Original Article

Correlation to Serum Ionized Calcium and Magnesium Concentration with Sympathetic Nerve Functions in Type 2 Diabetes Mellitus

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ARTICLE INFO

Article history:

Received : 21 October 2012
Accepted : 17 March 2013

Keywords:

Sympathetic,
Neuropathy,
Diabetes,
Nerve

ABSTRACT

Background : In type 2 DM, a metabolic disorder causes disturbances of different metabolic processes of the body including electrolyte imbalance. Therefore changes in serum level of Ca^{2+} and Mg^{2+} may have some relationship to the occurrence of neuropathy.
Objective : The study has been designed to observe the sympathetic nerve function status in type 2 diabetic subjects in order to assess the relationship between serum ionized calcium & magnesium.
Study design : This cross section study was carried out in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Forty seven type 2 diabetic subjects were included in two groups. Group B consisted of 25 recently diagnosed diabetic subjects aged 40-68 years and group C consisted of 22 subjects having duration of diabetes for 10-20 years. Twenty five (25) age and BMI matched healthy subjects were included in group A (non-diabetic) for control.
Methods : Sympathetic nerve function were assessed by two simple non-invasive cardiovascular reflex tests. These were systolic blood pressure, response to lying to standing up and diastolic blood pressure response to distain hand grip test. Ionized serum calcium and magnesium were measured by ion sensitive electrode method using NOVA electrode. Data were analyzed by 't' test, Pearson's correlation co-efficient.
Results: Serum ionized calcium levels were significantly higher in both the diabetic group B ($P<0.001$) and C ($P<0.001$) than that of control group A. Serum ionized magnesium level was significantly higher in diabetic group C ($P<0.001$) compared to that of control group A. Pearson's correlation of ionized calcium and magnesium with sympathetic nerve function parameters were done. Statistically significant relation was found in group B between sustained hand grip test with ionized calcium and in group C between systolic blood pressure response to standing with ionized magnesium.
Conclusion : Serum ionized calcium & magnesium level was increased in type 2 diabetic patients irrespective of duration and some correlation was found with sympathetic nerve function parameters

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Introduction

Calcium and magnesium play a key role in cellular metabolism. In vitro studies have shown the major role of magnesium ion in insulin action.[1,2] Magnesium has been suggested as second messenger for insulin action.[1] Resnick et al.[3] observed intracellular free calcium was elevated and magnesium was deficient in type 2 diabetic subjects. Olukoga et al.[1] and Smith et al.[5] reported that hypomagnesemia is a common finding in type 1 and type 2 diabetic subjects. In a study of Nigerian diabetic subjects it was shown that, fasting plasma magnesium was low, Again, hypomagnesaemia in diabetic subjects are often associated with increased urinary excretion of magnesium. This marked hypomagnesimia was correlated with poor metabolic control.[6]

The possible relationship of Ca^{2+}-Mg^{2+}- ATPase activity and serum ionized calcium in diabetic patients with peripheral neuropathy have investigated.[7] Migdalis et al.[7] also reported that diabetic neuropathy had significantly lower levels of the serum Ca^{2+}-Mg^{2+} ATPase, in patients with diabetic neuropathy there are abnormalities of Ca^{2+}-Mg^{2+} ATPase activity and serum Ca^{2+}. This provides further support for their role in microangiopathy in the pathogenesis of neuropathy. On the other hand, Mikhail and Ehsanipoor.[8] found serum ionized magnesium was significantly higher in diabetics than in control subjects.

Magnesium is one of the most abundant ions present in living cells and its plasma concentration is remarkably constant in health subjects. Plasma & intracellular magnesium concentration are strictly regulated by several factors. Among them, insulin seems to be the most important. In vitro and vivo studies have demonstrate that insulin increases the cellular permeability of magnesium and causes transport of the ion from extracellular to intracellular space. Intracellular Mg^{2+} concentration has been shown to be effective in modulating insulin action (mainly oxidative glucose metabolism) calcium related excitation-contraction coupling, and decrease smooth muscle cell responsiveness to stimuli. Again, poor intracellular magnesium concentration, may result in a defective tyrosine activity at the insulin receptor level and increased intracellular calcium ion concentration.[9]

As diabetes mellitus, a metabolic disorder causes disturbances of different metabolic processes of the body including electrolyte imbalance. Therefore, changes in serum levels some of the electrolytes e.g serum calcium and magnesium may have some relationship to the occurrence of neuropathy. In our country a large member of diabetic patients are suffering from neuropathy. Among them a quiet large member may have diabetic cardiac autonomic neuropathy which remain undetected unless complicated cardiac autonomic neuropathy is the clinically important form of diabetic autonomic neuropathy. Again, severity of cardiac autonomic neuropathy depend on the metabolic derangement. In addition, serum Ca^{2+} and Mg^{2+} may have some contribution in the development of cardiac autonomic dysfunction in patients with type 2 diabetes mellitus.

In the above context an attempt have been made to carry out a study on sympathetic nerve function parameters in type 2 diabetes subjects and in non-diabetic healthy subject in order to explore the role of serum ionized calcium and magnesium concentration.

Method

In this present work, 47 type 2 diabetic patients were taken and subdivided into two groups. Group B consisted of 25 recently diagnosed diabetic subjects and group C consisted of 22 subjects having diabetes for 10-20 years. 25 age and BMI matched healthy subjects were included in group A (non-diabetic) for control. Diabetic subjects were selected from out-patient department (OPD) of BIRDEM, Dhaka. Healthy controls were selected from the friends and relatives of the investigator and also from the patients. None of them had history of diabetes up to second degree relation. The test to be performed consents were taken from them. On the day of examination, fasting blood samples were collected in the morning after 10-16 hours of over night fast and then this sympathetic nerve function before the study. Estimation of all the biochemical test i.e. fasting plasma glucose (FPG) serum creatinine were done Biomedical Research Group Laboratory, BIRDEM and sympathetic nerve function tests

were measure in neurophysiological laboratory of the physiology Department, BSMMU, Dhaka. With all aseptic precautions 4 ml of venous blood was drawn from the antecubital vein with a sterilized plastic disposable syringe. After collection blood sample was centrifuged by 3000 RPM for 15 minutes and separated serum was immediately preserved at -70^{0}C for estimation of serum levels of FPG, creatinine, ionized calcium and ionized magnesium. The body weight in kilogram (Kg) was measured in patient with light clothing and height in centimeters (cm) by using appropriate scaled on bare foot (Detect-Medic, detect scales INC, USA). BMI of the subjects were calculated from the measured weight and height. Serum glucose was estimated by glucose oxidase (GOD/PAP) method (Bio-labo france). Serum creatinine was measured by Alkaline Picrate method (Ramdox laboratories, UK). Ionized serum calcium and magnesium were measured by ion sensitive electrode method using NOVA electrode (Chronelab, USA). sympathetic functions were assessed by two simple cardiovascular reflex tests.[10] These tests can be performed easily with minimal equipments. Instrument needed are sphygmomanometer and ECG machine, a mercury calcium attached to a mouth piece by a rigid flexible tube.

Data were expressed as mean±SD and range. To compare among groups one way ANOVA with Bonferroni 't' test was performed as the test of significance. The Pearson's correlation co-efficient were done to observe relationship among different variables.

Results

The mean(±SD) age and BMI of the study groups are shown in table 1. No statistically significant difference of age and BMI were observed among different study groups. Therefore all the group were matched for age and BMI.

Table-I: Mean±SD in different groups age and BMI (N=72)

Group	N	Age (years)	BMI (kg/m^2)
A	25	51.92±5.64 (45.00-67.00)	25.28±2.98 (19.56-31.40)
B	25	51.52±6.93 (40.00-68.00)	24.58±2.48 (18.29-28.35)
C	22	55.77±5.81 (45.00-68.00)	24.47±2.76 (19.70-30.45)
Statistical analysis			
Group		P value	P value
A vs B		>0.50^{NS}	>0.50^{NS}
A vs C		>0.10^{NS}	>0.50^{NS}
B vs C		>0.05^{NS}	>0.50^{NS}

Values in parenthesis indicate ranges
NS = Not significant
Group A = Control (Healthy non diabetic)
Group B = Recently diagnosed diabetic
Group C = Type 2 Diabetes mellitus for 10-20 years

Sympathetic nerve function parameters in different study group are shown in table II.

Table –II: Sympathetic nerve function parameters in different study group (N=72)

Group	N	DBP response to sustain hand grip test (mmofHg)	SBP response lying to standing (mmofHg)
A	25	27.84±7.83 (15.00-40.00)	1.04±6.25 (-10.00-10.00)
B	25	24.80±9.63 (10.00-40.00)	2.60±10.52 (-15.00-20.00)
C	22	19.96±10.82 (5.00-40.00)	8.18±10.41 (-5.00-25.00)
Statistical analysis			
Group		P value	P value
A vs B		>0.50^{NS}	<0. 50^{NS}
A vs C		<0.05*	<0.05*
B vs C		>0.10^{NS}	>0.10^{NS}

* = Significant
NS = Not significant
Group A = Control (Healthy non diabetic)
Group B = Recently diagnosed diabetic
Group C = Type 2 Diabetes mellitus for 10-20 years
SBP = Systolic blood pressure
DBP = Diastolic blood pressure

Serum levels of ionized calcium and magnesium in different study group are shown in table III. Serum ionized calcium levels were significantly higher in both the diabetic group B (P<0.001) and C (P<0.001) than that of control group A. No significant higher level of serum ionized calcium were observed between the diabetic group B & C (P>0.10). Serum ionized magnesium level was significantly higher in

diabetic group C (P<0.001) compared to that of group A.

Table III: Serum ionized calcium and magnesium levels in different study group

Group	N	Serum Ca^{2+} (mmol/L)	Serum Mg^{2+} (mmol/L)
A	25	0.93±0.10 (.74-1.12)	0.49±0.05 (0.38-0.60)
B	25	1.04±0.10 (0.82-1.24)	0.53±0.05) (0.48-0.62)
C	22	1.10±0.10 (0.96-1.34)	0.66±0.08 (0.48-0.78)
Statistical analysis			
Group		P value	P value
A vs B		>0.001***	>0.05^{NS}
A vs C		>0.001***	>0.001***
B vs C		>0.10^{NS}	>0.001***

N = Total number of subjects, values in parenthesis indicate ranges
* = Significant
NS = Not significant
Group A = Control (Healthy non diabetic)
Group B = Recently diagnosed diabetic
Group C = Type 2 Diabetes mellitus for 10-20 years

Relationship of sympathetic nerve function parameters with ionized calcium level were shown in table IV. In sustain hand grip test rise in diastolic blood pressure were positively correlated in group B and C and negatively correlated in group A. This relationship in group B was statistically significant, however in group A and C were not significant. In standing test fall in systolic blood were positively correlated with serum ionized calcium in group A and C and negatively correlated in group B. None of the correlation were statistically significant.

Table IV: Relationship of sympathetic nerve function parameters with Ca^{2+} with different study group (N=72)

Parameters	N	r value	P value
DBP response to sustain hand grip test			
Group A	25	-0.189	>0.10^{NS}
Group B	25	+0.519	>0.01**
Group C	22	+0.000	>0.50^{NS}
SBP response lying to standing			
Group A	25	-0.334	>0.10^{NS}
Group B	25	-0.076	>0.50^{NS}
Group C	22	+0.187	>0.10^{NS}

* = Significant
NS = Not significant
Group A = Control (Healthy non diabetic)
Group B = Recently diagnosed diabetic
Group C = Type 2 Diabetes mellitus for 10-20 years
SBP = Systolic blood pressure
DBP = Diastolic blood pressure

Relationship of sympathetic nerve function parameters with ionized magnesium level were shown in table V. In sustain hand grip test fall in diastrolic blood pressure were negatively correlated with serum ionized magnesium in group A and C and positively correlated in group B. None of the correlation were statistically significant. In lying to standing test, rise in systolic blood pressure were positively correlated in group A and C and negatively correlated in group B. These correlations in group C was statistically significant but in group A and B were not significant.

Table V: Relationship between sympathetic nerve function parameters in Mg^{2+} in different study group (N=72)

Parameters	N	r value	P value
DBP response to sustain hand grip test			
Group A	25	-0.081	>0.50^{NS}
Group B	25	+0.132	>0.50^{NS}
Group C	22	+0.349	>0.10^{NS}
SBP response lying to standing			
Group A	25	+0.193	>0.10^{NS}
Group B	25	-0.261	>0.10^{NS}
Group C	22	+0.429	<0.05^{NS}

* = Significant
NS = Not significant
Group A = Control (Healthy non diabetic)
Group B = Recently diagnosed diabetic
Group C = Type 2 Diabetes mellitus for 10-20 years
SBP = Systolic blood pressure
DBP = Diastolic blood pressure

Discussion

The present study has been undertaken to observe the relationship of sympathetic nerve function parameters with serum ionized calcium and magnesium. In this study all the parameters

in non-diabetic control are almost similar to those reported by other countries.[11,12,13,14] The non-diabetic (control) and the diabetic subjects were of compared age & BMI.

Serum ionized calcium level was significantly higher in recently diagnosed (P<0.001) and long duration diabetic groups (P<0.001) compared to that of control group. However no statistically significant changes in serum calcium level was found (P>0.10) between the two diabetic groups. Migdalis et al.[7] reported lower level of serum ionized calcium in diabetic neuropathic subjects (P<0.01). Pearson correlation coefficient between serum ionized calcium level with sympathetic nerve function parameters were observed except the positive relationship of sustained hand grip test in group B (r=+.519; P=<0.01) none of the relationships were found to be statistically significant.

Serum ionized magnesium level was significantly higher in long duration diabetic group (P<0.001) compared to that of non diabetic group and also between two diabetic groups (P<0.001). No statistically significant higher value of serum ionized magnesium level was observed (P>0.05) in recently diagnosed diabetic group in comparison to that of control group. Similar higher level of serum ionized magnesium was also reported by Mikhail and Ehsanipoor [8] but they did not mentioned about the duration of diabetes. In this study relationship between ionized magnesium with sympathetic nerve function test parameters were observed. Among these, positive correlation (r=+0.429; P<0.05) with SBP response to standing in long duration diabetic subjects. No significant correlation were observed in other sympathetic nerve function parameters with serum ionized magnesium concentration.

Diabetic autonomic neuropathy (DAN) is a common complication of diabetes. DAN can affect many organ system throughout the body. Several investigators mode various explanation about the autonomic neuropathy in diabetes mellitus.[15] In addition the factors such as metabolic insult to nerve fibers, neurovascular insufficiency, autoimmune damage neurohumoral growth factors deficiency are responsible to DAN.[16] Mecleron et al.[17] suggest that racial, nutritional and biochemical variations might play significant role in diabetic nerve dysfunction.

Conclusion

Significant correlation were observed for serum ionized magnesium with SBP response to standing in long duration diabetics subjects and sustained hand grip test in recently diagnosed diabetic patient.

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