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

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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 glyemia 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 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).

### Table 1. Descriptive statistics of followed parameters of Glucose1 first measurement, Glucose2 second measurement, Glucose3 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 measurement.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diabetics (Male)</th>
<th>Diabetics (Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose1 (mmol/L)</td>
<td>Valid N</td>
<td>Mean</td>
</tr>
<tr>
<td>40</td>
<td>61,1</td>
<td></td>
</tr>
<tr>
<td>Glucose2 (mmol/L)</td>
<td>40</td>
<td>9,93</td>
</tr>
<tr>
<td>Glucose3 (mmol/L)</td>
<td>40</td>
<td>9,26</td>
</tr>
<tr>
<td>AST1 (IU/L)</td>
<td>40</td>
<td>38,55</td>
</tr>
<tr>
<td>AST2 (IU/L)</td>
<td>40</td>
<td>26,17</td>
</tr>
<tr>
<td>AST3 (IU/L)</td>
<td>40</td>
<td>25,15</td>
</tr>
<tr>
<td>AL1 (IU/L)</td>
<td>40</td>
<td>31</td>
</tr>
<tr>
<td>AL2 (IU/L)</td>
<td>40</td>
<td>30,16</td>
</tr>
<tr>
<td>AL3 (IU/L)</td>
<td>40</td>
<td>29,73</td>
</tr>
<tr>
<td>gGT1 (IU/L)</td>
<td>38</td>
<td>31,44</td>
</tr>
<tr>
<td>gGT2 (IU/L)</td>
<td>40</td>
<td>31,16</td>
</tr>
<tr>
<td>gGT3 (IU/L)</td>
<td>39</td>
<td>28,56</td>
</tr>
<tr>
<td>AP1 (IU/L)</td>
<td>39</td>
<td>79,69</td>
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<tr>
<td>AP2 (IU/L)</td>
<td>39</td>
<td>73,33</td>
</tr>
<tr>
<td>AP3 (IU/L)</td>
<td>39</td>
<td>73,59</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>38</td>
<td>7,97</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>40</td>
<td>6,67</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>40</td>
<td>6,66</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diabetics (Male)</th>
<th>Diabetics (Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT1</td>
<td>Valid N</td>
<td>Mean</td>
</tr>
<tr>
<td>16</td>
<td>36,58</td>
<td>24</td>
</tr>
<tr>
<td>ALT2</td>
<td>16</td>
<td>33,58</td>
</tr>
<tr>
<td>ALT3</td>
<td>16</td>
<td>37,88</td>
</tr>
<tr>
<td>AST1</td>
<td>16</td>
<td>31,29</td>
</tr>
<tr>
<td>AST2</td>
<td>16</td>
<td>25,64</td>
</tr>
<tr>
<td>ALS3</td>
<td>16</td>
<td>27,08</td>
</tr>
</tbody>
</table>

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.
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 benefits 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 disease. 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.

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