Creatine Kinase Activity in Patients with Diabetes Mellitus Type I and Type II

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Abstract

Diabetes mellitus can be looked upon as an array of diseases, all of which exhibit common symptoms. While pathogenesis of IDDM (insulin dependant diabetes mellitus) is well understood, the same is not true for diabetes mellitus type II. In the latter case, relative contribution of the two factors (insulin resistance or decreased insulin secretion) varies individually, being highly increased in peripheral tissues and strictly dependant on insulin for glucose uptake. Moreover, in patients with diabetes mellitus type II, disbalance at the level of regulation of glucose metabolism as well as lipid metabolism has been noted in skeletal muscles. It is normal to assume that in this type of diabetes, these changes are reflected at the level of total activity of enzyme creatine kinase. This experimental work was performed on a group of 80 regular patients of Sarajevo General Hospital. Forty of those patients were classified as patients with diabetes type I and forty as patients with diabetes type II. Each group of patients was carefully chosen and constituted of equal number of males and females. The same was applied for adequate controls. Concentration of glucose was determined for each patient with GOD method, while activity of creatine kinase was determined with CK-NAC activated kit. Statistical analysis of the results was performed with SPSS software for Windows. Obtained results point out highly expressed differences in enzyme activity between two populations examined. Changes in enzyme activity are more expressed in patients with diabetes type II. Positive correlation between concentration of glucose and serum activity of the enzyme is seen in both categories of diabetic patients which is not the case for the patients in control group. At the same time , correlation between age and type of diabetes does exist . This is not followed at the level of enzyme activity or concentration of glucose.

KEY WORDS: Diabetes mellitus type I, Diabetes mellitus type II, Creatine kinase

Introduction

Diabetes mellitus, well known, as diabetes is a name usually used for the group of metabolic diseases, which have one symptom in common, namely hyperglycemia (2, 4). In the world today, 170 milllion people are affected by the disease. WHO and International Diabetic Federation estimate the number of people to be affected to increase by the year 2025 to 300 millions. In Bosnia and Herzegovina, in the year 2000 there was 111,000 of registered diabetics. In the year 2030, estimate amounts to 180,000(3). At the same time, number of newly detected diabetics increases in all age groups, which directly correlates with number of inhabitants, aging population, urbanization and its consequences: namely unhealthy nutrition and unhealthy life style (14). Incidence is high in the developing countries and prevalence is also different for different populations. The metabolic causes leading to this disease are well known and manifest themselves through disbalances in metabolism of glucose, lipids and proteins(7). This disease is characterized by decrease in concentration of glucose 6 phosphate in the cells, due to decreased activity of the enzyme heksokinase and at the same time decreased utilization of glucose in the cell. Under these conditions, the capacity of glycolisis is diminished as well as glycogenesis, capacity of glycogenolysis is also increased, metabolic pathway leading to synthesis of amino sugars being blocked (8). At the same time, inhibition of lypogenesis and increase in lypolisis are seen (7). Concentration of saturated fatty acids is increased. Due to these changes in lipid status, in diabetics hyperlipoproteinemias are very common (1). At the same time, capacity of protein synthesis is diminished, proteolysis is increased and capacity of Krebs cycle is highly decreased. Diminished capacity of Krebs cycle and respiratory chain is reflected at the level of functional capacity and morphologic appearance of mitochondria in muscle cells (10,11,12). Muscle cells can not function without energy. Within muscle cells, rich in mitochondria, this is achieved through activation of oxidative phosphorylation enzymes (9,10). The activity of oxidative enzymes in mitochondria is decreased for 40%; at the same time, these mitochondria have smaller dimensions and sometimes are destroyed (10). Relation between insulin resistance and diabetes type II is also related to the fact that in these patients metabolic muscle flexibility is lost (this assumes their capacity to choose their own metabolic substrates in order to obtain energy). So far, it has been found that the level of reduction of oxidative enzyme capacity correlates well with intensity of insulin resistance (11).Under these circumstances , it is normal to expect subsequent changes in the activity of creatine kinase, enzyme that is mostly present in muscle tissue. Since, the etiology of diabetes is quite different for the two populations of patients examined, in this work, it was presumed that the activity of creatine kinase would be different and reflect the metabolic status of each group of examined patients.

Materials and Methods

In this work, fresh samples of blood specimens were taken from 20 patients with diagnosis of diabetes mellitus type I and 20 patients with diagnosis of diabetes mellitus type II. Forty adequate controls with no evidence of muscle disease were analysed also. These patients were subdivided according to sex and age. In all the samples, activity of creatine kinase was examined as well as concentration of glucose. Glucose was analysed by spectrophotometric method (glucose oxidase-peroxidase, Bosnalijek). For each analysis, 10 μL samples were taken and treated with 1 mL of working solution. The absorbance of these samples as well as blank was measured on Janway spectrophotometer at 500 nm. Activity of creatine kinese was analysed by CK NAC activated test kit (Biosystems, Inc, kinetic uv test). After follow up of general procedure, enzyme activity was estimated according to formula:

$$A/min \times 10^6 \times TV / 6{,}3 \times 10^3 \times l \times V = U/l$$

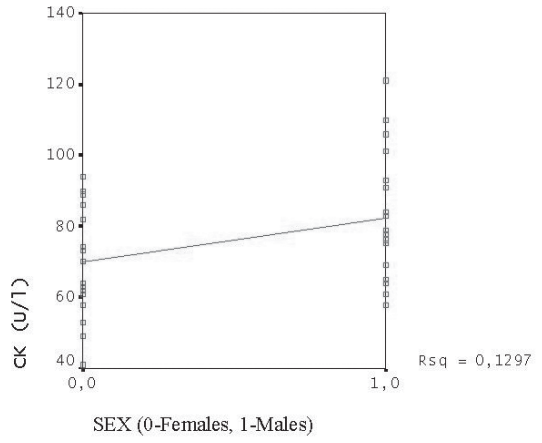
Results

Results of the study are presented in Table 1. As it can be seen from the table, concentrations of glucose are significantly higher in population of patients with diagnosis of diabetes type I and diabetes type II. At the same time, activity of creatine kinase, although it is kept within reference range is significantly higher in categories of patients with diabetes type I and diabetes type II. Two categories of diabetic patients exibit significant

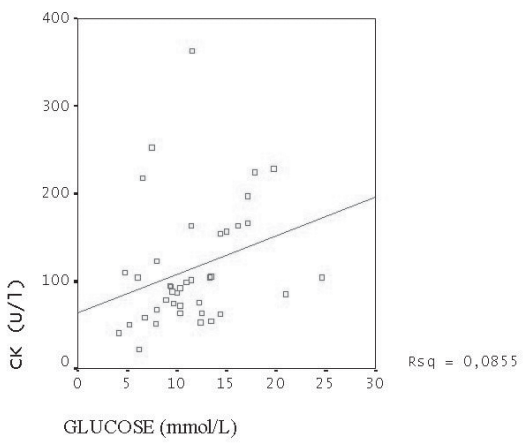
	Glucose (mmol/L)	Creatine kinase activity U/L
Controls	5,15 ± 0,08	76,15 ±2,76
Diabetic patients(type I)	12,25 ± 0,94*	132,90 ±17,90*
Diabetic patients(type II)	11,27±1,05*	95,10 ± 11,77**

* significant difference (p< 0,0005) with respect to controls
** significant difference (p< 0,0005) with respect to controls and significant difference (p = 0.0430) with respect to diabetes type I.

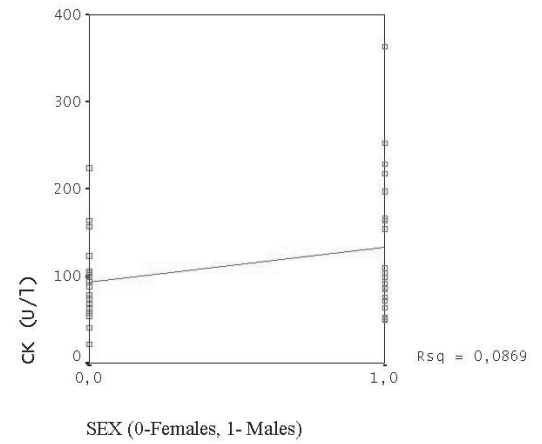
TABLE 1. Concentrations of glucose and creatine kinase activity in controls and patients with diabetes mellitus



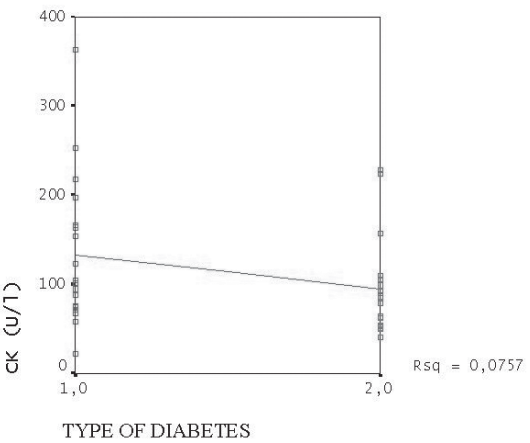
GRAPH 1. Correlation between sex and creatine kinase activity in controls



GRAPH 2. Correlation between glucose and creatine kinase activity in diabetic patients

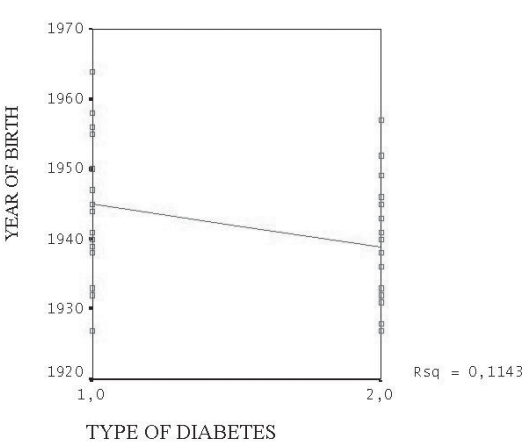


GRAPH 3. Correlation between sex and creatine kinase activity in diabetic patients



GRAPH 4. Correlation between type of diabetes and creatine kinase activity in diabetic patients

differences at the level of creatine kinase activity. In order to examine properly how measured parameters correlate with sex and age, Pearsson coefficients were determined and analysed separately for all examined populations. It was found out that within control group, there was no correlation between glucose and enzyme activity as well as between glucose and sex . Only existing correlation obtained was between sex and the activity of creatine kinase. This correlation is presented in Graph 1. When the values of Pearsson coefficient were examined for the group of diabetic patients, it was found out that the only correlation existing within this population was between concentration of glucose and the activity of creatine kinase. This correlation is shown in Graph 2. Correlation is also evident between type of diabetes and activity of creatine kinase. (correlations shown in Graph 4)



GRAPH 5. Correlation between type of diabetes and year of birth in diabetic patients

Correlation between type of diabetes and year of birth is also evident (Graph 5) as well as correlation between sex and the activity of the enzyme (Graph 3). For this population, correlation searched for but not discovered were: between sex and glucose concentration, type of diabetes and glucose concentration, age and glucose concentration and age and serum activity of the enzyme creatine kinase.

Discussion

Results obtained in this work can be explained at the level of metabolic differences between two examined groups . In diabetes in general, the alterations in glucose, lipid and protein metabolism are present and especially evident at the level of muscle cells (miocytes). Due do the disease, the utilization of glucose is decreased, phosphorylation of glucose is altered, synthesis of glycogen reduced, glycolisis suppressed. Due to smaller capacity of glycolisis, concentrations of oxal acetate and pyruvate are diminished also. Capacity of Krebs cycle is reduced as well as capacity of oxidative phosphorylation and respiratory chain. Depoes of ATP in muscle cells are diminished . Through stimulated glycolisis and further beta oxidation of fatty acids, organism tries to fight these processes. Due to the shortage of ATP, synthesis of creatine phosphate is decreased and the possibility of resynthesis of ATP from ADP is diminished. Situation can be further complicated through possible activation of AMP activated protein kinase (12,14). This enzyme becomes activated in cells when ratio of creatine phosphate to creatine falls and also due to ATP/AMP ratio. In healthy organism, this decrease is seen in muscle contractions and is due to utilization of ATP and creatine phosphate (13). It can be assumed that in diabetes, due to all the above mentioned disturbances and evident metabolic stress, this situation is further complicated during the period of muscle contractions. When activated, this enzyme would phosphorylate creatine kinase in muscle tissue (CK 3) and cause its inhibition. Through this activation, cell is trying to protect itself from depletion of ATP and by this means ensure sufficient amounts of ATP(14), while metabolic pathways that produce energy are again being activated. Since, these pathways are defficient in diabetics, this mechanism wont reflect results in this population. Sometimes, lack of synthesis of ATP can lead to total inhibition of creatine kinase activity. Therefore, it can be assumed that one of the potential mechanisms explaining observed small increase in creatine kinase activity in diabetics could be related to energy shortage(metabolic pathways leading to energy production are supressed, creatine phosphate is missing and due to potential inhibition of creatine kinase activated by AMP activated protein kinase), all of these facors being necessary for normal functioning of muscle cells. Due to these events, enzyme is leaking out of cytoplasm into blood. Evident positive correlation between concentration of glucose and the activity of creatine kinase can be easily attributed for. With higher concentration of glucose , disease is obviously not well controlled , uptake of glucose by the cells is lower , and the degree of metabolic disturbance higher leading to lower energy supply and therefore to a high degree of cell destruction. Since in this case one is speaking of autoimmune disease, which is accompanied by the appearance of macro CK type 1 activity, this can not be neglected as a cause of increased activity of creatine kinase in patients with this disease. Limited number of patients examined does not allow final conclusions about possible mechanisms of these changes.

Conclusion

Results of this study demonstrate that the activity of the enzyme creatine kinase is significantly higher in patients with diagnosis of diabetes mellitus type I and diabetes mellitus type II , when compared to activity of the enzyme observed in control population. Two categories of diabetic patients exibit significant differences at the level of enzyme activity which are reflected at the level of existing correlation coefficients for the examined groups of diabetic patients. Therefore, it can be concluded that the activity of this enzyme reflects the metabolic status in each category of examined patients. However,limitations related to number of examined patients point out the fact that further well designed investigations are needed in order to give definite answers related to possible mechanisms of these changes.

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