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Correlation between the Plasma Magnesium Levels and Glycated Haemoglobin in Sudanese Patients with Type 2 Diabetes Mellitus in Khartoum State

Tilal Abd El-Hameed Seedahmed[1], Samia Mahdi Ahmed[2,*]

[1]Chemical Pathology, University of Medical Science and Technology, Khartoum, Sudan
[2]Department of Clinical Chemistry, Sudan University of Science and Technology, Khartoum, Sudan
*Corresponding Author: samiama@sustech.edu

Abstract This is a cross sectional study aimed to evaluate the plasma levels of magnesium and HbA_{1C} of (60) patients with type 2 diabetes mellitus and (40) apparently healthy (non – diabetic) volunteers as control group, the patients were chosen from different hospitals and specific centers. Age and sex of the test group were matched with the control group. The plasma levels of magnesium were measured using a spectrophotometer (Bio system Company reagents). HbA_{1C} levels were measured using reagents and instruments from NycoCard company. Data was analyzed using SPSS. The results showed a significant reduction in the mean of the plasma levels of magnesium of the diabetic group when compared with the control group (p. value <0.05), whereas HbA_{1C} were increased in diabetic patients (type 2) compared to the control group (p. value <0.05). However there was a strong negative correlation between the plasma levels of magnesium and HbA_{1C} levels in diabetic patients, also there was a weak negative correlation between the plasma levels of magnesium and duration of disease. Plasma levels of magnesium have a strong negative correlation with HbA_{1C} levels, so results revealed that uncontrolled diabetic patients are at higher risk of hypomagnesaemia compared to controlled patients.

Keywords Diabetes Mellitus, Magnesium, Glycated Hemoglobin

1. Introduction

Diabetes mellitus is a major health problem worldwide. Diabetes is one of the leading causes of morbidity and mortality throughout the world. Diabetes mellitus is a major health problem worldwide. About 2.2% to 3% of the world`s population suffer from type 2 diabetes mellitus[1]. Diabetes mellitus (DM)is a chronic metabolic disorder that can lead to severe cardiovascular, retinal, neutological and rental complications[2,3].It is a serious debilitating and deadly disease that has now reached epidemic proportion and the prevalence rates are expected to go even higher in the future. Diabetic patients may reach End Stage Renal Disease (ESRD) if diabetes mellitus is not adequately controlled. In most countries diabetic nephropathy has become the single most frequent causes of ESRD[4-5].

The Glycosylated hemoglobin (HbA1c) is widely accepted and used as the most reliable test for assessment of chronic glycaemia[6].The HbA1c reflects the overall blood glucose levels over a period of 2-3 months and the major use of the HbA1c assay is to assess changes in metabolic control that follow an alteration in treatment[7].

The nephropathy is common in diabetic patients and usually associated with vascular complications. The long-term complications of diabetes have major consequences for individual and health care providers. The blood glucose was considered as a prime test for optimizing treatment of diabetes mellitus. But the HbA1c determination is the new better method to monitor the long term glucose control irrespective of glucose measurement for patient management. It would prevent or delay the further diabetic complications. Diabetic patients with oral hypoglycemic therapy should go for HbA1c test as recommended by the American diabetes association[8].

The role of magnesium in the body is widespread. It is an essential cofactor of more than 300 enzymesincluding those important in glycolysis, transcellular ion transport, neuromuscular transmission, synthesis of carbohydrates, proteins, lipid and nucleic acids and release of end response to certain hormones[9].

Diabetes mellitus has been suggested to be the most common metabolic disorder associated with magnesium deficiency, having 25 to 39% prevalence [10].Hypomagnesemia in diabetic patients result specifically from a reduction in tubular absorbtion of magnesium, as recently suggested by Garland[11]. Moles and McMulle have shown that low plasma magnesium concentration may contribute to insulin resistance[12].

2. Materials and Methods

2.1. Research Design

Study Design

This is an analytical, cross-sectional and hospital – based study.

Study Area

The study was done in Khartoum state, in specific centers.

Study Period

The study was carried during the period from May to August 2012.

Study Population

The main target population of this study is Sudanese patients with type 2 diabetes mellitus at age (40 -67) years; 36 females and 24 males, attending to specific centers.

Inclusion Criteria

Test group: Sudanese diabetic patients, type 2 diabetes mellitus.

Control group: Healthy volunteers.

Exclusion Criteria

Patients take magnesium supplementation, gout, hypertension, renal impairment and coronary heart diseases.

Sample Size

Sixty diabetic patients as (test group), and 40 normal healthy (non diabetic) as control group.

Ethical Consideration

Permission of this study was obtained from the local authorities in the area of the study.

The objectives of the study were explained to all individuals participating in the study.

An informed consent was obtained from each participant in the study.

Health education considering diabetes mellitus and its complications was provided to all participants.

2.2. Data Collection and Clinical Assessment

Interview and a Questionnaire

Interviews with a test group and the control group were done to obtain the clinical data and provide health education. Questionnaire designed specifically to obtain information which help in either including or excluding certain individuals in or from the study.

Blood Sampling

After informed consent, about 5 ml of venous blood were collected by standard procedure from each participants under complete a septic conditions, the blood withdrawal was followed national requirements. 2.5 ml were placed in lithium heparin container. Plasma was clear by low speed centrifugation, after separation the plasma samples were stored tightly closed at 2 – 8 ˚C and then used for magnesium analysis. The other 2.5 ml were placed in EDTA container and used for HbA1c analysis.

*(Icteric, lipemic, hemolyzed or bacterially contaminated sample was not used).

Biochemical Measurement

Plasma level of magnesium was measured using commercial reagent kits from Bio System Company, HbA1c level was measured using commercial reagent kits from NycoCard Company.

3. Methodology

Instruments

Spectrophotometer was used in this study for plasma magnesium measurement.

NycoCard Reader was used to measure HbA1c.

Plasma Magnesium Estimation

Principle: magnesium in the sample reacts with calmagite in alkaline media forming a colored complex that can be measured by spectrophotometer. Ethylene – glycol – Tetra acetic acid (EGTA) is included in the reagent to remove calcium interference.

Procedure:

	Blank	STD	Test
Reagent	1ml	1ml	1ml
STD		.o1ml	
Sample			.o1ml
D.W	.o1ml		

The contents of each tube was mixed well, incubated for two minutes at room temperature, then read at 520nm against blank.

Calculation

$$Conc.\ of\ magnesium\ (mg/dl) = \frac{Abs.\ Sample}{Abs.STD} \times\ conc.\ of\ STD\ (2mg/dl)$$

Linearity

This method is linear up to 4 mg/dl.

Measurement of Hba1c Levels

Principle

HbA1c is aboronate affinity assay. The kit contains test devices with a porous membrane filter, test tubes prefilled with reagent and washing solution. The reagent contains

agents that lyses erythrocytes and precipitate hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diols of glycated hemoglobin. When the blood is added to the reagent, the erythrocytes immediately lyses. All hemoglobin precipitate the boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction mixture is added to the rest device, and all the precipitate hemoglobin, conjugate bound and unbound, remains on top of the filters. Any excess of colored conjugate is removed with washing solution. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin), color intensity with the NycoCard reader II, the ratio between them being proportional to the percentage of HbA1c in the sample.

Procedures

5µL of whole blood was added to the test tube with reagent 1, mixed well and incubated for 3 minutes.

1. It was remixed to obtain a homogenous suspension. Reaction mixtures (25 µL) were applied to a test devise by holding the pipette approx. 0.5 cm above the test. The pipette was emptied quickly in the middle of the test. The reaction mixture was allowed to soak completely into the membrane for 10 seconds.
2. Washing solution (25 µL) was added to the test device. The washing solution was allowed to soak completely into the membrane for 10 seconds.
3. The test result was read within 5 minutes using NycoCard reader II.

Calculation

The test result was obtained immediately within 5 minutes.

Linearity

This method is linear up to 15%.

Quality Controls

The precision and accuracy of all methods used in this study were checked using commercially prepared control sera.

Statistical Analysis

All data collected in this study was analyzed using the SPSS computer programs, (t-test for mean and p. value for significance).

4. Results

Sixty patients with type 2 diabetes mellitus as a test group and forty apparently healthy Sudanese volunteers as control group were involved in this study. Both groups were matched for age and sex.

Plasma magnesium was found to be lower in test group compared to control group with P. value = 0.00 as shown in table (1).

Table 1. comparison between the mean of plasma levels of magnesium in diabetic group and control group.

Variable	Diabetic	Control group	P. value
	No = 60	No = 40	
	Mean ± SD	Mean ± SD	
Plasma Mg (mg/dL)	1.8 ± 0.2	2.3 ± 0.1	0.00
Range	(1.5 – 2.2)	– 2.6)	

The table shows the mean ± SD, range in brackets and probability (p.).
T-test was used for comparison.
P. value ≤ 0.05 is considered to be significant.

HbA1c level was found to be higher in test group compared to control group with p. value = 0.00 as shown in table (2).

Table 2. comparison between the mean of HbA1c levels in diabetic group and control group.

Variable	Diabetic	Control group	P. value
	No = 60	No = 40	
	Mean ± SD	Mean ± SD	
HbA1c (%)	7.9 ± 0.8	5.3 ± 0.4	0.00
Range	(5.2 – 10.3)	(4.5 – 6.1)	

The table shows the mean ± SD, range in brackets and probability (p).
T-test was used for comparison.
P. value ≤ 0.05 is considered to be significant.

The plasma levels of magnesium have a weak negative correlation with duration of diabetes in diabetic group. (r= -0.38, p. value = 0.00) as shown in table (3).

Table 3. correlation between the plasma levels of magnesium and duration of diabetes in diabetic group.

Variable	Statistics	Duration of disease(years)
Magnesium (Mg/dl)	Pearson Correlation	-0.38**
	p. value	0.00

** Correlation is significant at the 0.01 level.

The plasma levels of magnesium have a strong negative correlation with HbA1c levels in diabetic group. (r = -0.71, p. value = 0.00) as shown in table (4).

Table 4. correlation between the plasma magnesium levels and HbA1c levels in diabetic group.

Variable	Statistics	HbA1c (%)
Magnesium (mg/dl)	Pearson Correlation	-0.71**
	p. value	0.00

** Correlation is significant at the 0.01 level.

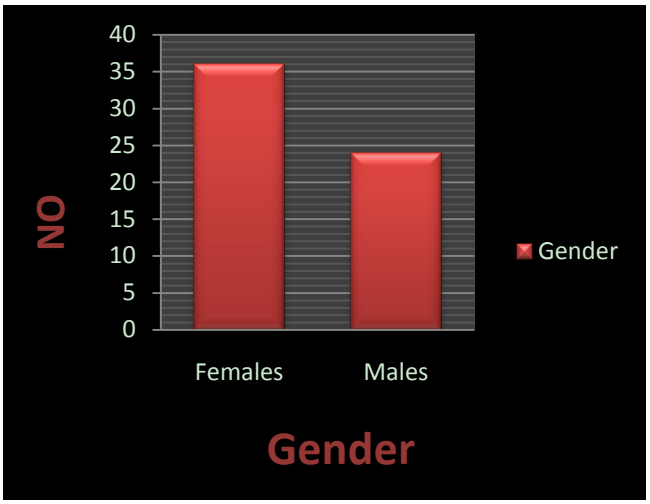


Figure 1. shows sex distribution in the diabetic group.

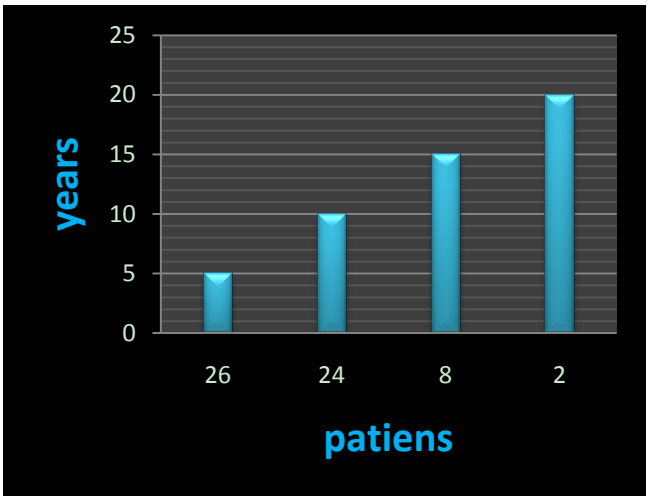
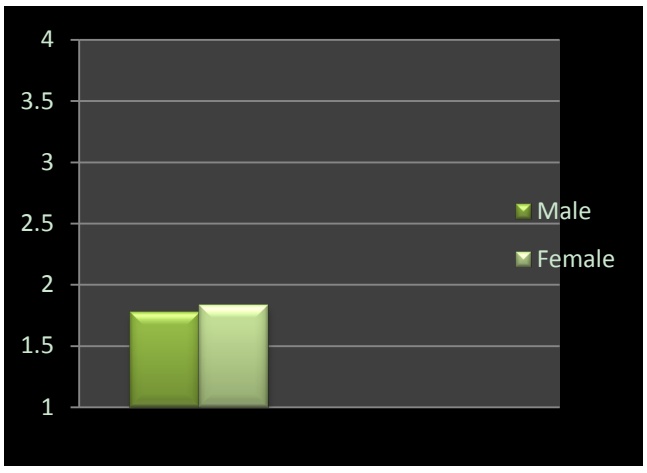


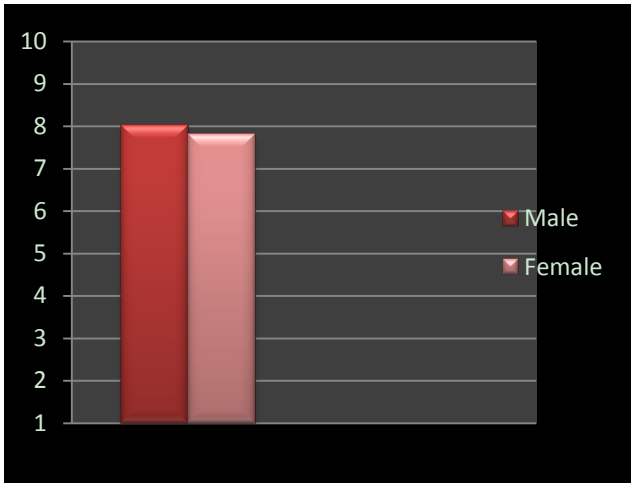
Figure 2. shows duration of disease in years and number of the patients.



magnesium(mg/dl)

Figure 3. shows the mean of magnesium in the diabetic patients according to gender.

The result revealed that the mean of magnesium level in the females (1.83mg/dl) was slightly higher than the mean of magnesium level in the males (1.77) as shown in figure 3 (statistically not significant, p. value > 0.05=0.3)



HbA1c(%)

Figure 4. shows the mean of HbA1c in the diabetic patients according to gender.

The result revealed that the mean of HbA1c level in the males (8.03%) was higher than the mean of HbA1c level in the females (7.81%) as shown in figure (4).This difference was found to be statistically insignificant (p. value > 0.05 =0.5)

5. Discussion

This study aimed to evaluate the plasma levels of magnesium and HbA1c in Sudanese patients with type 2 diabetes mellitus.

This study included 60 Sudanese patients with type 2 diabetes mellitus, their plasma magnesium and HbA1c levels were measured and compared with 40 non diabetic subjects as control.

Many studies done in the western countries show that the mean of plasma magnesium levels are lower in patients with type 2 diabetes mellitus than in general population.

In Sudan very few data is available considering the plasma magnesium levels in diabetic patients.

Sex distribution in figure (1) represents that the ratio of females is greater than males; Lester and west claimed that higher incidence and prevalence rate of type 2 diabetes mellitus were observed within females than in males [13-14].The results of this study showed a significant decrease in the mean of the plasma levels of magnesium of the test group compared to the control group table (1), this agrees with the previous studies [15,16 and 17], in study done by Henry [15] reported that low levels of plasma magnesium in type 2 diabetic patients, and suggested that, magnesium ions has a fundamental role in carbohydrate metabolism in general, and in the action of insulin in particular. Henry also stated that magnesium is a cofactor in the glucose transporting mechanism of cell membrane and for various enzymes in carbohydrate oxidation. On the other hand, we disagree with Masood*et al*[18] who reported no significant difference was

found in magnesium level when compared with control subject.

The present study showed a negative correlation between the plasma levels of magnesium and duration of diabetes (table 3), this result also agrees with a study done by Jeffery [19] who reported a negative correlation between the plasma levels of magnesium and duration of diabetes.

Since control of glycaemia has an effect on magnesium levels, the measurement of glycated hemoglobin not only shows promise of being a successful approach to the monitoring of diabetic patients but also provides a conceptual framework for the pathogenesis of secondary sequelae of diabetes. Statistically a significant correlation was found between the HbA1c level and the plasma level of magnesium (a negative correlation) table (4), this result agrees with the study done by Kudu *et al* [16], who reported that there was a negative correlation between magnesium level and HbA1c level. The result of the present study revealed that uncontrolled diabetic patients are at higher risk of hypomagnesaemia.

In this study, the level of magnesium was not affected significantly by gender (p. value > 0.05) as shown in figure (3).This result is in agreement with Masood et al [18] who reported there was no association between gender and magnesium level in type-2 diabetic patients. Also the level of HbA1c was not affected significantly by sex (p. value > 0.05) as shown in figure (4); this finding was in agreement with yang *et al*[22], who reported that HbA1c level is not affected by sex.

White and Campbell suggested a link between hypomagnesaemia and hyper glycaemia and they suggested that patients who have hypomagnes-aemia and diabetes mellitus should receive magnesium supplem-entation[23].

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