vsGGT	-0.041	0.80	0.275	0.02*
WC vs ALT	0.001	0.99	0.126	0.33
WC vs AST	0.014	0.93	0.147	0.29
WC vs GGT	-0.161	0.33	0.269	0.02*

elevation of liver enzymes were with abdominal obesity.

4. DISCUSSION

Although the difference of serum ALT and GGT level were statistically significant between diabetic patients and healthy controls, the means of ALT and GGT were within normal range. There was also increased mean AST level in diabetic patients than in healthy controls but the difference was not statistically significant. Our results support the finding of a study done by Tohidi et al. [18] among the general population in Tehran. In their result, AST was not raised or decreased significantly in T2DM but ALT and GGT were significantly elevated with ORs of 3.07 and 2.91, respectively [18]. Significant elevation of ALT but not AST may be due to the reason that ALT is more specific serum liver marker. AST is relatively not specific to liver and may also be elevated in other

tissue damage such as skeletal muscle, cardiac muscle [11].

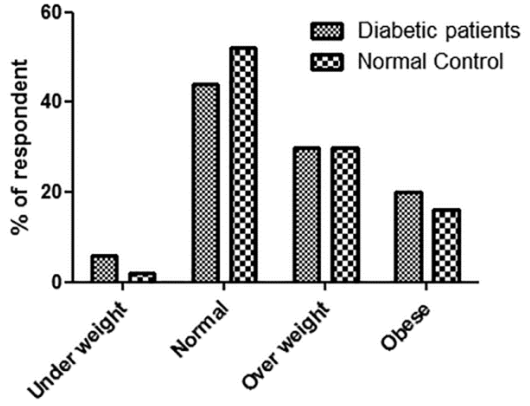

Fig. 1. Distribution of respondent according to BMI. There were higher numbers of diabetic patients than healthy individuals who are obese

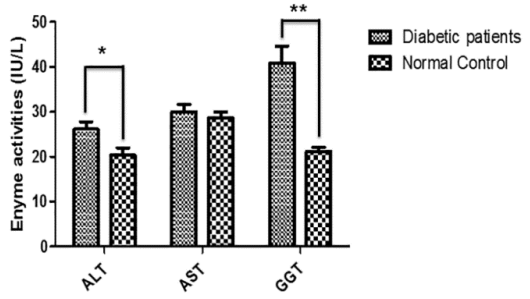

Fig. 2. Comparisons of liver enzymes activities in diabetic patients and healthy controls. The levels of ALT and ALT are significantly different, but AST activities are not significantly different between diabetic patients and healthy controls

*Data represent mean±SE. *p<0.05 and **p<0.01*

Our results also support the study done by Salmela et al. [19], where 57% of the 175 diabetic outpatients (100 subjects) had, at least, one abnormal LFT; 27% (48 subjects) had, at least, two abnormal tests. T2DM patients more frequently had elevated ALT (22.9 vs. 5.3%) and GGT (23.7 vs. 10.5%) levels than those with type 1 DM. West et al. [20] studied the status of ALT only in T2DM patients, which also showed the elevated level of ALT in diabetic patients compared with non-diabetic subjects. The prevalence of elevated ALT in type 1 (9.5%) and type 2 (12.1%) diabetic patients were considerably higher than 2.7% expected in the general population and higher than 5.6% reported at baseline in clinical trials.

In a larger study, Erbey et al. [21] analyzed 18,825 patients, of which, 4.1% had elevated ALT, and 6.7% have T2DM. Of those with T2DM, the prevalence of elevated ALT was 7.8%, compared to 3.8% prevalence in those without diabetes. There was 10.6% prevalence of elevated ALT in obese diabetic versus 6.6% prevalence in obese non-diabetic patients. In contrast to this study; our finding suggested that there was no significant association between BMI and liver enzymes. However, WC was significantly associated with GGT, but not with aminotransferases. It is true that more patients with marginal elevation of liver enzymes were with central obesity. According to International Diabetes Federation, the cut-off value of WC for south Asianis ≥90 cm for male and ≥80 cm for female [22].

NAFLD is replacing alcohol and viral hepatitis as the most common etiology of chronically elevated liver enzymes in the United States in both diabetic and non-diabetic individuals. Of those patients with NAFLD 60–95% are obese, 28–55% have T2DM, and 20–92% have hyperlipidemia [23]. Lim et al. [24] showed the strong interaction between serum GGT and obesity on the risk of prevalent T2DM. BMI was associated with prevalent diabetes only among persons with high normal serum GGT activity (P for interaction = 0.002). BMI was not associated with T2DM when GGT was low normal, suggesting the obesity itself may not be a sufficient risk factor for T2DM. Rather, in this view, to be a risk factor for diabetes, obesity must be coupled with other factors, such as serum GGT. Serum ALT is more sensitive marker of fatty liver than GGT [25], however, the study by Lim JS et al. [24] observed no BMI and serum ALT interaction associated with prevalent diabetes. The association between NIDDM and GGT was independent of serum glucose and of other predictors of diabetes. In the study done by Perry et al. [26], mean serum GGT was significantly higher in the NIDDM patients than in the rest of the cohort (20.9 [19.3-22.6] vs. 15.3 IU/l [15.0-15.6], p< 0.001). However, in the Tromso study, BMI was the dominant predictor of GGT levels in both men and women in multivariate analysis that included alcohol intake and major cardiovascular disease risk factor [27]. Ikai et al. [28] have reported significant association between hepatic steatosis and

elevated GGT, insulin resistance, and hyperinsulinemia. In this Japanese study, the well-described association between serum GGT levels and blood pressure was attenuated for plasma insulin level. Another Japanese investigation done by Nakanishi et al. [29] suggested that serum GGT may be an important predictor for developing T2DM [29]. Kim et al. [30] in South Korea have also suggested that increased serum ALT and GGT levels are risk factor for the development of T2DM. Lee et al. [31] established the significant association between GGT, obesity, and risk of T2DM. BMI appeared to be more strongly associated with T2DM in both men and women over age 50 years with GGT median or greater, compared with subjects with GGT less than median.

Our results support the another study done by Lee DH et al. [32], that have revealed that the associations between ALT or AST and diabetes were weaker than those of GGT and were mostly restricted to abnormal levels of liver enzymes. However, one prospective study, in Pima Indians, has reported that higher ALT, but not GGT, predicted T2DM [33]. Many studies support the significant relationship between elevated serum GGT and metabolic syndrome. The higher GGT levels are accompanied by insulin resistance and greater risk of developing T2DM [34]. Participants in higher GGT quartiles were older, had higher BMI and were more likely to have hypertension, and elevated lipids, fasting blood glucose, and CRP [35]. Another study from Taiwan also found the important association between GGT and metabolic syndrome, the higher GGT level occurs in obese person, particularly those with high WC [36]. In agreement with these studies, our result also showed elevated level of GGT in person with central obesity. Moreover, our results are in agreement with study done by Stranges et al. [37] in West New York, which showed the strong interaction between GGT levels and hypertension. Increased GGT levels are considered as a marker of alcohol abuse; however few moderate drinkers (8% of total subjects) have been included in the study. In our study, liver enzymes get elevated in the individuals with hypertension both in diabetic and non-diabetic subjects.

5. CONCLUSION

In conclusion, this study suggested that serum ALT and GGT are significantly elevated in T2DM patients compared to healthy individuals. In contrast, such association was not seen with serum AST level. The study also suggested the significant association between obesity and elevation of liver enzymes. Non-alcoholic fatty liver disease is highly prevalent in patients with T2DM. Analysis of this study also revealed an interaction between central obesity and elevation of liver enzymes. Because of marginal elevation of liver enzymes within normal range in diabetic patients, estimation of liver enzymes is highly recommended for patients with T2DM for early detection of liver dysfunctions.

6. LIMITATION OF STUDY

One limitation of this study is the small number of control compared to the T2DM. The control to case ratio in this study is 0.55. Future study with equal number of control and case can be conducted. However, we believe that our data of 58 healthy controls are still not too critically low to alter the conclusion.

ACKNOWLEDGEMENT

We are thankful for all staff of clinical laboratory of Nepal Medical College and Nobel College for their continuous supports and encouragement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. American Diabetes Association. Standards of Medical Care in Diabetes - 2016. Diabetes Care. 2016;39:S1-112.
2. Ono K, Limbu YR, Rai SK, Kurokawa M, Yanagida J, Rai G, et al. The prevalence of type 2 diabetes mellitus and impaired fasting glucose in semi-urban population of Nepal. Nepal Med Coll J. 2007;9(3):154-6.
3. Yadav BK, Adhikari S, Gyawali P, Shrestha R, Poudel B, Khanal M. Use of protein: creatinine ratio in a random spot urine sample for predicting significant proteinuria in diabetes mellitus. Nepal Med Coll J. 2010;12(2):100-5.
4. Shrestha S, Gyawali P, Shrestha R, Poudel B, Sigdel M. Serum urea and creatinine in diabetic and non-diabetic subjects. Journal of Nepal Association for Medical Laboratory Sciences P. 2008; 11:12.

5. Dahal S, Baral BK, Baral S, Shrestha R, Khanal M. Study of fasting serum lipid and lipoproteins profile in type-II diabetic patients attending NMCTH. Nepal Med Coll J. 2013;15(1):18-22.
6. Regmi P, Gyawali P, Shrestha R, Sigdel M, Mehta KD, Majhi S. Pattern of dyslipidemia in type 2 diabetic subjects in Eastern Nepal. JNAMLS. 2009;10(1):11-3.
7. Shrestha R, Jha SC, Khanal M, Gyawali P, Yadav BK, Jha B. Association of cardiovascular risk factors in hypertensive subjects with metabolic syndrome defined by three different definitions. JNMA J Nepal Med Assoc. 2011;51(184):157-63.
8. Sigdel M, Kumar A, Gyawali P, Shrestha R, Tuladhar ET, Jha B. Association of high sensitivity C-reactive protein with the components of metabolic syndrome in diabetic and non-diabetic individuals. J Clin Diagn Res. 2014;8(6):CC11-3.
9. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology. 2004;126(2):460-8.
10. Harris EH. Elevated liver function tests in type 2 diabetes. Clinical Diabetes. 2005; 23(3):115-9.
11. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: Prospective cohort study. BMJ (Clinical research ed). 2004; 328(7446):983.
12. 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):143-6.
13. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. Journal of Gastroenterology and Hepatology. 2004;19(8):854-8.
14. Kalra S, Vithalani M, Gulati G, Kulkarni CM, Kadam Y, Pallivathukkal J, et al. Study of prevalence of nonalcoholic fatty liver disease (NAFLD) in type 2 diabetes patients in India (SPRINT). The Journal of the Association of Physicians of India. 2013;61(7):448-53.
15. Prashanth M, Ganesh HK, Vima MV, John M, Bandgar T, Joshi SR, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. The Journal of the Association of Physicians of India. 2009;57:205-10.
16. Akbar DH, Kawther AH. Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic: Prevalence and general characteristics. Diabetes Care. 2003; 26(12):3351-2.
17. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 Suppl 1:S62-9.
18. Tohidi M, Harati H, Hadaegh F, Mehrabi Y, Azizi F. Association of liver enzymes with incident type 2 diabetes: A nested case control study in an Iranian population. BMC Endocrine Disorders. 2008;8:5.
19. Salmela PI, Sotaniemi EA, Niemi M, Maentausta O. Liver function tests in diabetic patients. Diabetes Care. 1984; 7(3):248-54.
20. West J, Brousil J, Gazis A, Jackson L, Mansell P, Bennett A, et al. Elevated serum alanine transaminase in patients with type 1 or type 2 diabetes mellitus. QJM: Monthly Journal of the Association of Physicians. 2006;99(12):871-6.
21. Erbey JR, Silberman C, Lydick E. Prevalence of abnormal serum alanine aminotransferase levels in obese patients and patients with type 2 diabetes. The American Journal of Medicine. 2000; 109(7):588-90.
22. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A consensus statement from the international diabetes federation. Diabetic Medicine: A Journal of the British Diabetic Association. 2006;23(5):469-80.
23. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: Summary of an AASLD single topic conference. Hepatology. 2003;37(5):1202-19.
24. Lim JS, Lee DH, Park JY, Jin SH, Jacobs DR, Jr. A strong interaction between serum gamma-glutamyltransferase and obesity on the risk of prevalent type 2 diabetes: Results from the Third National Health and Nutrition Examination Survey. Clinical Chemistry. 2007;53(6):1092-8.
25. Yu AS, Keeffe EB. Elevated AST or ALT to nonalcoholic fatty liver disease: Accurate predictor of disease prevalence? The American Journal of Gastroenterology. 2003;98(5):955-6.
26. Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care. 1998;21(5): 732-7.

27. Nilssen O, Forde OH, Brenn T. The tromso study. Distribution and population determinants of gamma-glutamyltransferase. American Journal of Epidemiology. 1990;132(2):318-26.
28. Ikai E, Ishizaki M, Suzuki Y, Ishida M, Noborizaka Y, Yamada Y. Association between hepatic steatosis, insulin resistance and hyperinsulinaemia as related to hypertension in alcohol consumers and obese people. Journal of Human Hypertension. 1995;9(2):101-5.
29. Nakanishi N, Nishina K, Li W, Sato M, Suzuki K, Tatara K. Serum gamma-glutamyltransferase and development of impaired fasting glucose or type 2 diabetes in middle-aged Japanese men. Journal of Internal Medicine. 2003;254(3):287-95.
30. Kim CH, Park JY, Lee KU, Kim JH, Kim HK. Association of serum gamma-glutamyltransferase and alanine aminotransferase activities with risk of type 2 diabetes mellitus independent of fatty liver. Diabetes/ Metabolism Research and Reviews. 2009;25(1):64-9.
31. Lee DH, Silventoinen K, Jacobs DR, Jr., Jousilahti P, Tuomileto J. gamma-Glutamyltransferase, obesity and the risk of type 2 diabetes: Observational cohort study among 20,158 middle-aged men and women. The Journal of Clinical Endocrinology and Metabolism. 2004; 89(11):5410-4.
32. Lee DH, Ha MH, Kim JR, Gross M, Jacobs DR Jr. Gamma-glutamyltransferase, alcohol, and blood pressure. A four year follow-up study. Annals of Epidemiology. 2002;12(2):90-6.
33. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002;51(6): 1889-95.
34. Andre P, Balkau B, Born C, Charles MA, Eschwege E. Three-year increase of gamma-glutamyltransferase level and development of type 2 diabetes in middle-aged men and women: The D.E.S.I.R. cohort. Diabetologia. 2006;49(11):2599-603.
35. Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease and mortality risk: The framingham heart study. Arteriosclerosis, Thrombosis and Vascular Biology. 2007;27(1):127-33.
36. Li TC, Liu CS, Lin CC. The relationship of liver enzyme abnormalities and obesity in aboriginal children in Taiwan. Journal of Gastroenterology. 2004; 39(12):1170-4.
37. Stranges S, Trevisan M, Dorn JM, Dmochowski J, Donahue RP. Body fat distribution, liver enzymes, and risk of hypertension: Evidence from the Western New York Study. Hypertension. 2005; 46(5):1186-93.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18188