ORIGINAL PAPER

Use of Databases for Early Recognition of Risk of Diabetic Complication by Analysis of Liver Enzymes in Type 2 Diabetes Mellitus

Maja Malenica[1], Besim Prnjavorac[2], Adlija Causevic[1], Tanja Dujic[1], Tamer Bego[1], and Sabina Semiz[1,3]

[1]Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina
[2]General Hospital Tesanj, Bosnia and Herzegovina
[3]Faculty of Engineering and Natural Sciences, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Corresponding author: prof Besim Prnjavorac, MD, PhD. General Hospital Tesanj. Tel.: +387 32 656 300; Fax.: +387 32 650 605. Mob.: +387 61 166 850. - ORCID ID: http//: www.orcid.org. 0000-0003-0331-055X E-mail: pbesim@bih.net.ba

ABSTRACT

Introduction: Because of increasing prevalence of T2MD worldwide, it's very important to recognize risk factors for diabetic complications, as soon as possible. Symptoms of complications appear a few or many years after tissue damage. So, it's imperative to establish surveillance of diabetics with laboratory and other diagnostic procedures for early recognition of diabetic complications. Follow up of clinical curs of diabetes, by using databases of patients, provide possibility for permanent analysis of important laboratory parameters and any changes could be registered. Although an emerging evidence suggests a strong association of ALT (alanine aminotransferase) and γGT (gamma glutamyl transferase) activity with type 2 diabetes mellitus (T2DM), only a limited number of studies have analyzed the association of AST (aspartate aminotransferase), ALT, γGT, and ALP (alkaline phosphatase) activities in controlled T2DM. **Material and Methods:** Gender differences are of special interest in trying to follow diabetes progression and development of its complications. Here the activities of ALT, AST, γGT, ALP were analyzed as well as levels of glycosylated hemoglobin (HbA1c) and fasting plasma glucose (FPG) in 40 T2DM patients and 40 age-matched healthy subjects. Blood samples were collected from all participants in regular 3-months intervals up to 6 months period. Standard IFCC enzyme protocols were used to determine enzyme activities. **Results and discussion:** In first measured interval, significantly higher activities of ALT (p= 0,050) and glucose levels (p=0,045) were shown in male. A significant correlation was shown between ALT and AST activity with FPG and HbA1c levels in first and third measured interval. ALT activity was much higher in the group of patients with poor glycemia control. Average levels of activities of enzymes stay nearly in normal limits, but changes of enzymes activities should be recognized as soon as possible, earlier than tissue changes and diabetic complications become irreversible.

Key words: aminotransferases, gamma glutamyl transferase, Type 2 diabetes, complication, databases.

1. INTRODUCTION

Diabetes mellitus represents a group of metabolic diseases, which have one symptom in common, namely hyperglycemia (1-3). In the case of type 2 diabetes (T2DM), the relative contribution of two main factors, insulin resistance and decreased insulin secretion, varies from individual to individual, and is particularly pronounced at the level of glucose uptake in the peripheral tissues (4). Disturbances at the level of triglyceride storage and lipolysis in insulin-sensitive tissues have been described as early signs of insulin resistance and could be detected much earlier than fasting hyperglycemia (5).

Changes related to insulin resistance are of multifactorial nature and so far are not well understood (6). Recently, the role of liver in the pathogenesis of T2DM and pre-diabetes attracted so much and appears to be directly related to insulin resistance, T2DM, and metabolic syndrome (2, 7, 8). A large number of clinical and experimental data imply an involvement of liver enzymes (ALT and γGT) in various processes that influence the risk of developing pre-diabetes, T2DM, and cardiovascular disease (11, 12).

Therefore, in this study "tracking" of liver enzyme activity within a "reference" range was done over defined periods of time in control subjects and patients with T2DM and prediabetes, with a particular emphasis on potential gender differences.

2. MATERIAL AND METHODS

Activity of liver enzymes (ALT, γGT, AST and AP) was analyzed in a group

of 40 patients with T2DM (16 male and 24 female patients) and 40 healthy controls, mean age of 61 years measured in three cycles, every two months. Thus, the first cycle was from 0-2 months, the second cycle from 2-4 months, and the third cycle from 4-6 months. All patients have signed written consent forms.

All research involving human subjects and material derived from human subjects in this study was done in accordance with the ethical principles outlined in World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects.

On the basis of cut-off values of HbA1c (6,3%), patients with T2DM were later on divided into diabetics with good glycemic control (HbA1c < 6,3%) and poor glycemic control (HbA1c > 6,3%).

Excluding criteria for patients with T2DM involved in the study were: insulin therapy, liver and kidney damages, cardiovascular complications, chronic pancreatitis and viral infections.

Control subjects were of approximately same age (40-60 years old), with normal results of glucose tolerance test (fasting plasma glucose less than 6,2 mmol/L, and two hours postprandial glycemia less than 7,8 mmol/L). Those patients did not have any history of kidney or liver diseases.

Statistical analysis was performed by SPSS 16.00 for Windows, by using Spearman correlation coefficient, test of multiple correlation and Student T test. A statistical significance was set as $p < 0.05$.

3. RESULTS

Based on these results, only glucose levels and ALT activity showed a tendency of significant difference in the first interval (Table 1).

Gender differences related to the liver enzyme activity and glucose levels are presented in Figures 1 and 2. As it shown in Figure 1, concentration of glucose was significantly higher in the diabetic male patients as compared to the female patients

Parameter	Diabetics		Healthy control	
	Valid N	Mean	Valid N	P value (Student)
Age	40	61,1		
Glucose1(mmol/L)	40	9,93	42	5,25	0,00001
Glucose2(mmol/L)	40	9,29	42	5,25	0,00001
Glucose3(mmol/L)	40	9,26	42	5,25	0,00001
AST1 (IU/L)	40	36,55	42	31,97	0,55
AST2 (IU/L)	40	26,17	42	31,97	0,0232
AST3 (IU/L)	40	25,15	42	31,97	0,00145
ALT1 (IU/L)	40	31	42	36,97	0,06
ALT2 (IU/L)	40	30,15	42	36,97	0,085
ALT3 (IU/L)	40	29,73	42	36,97	0,064
γGT1 (IU/L)	38	31,44	42	28,56	0,025
γGT2 (IU/L)	40	31,16	42	28,56	0,067
γGT3 (IU/L)	39	28,56	42	28,56	0,07
AP1 (IU/L)	39	78,69	42	64,83	0,0055
AP2 (IU/L)	39	73,33	42	64,83	0,073
AP3 (IU/L)	39	73,59	42	64,83	0,049
HbA1c1 (%)	38	7,97	42	4,52	0,0019
HbA1c2 (%)	40	6,67	42	4,52	0,00001
HbA1c3 (%)	40	6,66	42	4,52	0,00001

Table 1. Descriptive statistics of followed parameters of Glucose1 first measurement, Glucose2 second measurement, Glucose3 the third. AST (aspartate aminotransferase), ALT (alanine aminotransferase), gGT (gamma glutamyl transferase), AP (alkaline phosphatase), HbA1c (glycosylated hemoglobin). Number of any parameter show first, second or third mesurement.

Parameter	Diabetics (Male)		Diabetics (Female)		
	Valid N	Mean	Valid N	Mean	P value
ALT1	16	36,58	24	26,95	P=0,045
ALT2	16	33,58	24	27,00	P=0,241
ALT3	16	37,88	24	25,58	P=0,034
AST1	16	31,29	24	39,37	P=0,271
AST2	16	25,64	24	26,37	P=0,67
ALS3	16	27,06	24	23,46	P=0,81

Table 2. Descriptive statistics and result of comparision of ALT and AST in repeated measurement by gender.
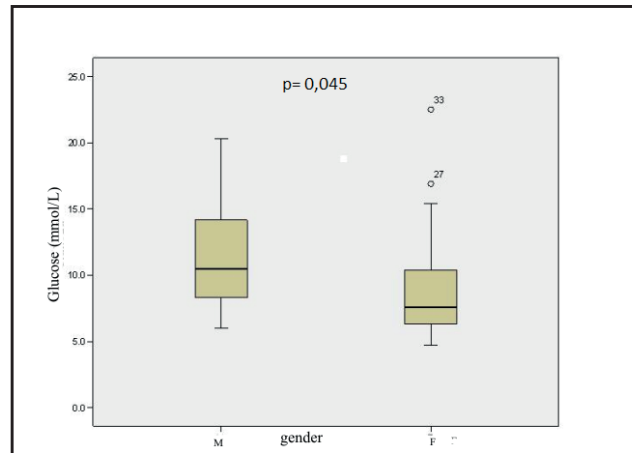

Figure 1. Comparison of median/means serum glucose concentrations between male (M) and female (F) T2DM patients in the first measured interval.
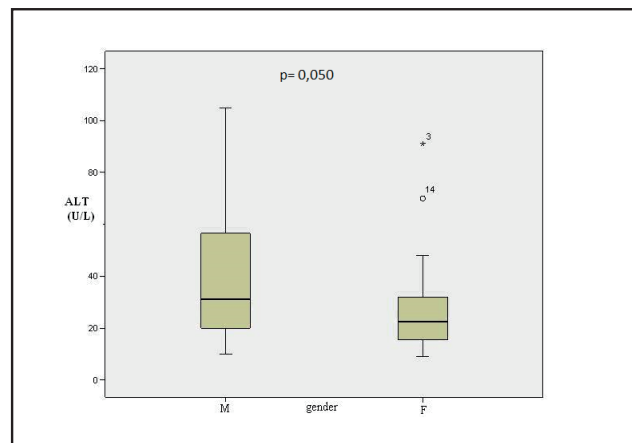

Figure 2. Comparison of median/means values of ALT activity between male (M) and female (F) T2DM patients in the first measured interval.

in the first measured interval. However, there were no significant differences in the glucose level between male and female diabetic patients during the other time intervals.

In order to monitor the effects of a proper glucose control on the liver enzyme activity, patients included in the study were subdivided into those with a good glycemic control (HbA1c< 6,3%) and those with a poor glycemic control (HbA1c > 6,3%).

In the group of diabetic patients with HbA1c > 6.3%, significant (p= 0,005) gender differences manifested at the level of the ALT activity in the third time interval only (4-6 months). However, the activity of all other liver enzymes did not significantly change throughout all periods of testing.

In the group of patients with HbA1c< 6,3% there were no statistical differences in all measured parameters in all tested time points. Furthermore, liver enzyme activities did not

change significantly in 2nd and 3rd interval of study as compared to the 1st interval, and there was no significant effect of gender on the activity of liver enzymes.

Test of multiple correlation showed significant correlation of male gender and ALT measurement in third time interval (p<0,05), and positive correlation between age and AST, but only upon first measurement.

In test of multiple regression, performed to compare value of ASP and ALT, statistical significance was shown between first measurement of ALT (p=0,0156) and duration of DM (given in years). Other measurement did not show statistical significance.

4. DISCUSSION

As liver is a very important organ in the regulation of normal, fasting and postprandial plasma glucose levels, a justification for analyzing the activities of liver enzymes within specified time intervals and correlation of their activity with the level of glucose control in diabetic patients is clearly evident. Namely, ALT and gGT activities are parameters that reflect liver fat content and represent indirect markers of hepatic insulin resistance, metabolic syndrome, and T2DM (13).

Results of the study demonstrated an effect of T2DM on ALT activity only. Although, still within the reference limits, ALT activity was significantly higher in diabetic patients compared to controls. This could suggest that proper monitoring of ALT activity could be potentially relevant in clinical practice, as previously reported (14, 15). These results are in compliance with some previous studies related to the effects of T2DM regarding of enzymes activities. In studies done by Vozarova et al. and Huang et al. (16, 17), elevated ALT baseline activity was associated with an increase in hepatic glucose output, suggesting that higher ALT activity is a risk factor for T2DM. On the other hand, study done by Nanipieri et al. (18) has associated higher ALT and gGT activity with both impaired glucose tolerance (IGT) and diabetes, whereas AP activity was associated with diabetes only and AST activity with IGT only. A potentially stronger association of gGT activity with diabetes observed in the previous studies (11, 19) might reflect its associations with several different processes relevant to diabetes pathogenesis, including oxidative stress implicated in the insulin resistance and diabetes (18).

In addition, our data showed a significant difference in ALT activity between male and female T2DM patients during the first follow-up interval. Namely, the ALT activity was significantly higher in the male patients with no adequate control of diabetes (HbA1c levels above 6.3

However, in recent publications can be seen even transaminase in normal range could be considered as risky factor for diabetes development and clinical course (8, 9, 10). In our study duration of diabetes was in significant correlation if AST enzyme in first measurement was analyzed. Other enzymes did not show statistical significance.

This study suggests the benefit of analyzing the activity of ALT enzyme through defined time intervals in patients with T2DM. A significance of routine ALT testing is specially emphasized in male population with HbA1c values above 6,3%. Namely, through proper follow up of ALT activity in this population, it is possible to monitor progression of this dis-

ease. Importantly, as our data demonstrated, the ALT activity could reflect how well controlled is diabetes, since it correlated with the HbA1c levels, as shown in Tables 1, with results of calculation of other parameters.

Result of statistical analyzes of differences of AST and ALT, by gender, is presented in Table 2.

In summary, this study demonstrated higher ALT activity (within reference limits) in male patients with diagnosed T2DM as compared to their female counterparts.

5. CONCLUSION

Average levels of activities of enzymes stay nearly in normal limits, but changes of enzymes activities should be recognized as soon as possible, earlier than tissue changes in diabetic complications become irreversible.

REFERENCES

1. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clinical chemistry. 2011; 57(6): e1-e47.
2. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes care. 2009; 32(4): 741-50.
3. Porepa L, Ray JG, Sanchez-Romeu P, Booth GL. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. CMAJ: Canadian Medical Association journal - journal de l'Association medicale canadienne. 2010; 182(11): E526-31.
4. Cerf ME. Beta cell dysfunction and insulin resistance. Frontiers in endocrinology. 2013; 4: 37.
5. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature reviews Molecular cell biology. 2008; 9(5): 367-77.
6. Lawlor DA, Sattar N, Smith GD, Ebrahim S. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. Am J Epidemiol. 2005;161(11): 1081-8.
7. Bonnet F, Ducluzeau PH, Gastaldelli A, Laville M, Anderwald CH, Konrad T. et al. Liver enzymes are associated with hepatic insulin resistance, insulin secretion, and glucagon concentration in healthy men and women. Diabetes. 2011; 60(6): 1660-7.
8. Lee DH, Silventoinen K, Jacobs DR, Jr., Jousilahti P, Tuomi-

leto 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.

9. Wannamethee SG, Shaper AG, Lennon L, Whincup PH. Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes care. 2005; 28(12): 2913-8.

10. Ahn HR, Shin MH, Nam HS, Park KS, Lee YH, Jeong SK, et al. The association between liver enzymes and risk of type 2 diabetes: the Namwon study. Diabetology & metabolic syndrome. 2014; 6(1): 14.

11. Cho NH, Jang HC, Choi SH, Kim HR, Lee HK, Chan JC, et al. Abnormal liver function test predicts type 2 diabetes: a community-based prospective study. Diabetes care. 2007; 30(10): 2566-8.

12. Nguyen QM, Srinivasan SR, Xu JH, Chen W, Hassig S, Rice J, et al. Elevated liver function enzymes are related to the development of prediabetes and type 2 diabetes in younger adults: the Bogalusa Heart Study. Diabetes Care. 2011; 34(12): 2603-7.

13. Forlani G, Di Bonito P, Mannucci E, Capaldo B, Genovese S, Orrasch M, et al. Prevalence of elevated liver enzymes in Type 2 diabetes mellitus and its association with the metabolic syndrome. Journal of endocrinological investigation. 2008; 31(2): 146-52.

14. Sattar N, Scherbakova O, Ford I, O'Reilly DS, Stanley 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.

15. Jiamjarasrangsi W, Sangwatanaroj S, Lohsoonthorn V, Lertmaharit S. Increased alanine aminotransferase level and future risk of type 2 diabetes and impaired fasting glucose among the employees in a university hospital in Thailand. Diabetes & metabolism. 2008; 34(3): 283-9.

16. 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.

17. Huang JF, Dai CY, Yu ML, Hsieh MY, Chuang WL. Abnormal liver function test predicts type 2 diabetes: a community-based prospective study: response to Cho et al. Diabetes care. 2008; 31(6): e53.

18. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM, et al. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. Diabetes Care. 2005; 28(7): 1757-62.

19. Schindhelm RK, Dekker JM, Nijpels G, Heine RJ, Diamant M. No independent association of alanine aminotransferase with risk of future type 2 diabetes in the Hoorn study. Diabetes care. 2005; 28(11): 2812.