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Association between Ca^{+2} / Mg^{+2}ATPase activity and Type 2 diabetic patients with nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is a major cause of sickness and death in people with diabetes. It can lead to the need for dialysis or a kidney transplant. Ca^{+2} / Mg^{+2}ATPase is an important regulator of intracellular calcium concentration and therefore, of erythrocyte deformability. So the present study was aimed to evaluate the erythrocyte Ca^{+2} / Mg^{+2}ATPase activity in type 2 diabetes patients and reveal any associations between enzyme activity and diabetic nephropathy. The study included sixty patients with type 2 diabetes who were subdivided into thirty with nephropathy (microalbuminuria and macroalbuminuria) and thirty without complications. Twenty healthy subjects, age- and sex-matched were included as control group. Patients and controls were assessed for FBG, PBG, glycosylated hemoglobin (HbA1c), serum creatinine and urea levels, lipid profile and fasting C-peptide level, microalbuminuria and Ca^{+2} / Mg^{+2}ATPase activity. The results showed that a significant decrease in Fasting C-Peptide of patients with diabetic nephropathy group as either compared with diabetic without complication or control group. Moreover there was a significant decrease in Ca^{+2}/Mg^{+2} ATPase activity among patients with type 2 diabetes and with or without nephropathy as compared with control group. Also enzyme activity did not differ among the degrees of nephropathy. Thus, alteration of Ca^{+2}/Mg^{+2} ATPase activity and Fasting C-Peptide may be recognized features of Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is associated with an increased risk of cardiovascular disease, retinopathy, nephropathy, neuropathy, sexual dysfunction and periodontal disease. Improvements in glycemic control may help to reduce the risk of these complications [(1)].

Diabetic nephropathy (DN) is the leading cause of end stage renal disease (ESRD). The classical definition of DN is a progressive rise in urinary albumin excretion (UAE), coupled with increasing blood pressure, leading to decline of glomerular filtration rate (GFR), and eventually end-stage renal failure [(2)].

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The Ca^{+2} / Mg^{+2}ATPase (EC 3.6.1.3) is the major mechanism for Ca^{+2} ion extrusion in erythrocytes, platelets , and smooth muscle cells and in these cells the Ca^{+2}-Mg^{+2}ATPase is calmodulin-dependent. The Ca^{+2} transporter translocates Ca^{+2} across the plasma membrane at the expense of the hydrolysis of ATP and has a high affinity for Ca2+ ions. The function of this transporter is to contribute to returning and maintaining the Ca^{+2} at submicromolar levels [3].

The intracellular calcium concentration is regulated by Ca^{+2} - Mg^{+2} ATPase of human red blood cells. So, the reduced calcium pump activity observed in diabetic patients with neuropathy, may be related to molecular mechanism, leading to an increased cellular calcium content, despite lower levels of plasma calcium. These abnormalities on cellular Ca^{+2} metabolism may contribute to reduced erythrocyte flexibility [4]. Therefore the aim of the study was to evaluate the erythrocyte Ca^{+2} / Mg^{+2}ATPase activity in type 2 diabetes patients and reveal any associations between enzyme activity and diabetic nephropathy.

SUBJECTS AND METHODS

This study included twenty control subjects and sixty patients with type 2 diabetes selected from those admitted to Diabetes and Endocrinology Unit, Internal Medicine Department, Tanta University Hospital, Egypt. Subjects were divided into:

Group I (Control group): Included 20 healthy volunteers.

Group II (Diabetic patients without complications): Included 30 type 2 diabetic patients without complications.

Group III (Patients with diabetic nephropathy):

A) Diabetic patients with microalbuminuria: Included 15 type 2 diabetic patients with microalbuminuria (Albumin/creatinine ratio 30 – 300 mg/g).

B) Diabetic patients with macroalbuminuria: Included 15 type 2 diabetic patients with macroalbuminuria (Albumin/creatinine ratio > 300 mg/g).

Exclusion criteria: Those with type 1 diabetes mellitus or type 2 diabetes mellitus having liver disease, end stage renal failure, heart diseases and cancer.

Informed consent was obtained from patients and controls after study approval by the Local Ethical Committee, Tanta University. Full history was taken for all patients with particular emphasis on the duration of diabetes, urinary symptoms, body mass index (BMI), Smoking habits, history of any other associated disease and therapeutic history. First morning urine samples were collected from each subject for complete urine analysis and estimation of microalbumin. Venous blood was drawn in the morning from each subject after an overnight fast; 0.5 ml blood was collected in sodium fluoride test tube for determination of fasting blood glucose (FBG). 2 ml blood were collected in a dry centrifuge tubes and were allowed to clot then centrifuged and the obtained serum was utilized for estimation of creatinine and urea levels, lipid profile and fasting C-peptide level. 2 ml blood were collected in EDTA tube for determination glycosylated hemoglobin (HbA1c). 5 ml blood were collected in sodium citrate test tube for determination of calcium - magnesium ATPase activity. (0.5ml) 2-h after breakfast was collected in sodium fluoride test tube for determination of postprandial blood glucose (PBG).

FBG and PBG were assayed by using commercial kit that was supplied by (Spinreact, Egypt) [5]**.** Glycosylated hemoglobin in whole blood was assayed by using the NycoCard READER® supplied by (Axis-Shield, Oslo, Norway).Creatinine [6] and Urea [7] levels were assayed by using commercial kit that was supplied by (BioSystems, Egypt)**.**The eGFR was calculated according to the simplified version of the Modification of Diet in Renal Disease (MDRD) formula as defined by [8]. Microalbumin in urine was assayed by using commercial kit that was supplied by (BioSystems, Egypt) [9]**.** Lipid profile was assayed by using commercial kit that was

supplied by (Human® company, Egypt) described by [10]. Fasting C-peptide was measured using human C-peptide ELISA kit (immunospec, Cat# E29-071).

Extraction of erythrocyte membranes: The hemoglobin - free ghost membranes were obtained according to the method of [11]. Protein determination by the method of [12]. The erythrocyte membrane Ca^{+2} / Mg^{+2}ATPase activity (nmole Pi/ mg protein/ h) was measured by the method of [13].

STATISTICAL ANALYSIS

The collected data were analyzed using software statistical computer package SPSS version 20. For quantitative data, mean and standard deviation were calculated. For comparison between more than two means of parametric data, the *P* value of analysis of variance (ANOVA) was calculated. Correlation between variables was evaluated using Person's correlation coefficient (r). Significance was adopted at P<0.05.

RESULTS

Table (1) shows a significant increase in BMI, FBG, PBG and HbA1c (P<0.001) among patients with type 2 diabetes and with or without nephropathy compared to control group. Patients with diabetic with Macro-albumin urea showed a significant increase, (P<0.001) versus both diabetic without complication and control group, in serum creatinine and urea. Moreover a significant decline in eGFR in diabetic with Macro-albumin urea versus both diabetic without complication and control group (P<0.001). In patients with diabetic nephropathy group; Total cholesterol, Triacylglycerol and LDL-C were significantly higher than control group.

Table (2) revealed that there was no significant difference between Microalbumin and Albumin/creatinine ratio of diabetic patients without complication compared with control group. However Microalbumin and Albumin/creatinine ratio were significantly higher in patients with diabetic nephropathy group than other groups (P<0.001).

Table (3) showed that a significant decrease in Fasting C-Peptide of patients with diabetic nephropathy group as either compared with diabetic without complication or control group (P<0.001). Moreover there was a significant decrease in Ca^{+2}/Mg^{+2} ATPase activity among patients with type 2 diabetes and with or without nephropathy as compared with control group (P<0.001). Also enzyme activity did not differ among the degrees of nephropathy.

DISCUSSION

In type 2 diabetics, more patients have DN at the time of diagnosis of diabetes as type 2 diabetes can go unrecognized for years. So albuminuria is common among patients with established diabetes, is present before the onset of diabetes, and becomes more prevalent with worsening glucose tolerance. About 20%–40% of type 2 diabetics with microalbuminuria progress to overt nephropathy; and about 20% will develop ESRD after the development of overt nephropathy [14].

In the present study patients with type 2 diabetes and nephropathy showed a significant increase in serum creatinine, BUN, as well as microalbuminuria when compared to those without complications. These results revealed excellent predictive parameter for DN. Our findings were consistent with **Buch et al., 2012** who concluded that microalbuminuria is essential for early detection of DN in patients with type 2 diabetes.

In the present study patients with type 2 diabetes without complications showed a significant decrease in Ca^{+2}/Mg^{+2} ATPase activity and Fasting C-Peptide when compared to control group. However, Ca^{+2}/Mg^{+2} ATPase activity were no significant difference in diabetic nephropathy group compared with diabetic patients without complications or between subgroups diabetics with micro albumin urea and macro albumin urea. Thus it is conceivable to say that the decrement of Ca^{+2}/Mg^{+2} ATPase activity not related to DN.

This results are in agreement with **Koc et al., 2003** reported that Ca^{+2}/Mg^{+2} ATPase activity was no difference in diabetic nephropathy groups compared with diabetic patients. Also, **Migdalis et al., 2000** stated that significantly

lower level of Ca^{+2}/Mg^{+2} ATPase activity in type 2 diabetic patients compared with control group. The cause of decreased Ca^{+2}/Mg^{+2} ATPase activity in our diabetic patients cannot be determined from this study. Glycosylation of proteins may alter their physiological properties, particularly their binding affinities. Therefore, it can be postulated that either glycosylation of the enzyme or glycosylation of the activator protein is responsible for impaired activation of the ATPase.

CONCLUSION

Our results of the present study showed that impairment of erythrocyte membrane Ca^{+2}/Mg^{+2} ATPase activity Type 2 diabetes mellitus. These changes were, also, accompanied by decrease in Fasting C-Peptide. Moreover, no changes in Ca^{+2}/Mg^{+2} ATPase activity in diabetic nephropathy.

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Table (1): Demographic, clinical data and biochemical parameters of the studied groups.

Groups / Parameters	Group 1 Control (n=20)	Group 2 (Diabetic without complication) (n= 30)	Group 3 (Patients with diabetic nephropathy)	
			(A) Diabetic with Micro-albumin urea (n= 15)	(B) Diabetic with Macro-albumin urea (n= 15)
Age (years) Mean ±S.D	**36 ± 3.2**	**50^{a***} ± 5.8**	**53^{a***} ± 4.4**	**55$^{a***\ b*}$ ± 7.1**
Sex (M/ F)	**9/ 11**	**13/ 17**	**6/ 9**	**7/ 8**
Diabetes duration (Years) Mean ±S.D		**5.5 ± 1.7**	**8^{b***} ± 2.1**	**10 ^{b***} ± 2.2**
BMI (kg/m^2) Mean ±S.D	**23.54 ± 1.07**	**32.8 ^{a***} ± 1.9**	**33.5 ^{a***} ± 0.9**	**33.6 ^{a***} ± 1.45**
Smoking habits N (%)	**4 (20%)**	**8 (26.7%)**	**4 (26.6%)**	**5 (33.3%)**
FBG (mg/dl) Mean ±S.D	**88.6 ± 4.3**	**163 ^{a***} ± 6.2**	**236 ^{ab***} ± 8.0**	**267 ^{abc***} ± 8.1**
PBG (mg/dl) Mean ±S.D	**108.8 ± 6.4**	**249 ^{a***} ± 7.9**	**324 ^{ab***} ± 8.4**	**357 ^{abc***} ± 9.9**
Hb_{A1c}% Mean ±S.D	**5.6± 0.53**	**7.0 ^{a***}± 0.53**	**8.8 ^{ab***} ± 0.73**	**9.6 ^{abc***} ± 0.83**
Creatinine (mg/ dl) Mean ±S.D	**0.86 ± 0.11**	**1 ± 0.17**	**1.2 $^{a***\ b**}$ ± 0.31**	**1.4 $^{ab***\ c*}$ ± 0.37**
Urea (mg/dl) Mean ±S.D	**30.8± 4.6**	**31.3 ± 4.1**	**45 ^{ab***} ± 4.8**	**63.7 ^{abc***} ± 4.7**
eGFR (ml/min/1.73m^2) Mean ±S.D	**95.13 ± 16.9**	**80.1 ± 20.7**	**63.4 ^{a***} ± 19.9**	**50.4 ^{ab***} ± 11.5**
Total cholesterol TC (mg/ dl) Mean ±S.D	**170.7 ± 16.1**	**211.5 ^{a***} ± 18.23**	**230.1 $^{a***\ b**}$ ± 15.2**	**236.3 ^{ab***} ± 14.9**
Triacylglycerol TGs(mg/dl) Mean ±S.D	**120.1 ± 23.3**	**184.3 ^{a**} ± 55.05**	**221.1 ^{a***} ± 67.5**	**236.7 $^{a***\ b*}$ ± 61.7**
HDL-C (mg/dl) Mean ±S.D	**67.2 ± 4.9**	**52.2 ^{a***} ± 3.5**	**45.3 ^{ab***} ± 3.6**	**43.6 ^{ab***} ± 1.9**
LDL-C (mg/dl) Mean ±S.D	**81.6 ± 5.5**	**118.7 ^{a***} ± 3.7**	**147 ^{a***}± 3.5**	**148.9 ^{a***} ± 5.8**

Data are presented as mean ± SD

a: Significant vs. control.

b: Significant vs. Diabetic without complication.

c: Significant vs. Diabetic with Micro-albumin urea.

* p<0.05,** p<0.01, *** p<0.001

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Table (2): Microalbumin and Albumin/creatinine ratio (mg/g) in the studied groups.

Groups / Parameters	Group 1 Control (n=20)	Group 2 (Diabetic without complication) (n= 30)	Group 3 (Patients with diabetic nephropathy)	
			(A) Diabetic with Micro-albumin urea (n= 15)	(B) Diabetic with Macro-albumin urea (n= 15)
Microalbumin (mg/ L) Mean ±S.D	11.67 ± 3.7	20.6 ± 4.5	124.4 ^{ab***} ± 35.3	718.8 ^{abc***} ± 144.8
Urine creatinine (mg/ dl) Mean ±S.D	91.76 ± 33.5	112.9 ± 30.22	106.6 ± 22.75	126 ± 28.73
Albumin/creatinine ratio (mg/ g) Mean ±S.D	13.5 ± 2.7	18.8 ± 4.2	111 ^{ab***} ± 37.7	547.9 ^{abc***} ± 88.9

Data are presented as mean ± SD

a: Significant vs. control.

b: Significant vs. Diabetic without complication.

c: Significant vs. Diabetic with Micro-albumin urea.

* p<0.05,** p<0.01, *** p<0.001

Table (3): Fasting C-Peptide and Ca^{+2}/Mg^{+2} ATPase activity in the studied groups.

Groups / Parameters	Group 1 Control (n=20)	Group 2 (Diabetic without complication) (n= 30)	Group 3 (Patients with diabetic nephropathy)	
			(A) Diabetic with Micro-albumin urea (n= 15)	(B) Diabetic with Macro-albumin urea (n= 15)
Fasting C-Peptide (ng/ml) Mean ±S.D	3.2 ± 0.64	2.2 ^{a***} ± 0.31	0.9 ^{ab***} ± 0.27	1.1 ^{ab***} ± 0.22
Ca^{+2}/Mg^{+2} ATPase activity (nmol Pi/mg protein/h) Mean ±S.D	396± 18.7	332.6 ^{a***} ± 26.9	323.6 ^{a***} ± 14.8	319.2 ^{a***} ± 16.5

Data are presented as mean ± SD

a: Significant vs. control.

b: Significant vs. Diabetic without complication.

c: Significant vs. Diabetic with Micro-albumin urea.

* p<0.05, ** p<0.01, *** p<0.001